MOLECULAR EPIDEMIOLOGY OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN UNIVERSITY OF JOS COMMUNITY

BY

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DECLARATION

I declare that the work reported in this dissertation entitled 'Molecular Epidemiolgy of Methicillin Resistant *Staphylococcus aureus* in University of Jos Community' was carried out by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University under the supervision of Prof. J. A. Onaolapo, Prof. Y. K. E. Ibrahim and Prof. G. O. Adeshina

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been presented in any previous application for another degree or diploma at any other University.

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…… ……. …….....

Name Signature Date

CERTIFICATION

This dissertation entitled 'Molecular Epidemiology of Methicillin Resistant *Staphylococcus aureus* in University of Jos Community', meets the regulations governing the award of the Degree of Doctor of Philosophy (Ph.D) of the Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the glory of God Almighty

And to

Prof. T. A. Iranloye

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Life is a journey and as one goes along the way, there are people one meets who give helping hands to make the journey less burdensome. Indeed this programme is one of such journeys. Therefore, I want to use this opportunity to appreciate my helping hands.

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ABSTRACT

Staphylococcus aureus is a virulent pathogen that is currently not only the most common cause of infections in hospitalized patients worldwide but increasingly also as a community associated pathogen. This study was aimed at determining the prevalence of methicillin resistant *S. aureus* among apparently healthy student population of the University of Jos, Jos Nigeria. Two hundred and seventeen (217) urine samples were screened for *Staphylococcus aureus*. Isolates were characterized by conventional cultural and biochemical methods including rapid test kits (MicrogenID test kit). Their susceptibility profiles were determined against a panel of eleven antibiotics including cefoxitin and oxacillin. The MRSA status were confirmed by the phenotypic test using disc diffusion test method and molecular analysis employing multiplex PCR for the detection of the *mecA, nuc* genes and *spa* typing. Of the 217 samples collected 171 (78.8%) were positive for Staphylococcal species while 73(54.1%) of 135 screened with the MicrogenD test kit were identified as *S. aureus*. The susceptibility test showed that the highest level of resistance was demonstrated against penicillin (91.8%) followed by cefotaxime (80.7%), ofloxacin (79.2%), tetracycline (64.9%) and oxacillin (60.8%) in that order. None of the 33 isolates subjected to E-test was Vancomycin resistant. Phenotypic detection of methicillin resistance with the use of cefoxitin and oxacillin discs gave a prevalence of 79.5% (136/171) of the Staphylococcal isolates and 47.9% of *S. aureus*. These isolates were resistant to the betalactam antibiotics and were multidrug resistant. They were, however largely susceptible to ciprofloxacin, vancomycin, erythromycin, gentamicin and cotrimoxazole. 88.30% had MAR index greater than 0.3. Sixteen of the *S. aureus* isolates had the *mecA* and *nuc* genes. The nucleotide sequence of *16S rRNA* gene of the 16 isolates showed that 12 of them had 77-92% identity with *S. aureus* strain N315. Three isolates (5, 9 and 16) could not be typed as there was no similarity with GenBank data base. Four of the isolates have common lineage with 8 BWI (2), S1123 and 363LF (1) and CLRSA1 (1) strains. The study shows that *mecA* gene is present in the study population. *mecB* gene was also detected in the 16 isolates. This is the first time this gene is being reported in *S. aureus* and this may increase the level and magnitude of resistance by MRSA. The identification of *mecA* and *mecB* genes in community isolates implies that any outbreak of infections caused by these strains may pose a threat to public health as these strains are resistant to all antibiotics tested except ciprofloxacin, vancomycin and co-trimoxazole.

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CHAPTER ONE

1.0 INTRODUCTION

The discovery of antimicrobial agents (antibiotics and related medicinal drugs) has substantially reduced the threat posed by infectious diseases. Their use coupled with improvements in sanitation, housing, nutrition, and the advent of widespread immunization programmes has led to a dramatic drop in deaths from these diseases which were previously widespread, untreatable, and frequently fatal, thereby contributing immensely to saving lives and eased the sufferings of millions of people (Gelband *et al.*, 2015; WHO, 2015). Their role has expanded from treating serious infections to preventing infections in surgical patients, protecting cancer patients and people with compromised immune systems, and promoting growth and preventing disease in livestock and other food animals (Gelband *et al.*, 2015).

However, the overuse and misuse of these antimicrobial agents and the emergence and re-emergence of some infectious diseases is hampering efforts towards effective control and eventual elimination of these most dangerous infectious diseases. These gains of antibiotic therapy are now seriously jeopardized by the emergence and spread of microbes that are resistant to cheap and effective first-choice or "first-line" drugs. Once-treatable infections are becoming difficult to cure, raising costs to healthcare facilities, and patient mortality is rising, with costs to both individuals and society. Decreasing antibiotic effectiveness has risen from being a minor problem to a broad threat, regardless of a country's income or the sophistication of its healthcare system (Gelband *et al.*, 2015).

The consequences are severe. Infections caused by resistant microbes fail to respond to treatment, leading to prolonged illness and greater risk of death; longer periods of infectivity which increase the numbers of infected people moving in the community and thus exposing the general population to the risk of contracting a resistant strain of infection (WHO 2017).

Resistance to “first-line” drugs results in switch to second- or third-line drugs which are generally much more expensive and sometimes more toxic as well (WHO, Fact sheet, 2015). Resistance to antimicrobials is a natural biological phenomenon due to “selective pressure”. The microbes which adapt and survive carry genes for resistance, which can be passed on. Bacteria are particularly efficient at enhancing the effects of resistance, not only because of their ability to multiply very rapidly but also because they can transfer their resistance genes, when they replicate or to related species through processes like conjugation, transduction, and transformation. Resistance to a single drug can thus spread rapidly through a bacterial population. Given the ease and frequency with which people now travel, antibiotic resistance is a global problem, requiring efforts from all nations and many sectors.

In the early to late twentieth century, medicine and science were able to stay ahead through the discovery of potent new classes of antimicrobials. This process has since slowed down to a virtual standstill, partly because of misplaced confidence that infectious diseases had been conquered (at least in the industrialized world), increase in the number of infections, and the dearth of new discoveries probably due to poor funding.

The WHO launched the first global strategy for combating the serious problems of the emergence and spread of antimicrobial resistance in 2001. The strategy builds on a number of activities which include monitoring the global emergence and spread of antimicrobial resistance by establishing laboratory-based networks for the surveillance of antimicrobial resistance by microorganisms. Also in May 2015, the World Health Assembly endorsed a global action plan aiming to ensure prevention and treatment of infectious diseases with safe and effective medicines (WHO, Fact sheet, 2016).

Evidence from around the world as reported by the WHO indicates an overall decline in the total stock of antibiotic effectiveness: resistance to all first-line and last-resort antibiotics is rising (WHO, 2016). The report noted that while there are some new antibiotics in development, none of them are expected to be effective against the most dangerous forms of antibiotic-resistant bacteria. The patterns of bacteria resistance to specific antibiotics differ regionally and by country, mirroring patterns of infectious disease and antibiotic use. The U.S. Centers for Disease Control and Prevention (CDC) estimates that antibiotic resistance is responsible for more than 2 million infections and 23,000 deaths each year in the United States, at a direct cost of $20 billion and additional productivity losses of $35 billion (CDC 2013). In Europe, an estimated 25,000 deaths are attributable to antibiotic-resistant infections, costing €1.5 billion annually in direct and indirect costs (EMA and ECDC 2009). Although reliable estimates of economic losses in the developing world are not available, it is estimated that 58,000 neonatal sepsis deaths are attributable to drug- resistant infections in India alone (Laxminarayan et al. 2013). Studies from Tanzania and Mozambique indicate that resistant infections result in increased mortality in neonates and children under five (Kayange *et al.* 2010; Roca *et al.* 2008).

The bacterial infections which contribute most to human diseases are also those in which emerging antimicrobial resistance is most evident: diarrhoeal diseases, respiratory tract infections, meningitis, sexually transmitted infections, and hospital acquired infections. Some important examples include penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, multi-resistant salmonellae, and multi-resistant *Mycobacterium tuberculosis* and HIV/AIDS virus (WHO, 2000).

Staphylococcus aureus has been known to be a major pathogen causing a wide spectrum of clinical manifestations, such as wound infections, pneumonia, septicaemia, and endocarditis

with beta-lactam antibiotics being the drugs of choice for therapy (Grisold *et al*., 2002; Onanuga *et al*, 2006). Since the introduction of methicillin into clinical use in 1961, the occurrence of methicillin-resistant *S. aureus* (MRSA) has steadily increased and nosocomial infections caused by such isolates have become a serious problem worldwide (Benner and Kayser, 1968; Lowy 1998; Boucher *et al.*, 2010). Considerable research efforts have been put forward to improve our understanding of its complex pathogenesis. In spite of these efforts, the burden of staphylococcal infections is still on the rise. The unresolved questions regarding this pathogen, include: (i) the nature of the driving forces behind the rise and decline of methicillin-resistant *S.aureus* (MRSA) clones; (ii) the mechanisms by which a commensal becomes a pathogen; (iii) the molecular underpinnings of toxin overexpression in hypervirulent MRSA clones such as USA300; and (iv) the repeated failures of anti-*S.aureus* vaccine approaches (Rasigade and Vandenesh, 2014).

1.1 Statement of Researh Problem

Staphylococcus aureus is a virulent pathogen that is currently the most common cause of infections in hospitalized patients worldwide. *S. aureus* infection can involve any organ system. The core resistance phenotype that seems to be most associated with the persistence of *S. aureus* in the hospital is methicillin resistance. The increase in the resistance of this virulent pathogen to antibacterial agents, coupled with its increasing prevalence not only as a nosocomial pathogen but also in community infections without the usual risk factors associated with hospital environment is of major concern and documented in several reports (Archer, 1998; Bukharie *et al.*, 2001; Rasigade and Vandenesh, 2014). The patterns of bacteria resistance to specific antibiotics differ regionally and by country, mirroring patterns of infectious disease and antibiotic use. Methicillin-resistant *Staphylococcus aureus* (MRSA) has declined in incidence in Europe, the United States and Canada over the past eight years, to 18 percent, 44 percent, and 16 percent, respectively. It also has begun to decline in South Africa

(to 28 percent), where antibiotic stewardship is taking hold. In sub-Saharan Africa, India, Latin America, and Australia, it is still rising (Gelband *et al.*, 2015). In Nigeria, studies on methicillin-resistant Staph aureus have been conducted, particularly in southwestern zone (Adetayo *et al.*, 2014). In their review, Falagas *et al*. (2013) observed that the prevalence of hospital associated MRSA appears to be different in the northern compared with the southern part of the country. For the northern part of Nigeria, the prevalence of MRSA varied from 9% in north-central to 28% in north-east, while in the south-west part of the country it was between 20% and 41%. Despite this epidemiological data on MRSA in Nigeria, available data are still relatively limited when compared to information from developed countries which may be attributable to high level of awareness of MRSA infections and its clinical and societal consequences (Adetayo *et al.*, 2014). These data though limited have been mainly on hospital associated MRSA. The few community associated MRSA studies documented in Nigeria are from the Southern part of the country (Owolabi and Olorioke, 2015; Ibe *et al.*, 2013; Iroha *et al.*, 2014; Ike *et al.*, 2016; Akerele *et al.*, 2015). No similar studies have been carried out in the Northern part of the country, of which Jos metropolis is part. University of Jos is located in the heart of Jos metropolis, with a large student population. Considering the fact that MRSA prevalence varies based on geographical location, type of hospital and study population, it becomes necessary to determine the prevalence of CA-MRSA in a section of Jos metropolis.

1.2 Justification for the Research

Among the strategies recommended by the WHO for curbing antibiotic resistance is promoting regional antibiotic resistance surveillance in addition to encouraging new product development (WHO 2016). *S. aureus* though not a threat to healthy individuals, causes serious infections in immune compromised individuals such (HIV positive, traumatized individuals, etc.). The *SCCmec* resistant island of the MRSA carries genes that codes for resistance to other antibiotics. A knowledge of the presence and prevalence of MRSA carrying the *mecA* gene in

this environment especially in community isolates will promote the understanding of the resistance problem and the best approach to tackling it. Moreover, surveillance studies on the prevalence of MRSA in this environment are limited. This study will therefore contribute to the data of knowledge available on this subject.

1.3 Aim and objectives:

The aim of this work is to determine the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in sample population of healthy individuals of the University of Jos community who are not on antibiotics, using molecular techniques.

1.3.1 Specific objectives*:*

1. Isolate, purify and characterise Staphylococci spp. from urine specimens from the University of Jos community
2. Determine the antibiotics susceptibility profiles of the isolates by agar diffusion method and the multiple antibiotic resistance (MAR) indexing.
3. Phenotypically identify methicillin resistant isolates from the diffusion test and by the oxacillin breakpoint test.
4. Determine the incidence of vancomycin resistance by the dilution and break-point test.
5. Determine the presence of beta-lactamase and plasmids among the resistant *S. aureus* isolates.
6. Molecularly confirm the MRSA using PCR (*mecA*, 16SrRNA, *spa*, *nuc*).

1.4 Hypothesis

1.4.1: **Null Hypothesis:** There is no incidence of Methicillin resistance *Staphylococcus aureus* in healthy individuals of the University of Jos community.

1.4.2: **Alternate Hypothesis:** Methicillin resistance *Staphylococcus aureus* is prevalent among healthy individuals of the University of Jos community.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. History of antimicrobial chemotherapy

Antimicrobial chemotherapy describes the use and administration of a substance to a patient in order to exert a systemic antimicrobial action. There are three distinct eras in the history of chemotherapy. The first era was the use of alkaloids dating back to 1619 when cinchona bark extract was used to treat malaria. Thereafter, ipecacuanha root was found to be effective against dysentery in South America (Lee, 2008; Raju and Reddy, 2005). In this era, alkaloids, quinine and emetine were mainly the substances used.

Synthetic products initiated the second era and began with the discovery of salvarsan by Paul Ehrlich in 1909 which treated early stage syphilis. The notion that bacteria are not susceptible to any substance which is not toxic to humans was debunked by the discovery of prontosil. Prontosil was synthesized first by Klarer and Mietzsch in 1932. Domagk administered it for haemolytic streptococcus infections and found it effective. In 1936, Colebrook and Kenny found prontosil effective in puerperal fever. The mechanism of action of prontosil against organisms was found to be due to the release of para-aminobenzene sulfonamide from it. (Spring, 1975; Amunom *et al.*, 2011; Boulos, 2013). This finding precipitated the development of sulpha drugs starting with sulfanilamide which exhibited inhibitory action against streptococci *in vitro.* Thereafter, in 1938, sulphapyridine which was used to treat pneumococcal pneumonia, followed by sulfathiazole discovered in 1939 and sulfadiazine in 1941 (Abu-Bakar *et al.*, 2011; Becker *et al.*, 1996). Since then synthesis and development of antibacterial agents with improved pharmacological activities have continued to date.

The third era was the discoveries of antibiotics. The term ‘antibiotic’ given by Selman Waksman in 1942 was used to refer to substances produced by micro-organisms but

antagonistic to the growth of other micro-organisms when in high dilution (Spring, 1975; Singh, 2015). One of the early pioneers of inter-microbic antagonism was Pasteur. One of the best known products then was pyocyanase, an extract from *Pseudomonas aeruginosa.* Other researchers obtained extracts from *Penicillium* and *Aspergillus* which however, were too low in concentration to be effective. Dubo's quest to fight pyogenic cocci led to the discovery of tyrothrycin from *Bacillus breves*. However, tyrothrycin was toxic in humans though it has a systemic curative effect in mice (Spring, 1975; Aminov, 2010; Greek and Hansen, 2013). The positive outcome of this discovery was that Howard Florey and his colleagues were motivated to embark on a study of naturally formed antibacterial substances and penicillin was second on their list. Accidental sneezing on a petri dish led to the discovery of the antibacterial activity of the enzyme, lysozyme (Khardori, 2006). Sir Alexander Fleming discovered that bacteria formed colonies on the plate but none was found in the spots occupied by the mucus. However, further analysis indicated that lysozyme acted mostly against harmless organisms. Fleming's discovery of penicillin from cultures *Penicillium notatum* was another accidental finding. He went on vacation leaving a culture of staphylococci exposed in his laboratory. When he got back to his laboratory, he observed a mold in the petri dish along with a sizeable empty space between the mold and the staphylococcus growth. On observation under the microscope, he noted that something within the mold was attacking the bacteriium. He cultured the mold in broth and filtered. He noticed the filtrate had a substance that affects bacteria (Okonko *et al.*, 2010). However, it took several years for penicillin to be purified, its structure ascertained and its large scale production achieved. The first sample of penicillin yellow-brown powder was made by Florey and Chain and it was found to be a million times more potent than Fleming's original filtrate (Khardori, 2006). In 1941, the Fermentation Division of the newly created Northern Regional Research Laboratory in Peoria, Illinois was the first site for commercial production of penicillin. Staphylococcus, resistant to sulfonamides and lysozyme was found

sensitive to penicillin, hence, 95% of the wounded during the World War II lived after the administration of penicillin. Subsequently over time, penicillin molecule was modified to produce ampicillin, keflin and other drugs. Furthermore, the discoveries of the mycins followed: streptomycin in 1944, erythromycin in 1952. Streptomycin was invented after it was observed that penicillin could not help tuberculosis patients. Waksman and his students undertook a study of thousands of soil cultures to identify an antibiotic that would treat tuberculosis. Finally, the dirt and mold found on the neck of a sick chicken has the substance. Streptomycin was administered to a woman with advanced pulmonary tuberculosis and right lung surgically removed and it saved her life. As discoveries were underway, resistance to sulfonamide surfaced in gonococci, pneumococci and haemolytic streptococci (Spring, 1975; Aminov, 2010).

2.2. Progressive trends in antimicrobial chemotherapy

After the discovery of streptomycin, there was an exponential growth of pharmaceutical research and development. A variety of compounds were introduced into the market. More than 5000 antibiotics are presently known, about a thousand were carefully investigated and about 100 are currently used to treat infections (Khardori, 2006). Most of these antibiotics were extracted mainly from actinomycetes and bacteria. Most of the antibiotics are semi-synthetic while the others are synthetic. Table 2.1 shows the progressive discovery or publications of some antimicrobial agents from 1912 to 1983.

Table 2.1: Discovery or publication of some important antimicrobial agents

S/No	Antimicrobial Agents	Year discovered or published
1	Isoniazid	1912 - 1952
2	Prontosil	1934
3	Penicillin	1929 - 1940
4	Streptomycin	1944
5	Bacitracin	1947
6	Chloramphenicol	1947
7	Chlortetracycline	1948
8	Cephalosporin C, N and P	1948
9	Neomycin	1949
10	Nystatin	1950
11	Erythromycin	1952
12	Amphotericin B	1954
13	Vancomycin	1956
14	Rifamycin	1957
15	6APA	1957
16	Fusidic acid	1960
17	Lincomycin	1962
18	Gentamicin	1963
19	Josamycin	1964
20	Cephalothin	1964
21	Cefotaxime	1977
22	Clavulanate	1977
23	Carbapenems	1983
24	Quinupristin/dalfopristin	1999
25	linezolid	2000
26	tigecycline	2005
27	telithromycin	2005

(Source: Neu, 1994)

The β-lactams were explored and introduced from the 1940s to 1980s. The first aminoglycoside, streptomycin was introduced in 1942 followed by neomycin which is toxic and not suitable for system administration. Thereafter, kanamycin was introduced in 1957 and became the aminoglycoside of choice until the entrance of gentamicin in 1963. Gentamicin was a breakthrough for infections caused by *Pseudomonas aeruginosa* and other aerobic Gram-negative bacilli. Other aminoglycosides that were introduced include Tobramycin, amikacin, and netilmicin in 1968, 1972, and 1975, respectively.

Also in the 1950s, a prototype macrolide, erythromycin was introduced. Macrolides are mainly bacteriostatic, though bactericidal actions may take place under some conditions or against specific organisms (Khardori, 2006). In addition, clarithromycin and azithromycin, were made available. They are more stable, have a longer half-life, better tolerated and have broader spectrum than erythromycin. Then the ketolides such as telithromycin, a new class of macrolides was available in 2005. It is a semi-synthetic derivative of erythromycin which has a higher (10- to 100-fold) affinity for the ribosome binding site (domain V). Telithromycin is used in the treatment of community-acquired pneumonia, acute exacerbation of chronic bronchitis, and acute sinusitis.

The first tetracycline, aureomycin was discovered in 1948 (Smilack, 1999). The tetracyclines remain the most widely prescribed antibiotics after amoxicillin and erythromycin (Khardori, 2006). This class of antibiotics includes Tetracycline, oxytetracycline, doxycycline, minocycline, and demeclocycline. Doxyclycline is the antibiotic of choice amongst tetracycline due to its low cost, reduced toxicity, and superior pharmacokinetic properties.

Lincomycin, a lincosamide was first isolated from *Streptomyces lincolnesis* in 1962. In 1966, clindamycin derived from lincomyin was introduced having better absorption and improved antibacterial activity. The lincosamides have similar mechanism as macrolides which is the

inhibition of bacterial protein synthesis by reversible binding to 50S ribosomal subunit. The implication of this is that clindamycin susceptible organisms that are resistant to erythromycin may subsequently be resistant to clindamycin. However, in addition, Clindamycin facilitates opsonisation, phagocytosis, and intracellular killing of bacteria (Khardori, 2006). Clindamycin's activity is against anaerobic gram-positive and gram-negative organisms, except Clostridium difficile and Fusobacterium varium; and aerobic gram+ve bacteria except enterococci.

Trimethoprim-sulphamethoxazole (co-trimoxazole) is one of the most widely used antibacterial combination therapy. Co-trimoxazole is active against many aerobic gram-positive and gram-negative bacteria encountered in community-acquired infections. In addition, some nosocomial pathogens, such as *Stenotrophomonas maltophilia, Burkholderia cepacia, and Serratia marcescens,* are also susceptible to co-trimoxazole as well as *Nocardia asteroides, Listeria monocytogenes, Pneumocystis jirovecii, Isospora belli*, and cyclospora.

Vancomycin was the first glycopeptide isolated in the 1950s from *Streptomyces orientalis*. It was used to treat infections caused by penicillin-resistant *Staphylococci* and other Gram-positive bacteria. However, with the advent of oxacillin and naficillin, the use of vancomycin decreased until the emergence of methicillin-resistant *Staphylococci* in the 1980s. The bactericidal activity of vancomycin against all common Gram-positive bacteria persisted for more than 3 decades before emergence of vancomycin–intermediately susceptible *S. aureus* and vancomycin-resistant *S. aureus* which has created a need for alternatives for the management of serious infections caused by resistant Gram-positive organisms (Khardori, 2006). Another glycopeptide, teicoplanin was derived from the fermentation products of *Actinoplanes teicnomyceticus.* Although, teicoplanin is chemically similar to vancomycin, it is more lipophilic leading to rapid and excellent tissue penetration as well as intracellular

concentration. At physiological pH, it is water soluble; it releases slowly from the tissues and has long terminal half-life of 150 hours.

Fluoroquinolones are synthetic antibacterial agents that were made available in the mid-80s to optimize antibacterial activity against Gram-negative and Gram-positive bacteria as well to improve pharmacokinetic properties and safety (Walker, 1999). The prototype quinolone was nalidixic acid and considered as the first generation fluoroquinolone. The second-generation fluoroquinolones, such as norfloxacin, ciprofloxacin, and ofloxacin, have mainly Gram-negative activity. The most active fluoroquinolone against *P. aeruginosa* remains ciprofloxacin. On contrary, the third- and fourth-generation fluoroquinolones, such as levofloxacin, gatifloxacin, and moxifloxacin, have better activity against Gram-positive organisms, especially *S. pneumoniae*, including penicillin-resistant strains (Khardori, 2006).

Pristinamycin, was the first streptogramin antibacterial extracted from *Streptomyces pristinaespiralis* about 41 years ago in France and used primarily in the treatment of respiratory and skin structure infections. Quinupristin/dalfopristin (30:70) was made available in United States in 1999 for the treatment of infections caused by vancomycin-resistant *Enterococcus faecium* and other Gram-positive organisms for which the other available antibacterial agents are not effective or tolerated (Nadler *et al.*, 1999). The combination is also active against *Neisseria meningitidis, Moraxella catarrhalis, Legionella pneumophilia,* and *Mycoplasma pneumonia.*

Initially, oxazolidinones were developed in the 1970s for antibacterial activity against bacteria and fungi in tomatoes and other plants. They were explored in the 1980s for human infections but due to monoamine oxidase inhibition and bone marrow toxicity, they were not developed for human use (Khardori, 2006). However, chemical modifications led to linezolid, a safer agent with superior pharmacokinetics was made available in United States in the year 2000.

Linezolid was introduced for treatment of infections caused by vancomycin-resistant enterococci and respiratory and skin structure infections caused by Gram-positive bacteria.

In recent times, the global anti-infective market exceeds $20 billion dollars annually with antibacterial agents comprising the bulk of the trade (Okonko *et al.*, 2010). Furthermore, the emerging microbial resistance led to the study of how multicellular organisms defend themselves against infections from opportunistic pathogens. The study led to the exploration of macromolecular compounds such as peptides and lipopeptides as antibacterial agents. Daptomycin, a cyclic lipopeptide that exhibits rapid, concentration-dependent bactericidal activity in vitro against a broad spectrum of Gram-positive pathogens was approved in 2004 (Arbeit *et al.*, 2004). Daptomycin was used in infections caused by organisms resistant to currently available agents such as oxacillin, vancomycin, and linezolid. The study also led to the discovery of antimicrobial peptides from the humoral immune system of silk moth (*Hyalophora cecropia*) (Engler *et al.*, 2012). These antimicrobial peptides are broad spectrum agents which attack Gram-negative and Gram-positive bacteria and fungi. Actually the discovery of antimicrobial peptides began in the 1980s and currently there are more than a thousand of them in the antimicrobial peptides database. However, only a few of them have gone into clinical application.

As chemical modifications are undertaken in the quest to find new antibacterial agents to combat bacterial resistance, tigecycline, a broad-spectrum glycylcycline antibiotic was derived from tetracycline minocycline (Noskin, 2005). It was made available in 2005 for the treatment of treatment of complicated intra-abdominal infections and complicated skin and skin structure infections (Babinchak *et al.*, 2005; Grosse *et al.*, 2005).

2.3. The beta-lactam antibiotics

Penicillin is a protype β-lactam and other antibiotics with a β-lactam ring include cephalosporins, carbapenems, and monobactams. They inhibit a group of enzymes - transpeptidase, carboxypeptidase, and endopeptidase – collectively referred to as penicillin binding proteins. The enzymes inhibit the development of the peptidoglycan structure and consequently initiate cell lysis and death (Tomasz, 1979; Khardori, 2006).

2.3.1. Penicillins

The initial utilization of penicillin in the early 1940s was followed by the emergence of penicillinase-producing *Staphylococcus aureus.* This motivated the development of penicillinase-resistant penicillins which include methicillin, oxacillin, and nafcillin. The acyl side chain prevents the disruption of the β-lactam ring by penicillinase. Aminopenicillins such as ampicillin, amoxicillin, and bacampicillin were developed because of the need to have antibiotics that have activity against Gram-negative bacteria. Carboxypenicillins (carbenicillin and ticarcillin) and ureidopenicillins (mezlocillin, azlocillin, and piperacillin) broadened antibacterial activity to include Enterobacteriaceae, such as *Klebsiella spp*, *Enterobacter spp*, and *Pseudomonas aeruginosa*. Gram-positive bacteria became resistant to penicillins due to the production of β-lactamase. B-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) were developed and administered in combination with penicillins. Such combinations included amoxicillin-clavulanate, ampicillin-sulbactam, ticarcillin-clavulanate, and piperacillin-tazobactam (Khardori, 2006).

2.3.2. Cephalosporins

Cephalosporins are a large family of broad spectrum β-lactam agents introduced in the 1960s. They were favoured because of their low level of drug-associated toxicity and good pharmacokinetic profiles. The first generation cephalosporins such as cefazolin, cephalexin are

active against aerobic, Gram-positive cocci, including methicillin-susceptible *S aureus*. Second generation cephalosporins (e.g. cefuroxime, cefamandole, cefoxitin) in comparison to the first, are more active against selected Gram-negative organisms, such as *Klebsiella spp, E coli,* and *Proteus spp* while the third generation cephalosporins (e.g. cefotaxime, ceftriaxone), though broadspectrum, are most active against Gram-negative aerobic organisms. Cefepime, a fourth generation cephalosporins, has an extended spectrum against both Gram-positive and Gram-negative organisms, including P aeruginosa and are resistant to betalactamases. In addition, the antibiotics cefoxitin and cefotetan (technically cephamycins) are active against anaerobic bacteria as well (Khardori, 2006).

2.3.3. Carbapenems

Carbapenems, are β-lactams antibiotics that have a broad spectrum activity against aerobic and anaerobic Gram-negative and Gram-positive bacteria. However, they are not active against *Stenotrophonomas maltophilia.* This class of β-lactam antibiotics was introduced in the 1980s. Imipenem in combination with cilastatin was made available in 1985 while meropenem was introduced in 1996. Carbapenems are bactericidal against susceptible bacteria except enterococci. While Imipenem is more active against gram-positive cocci *in vitro,* meropenem has better in vitro activity against gram-negative bacilli. In addition to the two drugs in this group, ertapenem was recently introduced. However, it does not have activity against enterococci or *Pseudomonas aeruginosa* (Khardori, 2006; Zhanel *et al.*, 2007).

2.3.4. Monobactams

Monobactams are synthetic beta-lactam compounds wherein the beta-lactam ring is alone and not fused to another ring (in contrast to most other beta-lactams, which have at least two rings). Aztreonam is the only available drug in this class, monobactams and it is synthetic. The β-lactam ring is activated by its sulfonic acid group. The antibacterial activity of aztreonam is

limited to aerobic gram-ve bacilli because of its lack of affinity for the penicillin-binding proteins of gram-positive and anaerobic bacteria. In comparison to aminoglycosides, it is non-nephrotoxic and weakly immunogenic (Moore *et al.*, 1992). Consequently, aztreonam is a useful non-nephrotoxic substitute to aminoglycosides in susceptible bacteria but administered with caution to patients with significant penicillin hypersensitivity (Khardori, 2006).

2.4. Bacterial drug resistance

Bacterial resistance is the ability of bacteria to continue to grow and multiply in the presence of an antibiotic to which it had previously been susceptible (WHO 2016). Bacterial resistance emerged between 1930s and 1940s after the introduction of antibacterial agents. For instance, resistance to trimethoprim/sulphamethoxazole by streptococci was described in *in vitro* and animal experiments in early 1930s. In addition, the bacterial enzyme penicillinase that inactivates penicillin was discovered after the introduction of penicillin (Virk and Steckelberg, 2000). However, because there was progressive development of newer antibacterial agents, bacterial resistance was overshadowed then. *S. pneumonia* resistance to penicillin was first reported by researchers in Boston in 1965, then in Australia and South Africa in 1967 and 1977 respectively (Okonko *et al.*, 2010). Since these reports, there has been increasing resistance of *S. pneumonia* to penicillin over the world. In addition, a few years after penicillin was published, resistant staphylococci were identified. Bacterial resistance to antibacterial agents was anticipated; however, what was not is the global indiscriminate use of the antibacterial agents which has helped to escalate the rate of resistance. Presently, resistance is exhibited against all available classes of antibacterial agents posing a grave medical problem. Even more challenging is that the economy and regulation has served to reduce the development and introduction of newer antibacterial agents suggesting that the antibacterial agents currently in the market may be all that will be available in the coming decade (Rice, 2012). Furthermore, the multi-resistance nature exhibited by many pathogens and the varied mechanisms of

resistance make identifying and developing safe new agents with broad activity extremely difficult (Rice, 2012).

2.4.1. Mechanisms of bacterial resistance to drugs

Resistance of bacteria to antibacterial agents can be intrinsic or acquired. The naturally occurring intrinsic resistance of bacteria to any antibacterial agent determines the spectrum of activity of the agent when it is made available. For instance, *P. aeruginosa* was resistant to vancomycin from the onset when vancomycin was introduced and anaerobic bacteria were resistant to aminoglycosides (Khardori, 2006). Acquired resistance is achieved when bacteria which has been susceptible and exposed to an antibacterial agent develop resistance. Acquired resistance is attained by gene mutation or through the acquisition of exogenous resistance determinants (Rice, 2012).

Micro-organisms often become resistant simply by:

- Preventing entrance of the drug into the envelope's membrane.
- Pumping the drug out of the membrane after it has entered (translocases).
- Inactivating drugs through chemical modification (hydrolysis).
- Modification of target enzyme or organelle so that it is no longer susceptible to the drug.
- Using an alternate pathway to bypass the sequence inhibited by the agent or
- Increasing the production of target metabolite (Okonko *et al.*, 2010).

Actually, there are many mechanisms by which bacteria protect themselves and can be classified into 4 basic types as shown in Figure 2.1

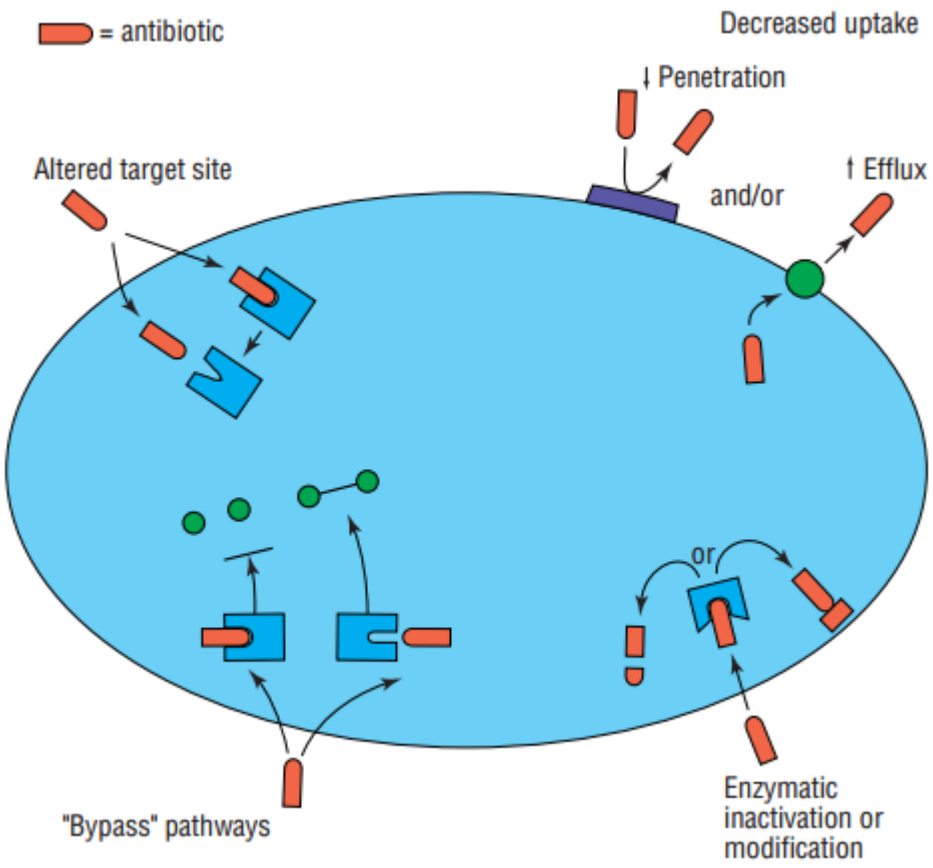


Fig 2.1: Four major biochemical mechanisms of antibiotic resistance. (Source: Hawkey, 1998)

With antibacterial agent modification, the bacteria resistant to the antibacterial agent retain the same sensitive target as the sensitive bacteria but the antibacterial agent is prevented from reaching the resistant bacteria (Hawkey, 1998). This is typical in the case of the β-lactams. The β-lactamase splits the four membered β-lactam ring rendering the antibiotic inactive. β-lactamases are widely distributed in many bacteria – Gram-negative and Gram-positive. However, the degree of inhibition varies amongst the β-lactamase inhibitors, such as clavulanic acid.

The second mechanism by which bacteria protect themselves is by preventing the antibacterial agent from penetrating into the cell or pumping it out faster than it can flow in. Access to gram-ve bacterial cell by β-lactams is through a water filled hollow membrane protein called porin. Figure 2.2 depicts the interaction of β-lactams with Gram-negative and Gram-positive bacterial cells.

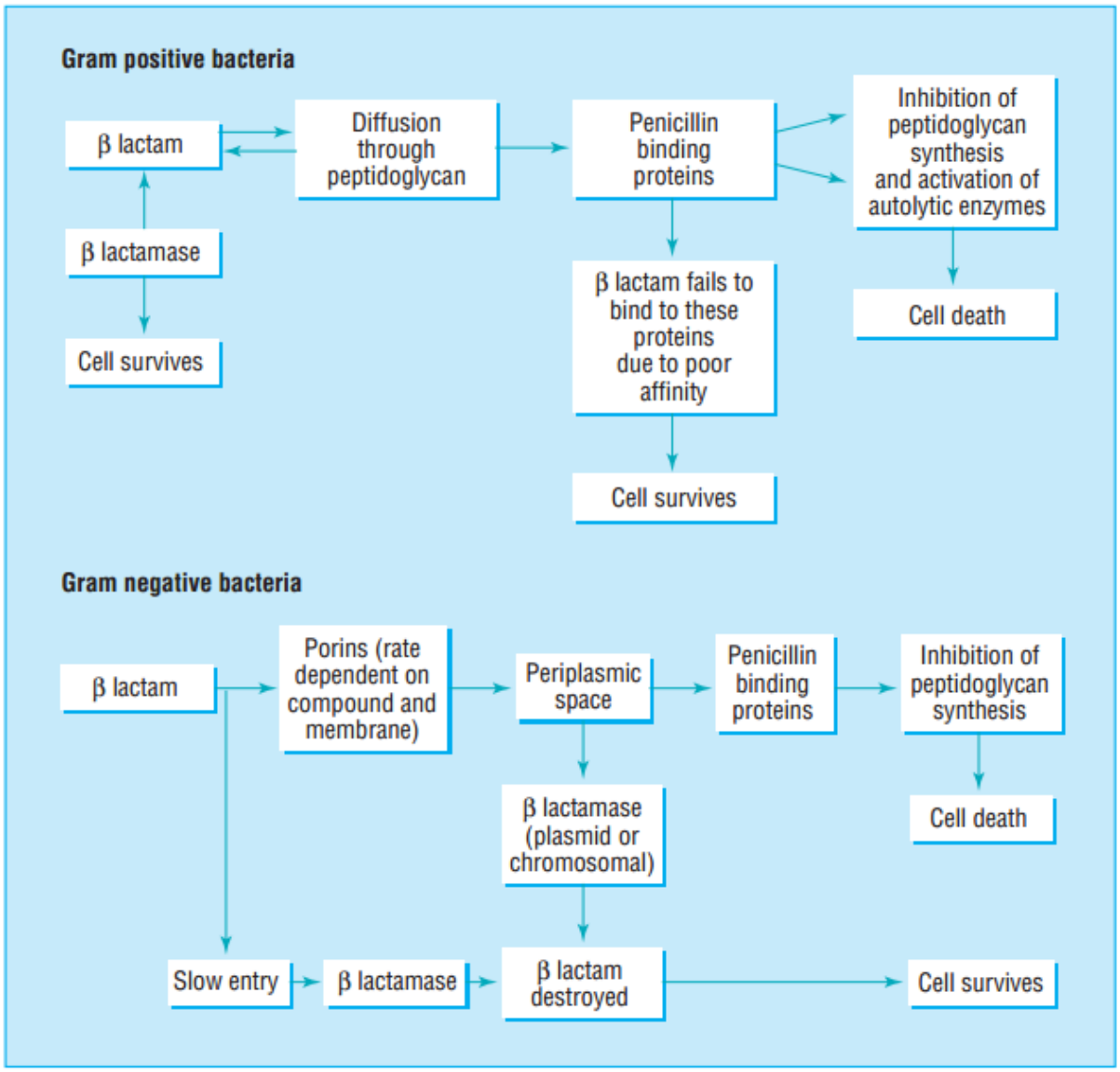


Fig 2.2: Interplay of β-lactams with Gram positive and Gram negative bacteria. (Source: Hawkey, 1998)

The third mechanism, the alteration of primary site of action may be due to structural changes at the target site. The antibacterial agent penetrates the cell, arrives at the target but is unable to inhibit the activity of the target due to structural changes. For instance, most of *Streptococcus pneumoniae* which were susceptible to penicillins and cephalosporins can acquire DNA from other bacteria which change the enzyme responsible for cell wall synthesis such that they develop low affinity for the antibacterial agents and hence, become resistant. However, the enzyme, although structurally changed is still able to synthesize peptidoglycan.

The fourth mechanism of bacterial protection is the production of an alternative target (often enzyme) that is resistant to inhibition while continuing to produce the original sensitive target (Hawkey, 1998). An example is the production of an alternate penicillin binding protein (PBP2a) in addition to the 'normal' penicillin binding protein by methicillin resistant *Staphylococcus aureus* (MRSA). Hence because PBP2a is not inhibited by antibacterial agents such as flucloxacillin, the cell continues to synthesize peptidoglycan which ensures that the cell survives.

2.4.2. Transferable resistance

One plausible and one of the most likely factors promoting the exhibition of resistance against all classes of antibacterial agents is due to transferable resistance. Transferable resistance was first identified in 1959, when a study showed that plasmids transfer resistance genes from Shigella to E coli (Khardori, 2006). The various mechanisms by which plasmids and other genetic materials are transferred between bacteria are:

- **Transduction:** Bacteriophages are used to enclose DNA plasmids and transferred to another organism of the same species. This mechanism is observed in the transfer of β-lactamase production from a penicillin-resistant to a penicillin-susceptible staphylococcus.

- **Transformation:** From dead bacteria, naked DNA can be taken up by certain bacteria species resulting in an altered genotype including antibiotic resistance. The mechanism of transformation is the principle in the laboratory behind DNA recombinant technology, in which this process is facilitated by various experimental steps. It is envisaged to be the main route by creation of a ‘‘mosaic penicillin-binding protein gene” for the spread of penicillin resistance in *S pneumonia* (Khardori, 2006).

- **Conjugation:** This refers to a unilateral transfer of genetic material which may occur between bacteria of the same or different genera. A class of plasmids referred to as resistance (R) factors carry genes for resistance to antibacterial agents and heavy metals and the transfer occurs through conjugation (mating) facilitated by fertility (F) factor. This is accomplished by the extension of sex pili (fimbriae) from the donor (F+) cell to the recipient. The transfer to the recipient cell of plasmids or other extrachromosomal DNA particles is undertaken through these protein tubules. Hence, a series of closely linked genes may transfer resistance to multiple antibacterial agents and such resistance transfer factors (RTF) are the most regular method of propagation of multi-drug resistance among Gram-negative bacteria. Resistance is acquired to penicillin and cephalosporins by plasmids carrying genes for the formation of β-lactamase. Plasmids also code for acetyltransferase that inactivates chloramphenicol and for enzymes that acetylate, adenylate, or phosphorylate various aminoglycosides. In addition, they determine the active transport of tetracyclines across the cell membrane and code for many other enzymes that inactivate antibacterial agents.

- **Transposons:** This method of acquiring resistance is undertaken by transposons. Transposons also referred to as transposable elements or jumping genes are DNA sequences that migrate from one location on the genome to another. DNA sequences are transferred between plasmids or between a plasmid and a portion of a bacterial

chromosome. Transposition extends the range of bacteria to which plasmids spread. It is envisaged that MecA gene discovered in methicillin resistant Staphylococcus aureus (MRSA) may have been acquired by transposition.

2.4.3. The genesis of resistance genes

The studies of pre-antibiotic bacterial populations and the analysis of the development of bacterial resistance to antibiotics such as tetracycline (after their introduction into the environment) suggested that antibiotic resistant microbes were absent from human and animal flora (Mazel and Davies, 1999). The studies therefore suggested that there is a direct correlation over time with the use of antibacterial agents and the bacterial resistance. Initially, during the early introduction of antibacterial agents, the biochemical and molecular mechanisms of resistance were not understood. Antimicrobial resistance was identified in the early 1940s in streptococci, gonococci, and staphylococci and the concept of multi-drug therapy was introduced for tuberculosis after the recognition of mutation of target genes for streptomycin in *M. tuberculosis*. In 1950s, during epidemics of intestinal infection in Japan, there was a rapid resistance of *Shigella sp* to antibiotics. This incident was attributed to the appearance and dissemination of plasmids encoding antibiotic resistance (R plasmids). Understanding the evolution and dissemination of bacterial resistance requires the appreciation of the rapidity of the constant exchange of bacteria among human, environmental, animal, and agricultural sources throughout the world (Khardori, 2006). It is perceived that the currently observed bacteria host harbouring the resistance plasmid may not have been the bacteria from which determinants of antibiotic resistance were derived (Hawkey, 1998). This is buttressed by the DNA sequencing studies of β-lactamase and aminoglycosides inactivating enzymes, though they show similarities in the protein studies of the two families, there are significant sequence differences. It would be challenging to derive a model by which bacteria evolution could have occurred by mutation alone from common ancestral genes. This is due to possibilities of

derivation being from a large and diverse gene pool which is presumed to be occurring with environmental bacteria already. Resistance determinants found in bacteria and fungi that produce antibiotics are similar to those found in clinical bacteria. Exchange of genes may occur in soil or even more in the GIT of humans or animals. In addition, it has been discovered that commercial antibiotics are contaminated with chromosomal DNA from the producing industrial microbes. Such DNA contains identifiable antibiotic resistance gene sequences that can be amplified and identified by the polymerase chain reaction (Webb and Davies, 1993). The study by Webb and Davies (1993) suggested that the development of resistance in clinically important bacteria may have been arisen from the presence of specific DNA sequences in certain antibiotics used as growth promoters in animals; and therapy and prophylaxis in humans. This is in addition to the heavy selective pressure and indiscriminate use of antibacterial agents in medical practice. Hence, antibiotic restriction wherever attainable is being suggested (Österblad *et al.*, 2001).

2.4.4. Resistance to β-lactams

β-lactams antibiotics functions by binding to penicillin-binding proteins (PBPs) – cell wall synthesis enzymes. The binding of β-lactams to PBPs causes the inhibition of the synthesis of peptidoglycan resulting in the weakening of the cell wall and subsequent inhibition of cell growth and frequent cell death (Rice, 2012). The three mechanisms by which bacteria are resistant to β-lactams are reduced access to the PBPs, reduced PBP binding affinity, and destruction of the antibiotic through the expression of β-lactamase. β-lactamase are enzymes that bind and hydrolyse β-lactams. In Gram-positive bacteria, because they do not have outer bacterial membrane, the antibiotics have free access to the bacterial cytoplasmic membrane which harbours the PBPs. The presence of outer membrane in Gram-negative bacteria restricts the entry of β-lactams as well as concentrates the β-lactamase molecules.

2.4.4.1. Resistance to β-lactams in Gram-positive bacteria

Clinically significant β-lactam resistance in Gram positive bacteria occur mainly through the expression of PBPs that bind β-lactams with low affinity with exception of *staphylococci* which produces narrow spectrum penicillinase (Rice, 2012). In *Staphylococcus aureus*, the resistance to methicillin is due to the production of PBP2 which has low affinity for β-lactams. The resistance of *Enterococcus faecium* to ampicillin is attributed to production of low affinity PBP5 and resistance is transferable between *Enterococcus faecium* strains (Rice, 2012). In addition, the dissemination of ampicillin-resistant *Enterococcus faecium* throughout the world was attributed to clonal complex 17 (Top *et al.*, 2008), a loosely associated group of strains that have undergone a high degree of gene acquisition. *Streptococcus pneumoniae* become resistant to β-lactams by its ability to take up DNA. Resistant strains display a variety of "mosaic" genes derived from recombination between native pneumococcal PBP genes and those from less susceptible viridans streptococci. However, this mechanism of resistance is narrowed by the levels of resistance expressed by the native PBPs that contribute to the mosaic and generally remains at low levels that affect the efficacy of intravenous antibiotics only in the cerebrospinal fluid (Rice, 2012).

2.4.4.2. Resistance to β-lactams in Gram-negative bacteria

Expression of β-lactamase is the main mechanism of resistance in Gram-negative bacteria. The explosion in the number of named β-lactamases in the past decade has been due to a combination of increase in β-lactams and the widespread access to molecular biological techniques (Rice, 2012). As at July 2011, the number of named β-lactamases was 927 from 24 different β-lactamase classes (Table 2.2). Class A are extended spectrum beta-lactamases, classes B and D are carbapenemases while class C are plasmid-mediated cephalosporinases (Cuzon *et al*, 2012).

Table 2.2: Named β-lactamases from clinical isolates

Ambler class[b] (No.)			
A	B	C	D
TEM (190)	IMP (30)	CMY (73)	OXA (224)
SHV (141)	VIM (3)	FOX (10)	
CTX-M (120)	IND (8)	ACT (9)	
GES (17)	NDM (6)	DHA (8)	
KPC (11)		MOX (8)	
PER (7)		MIR (5)	
VEB (7)		ACC (4)	
SME (3)		CFE (1)	
PCI (1)[c]		LAT (1)	

[a]Based on information obtained from Lahey Clinic Web site (http://www.lahey.org/studies) on July 25, 2001.

[b]Ambler classification is based on DNA sequence similarity and does not directly correlate with function or spectrum.

[c]PCI is the only B-lactamase of clinical importance found in Gram-positive bacteria. At this time, it is virtually uniformly present in pathogenic staphylococcal species and has never been shown to extend is spectrum to include broad-specrum cephalosporins.

(Source: Rice,2012).

To comprehend the challenge of β-lactamase induced resistance in Gram-negative bacteria, Rice (2012) discussed them as occurring in 4 waves (Figure 2.3). The first wave described a few different narrow spectrum penicillinases that arose in association with the administration of ampicillin in the treatment of Gram-negative bacterial infections. The prevalence of these enzymes necessitated the development and introduction of newer β-lactams such as cephalosporins, carbapenems, and aztreonam that were resistant to hydrolysis.

The second wave was the emergence of resistance of Gram-negative bacteria to the extended-spectrum cephalosporins and this resistance concentrated in *K pneumonia*. Overlapping with the second wave was the third wave which involved the emergence and propagation of the CTX-M family of β-lactamases while the fourth wave was the emergence of the carbapenemases.

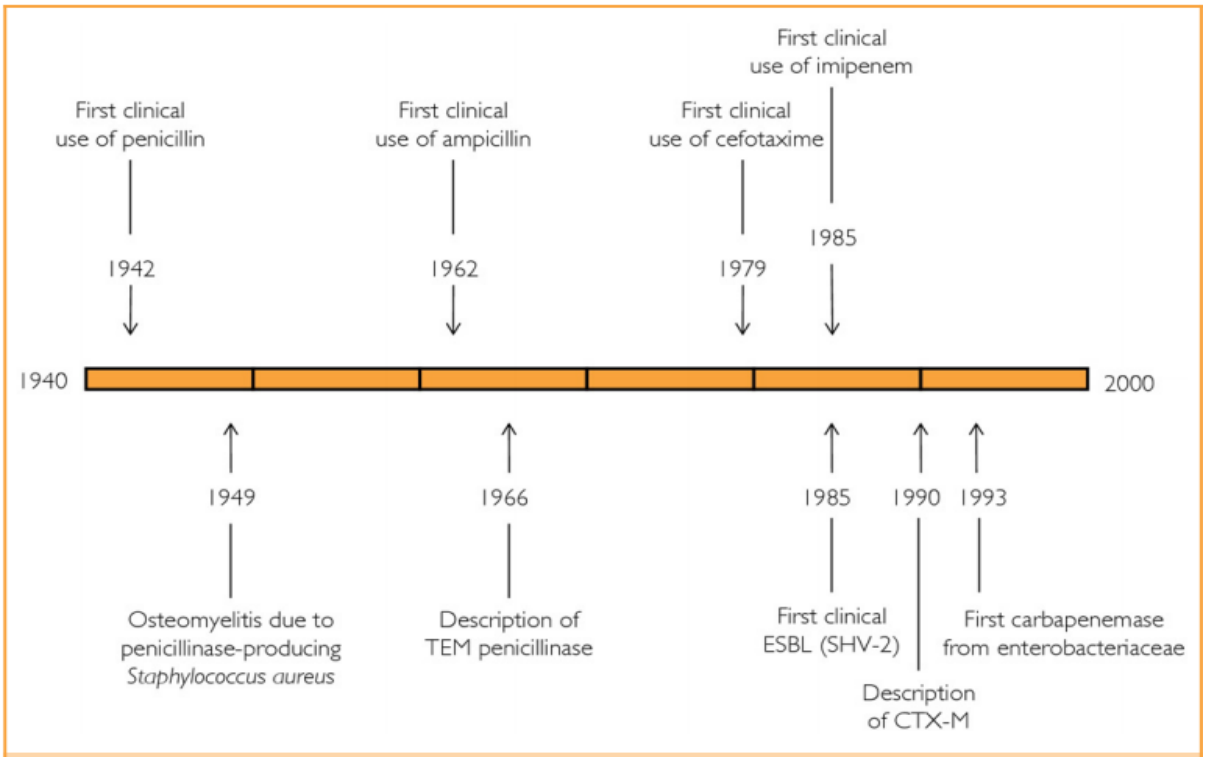


Fig 2.3: Time line showing the use of different classes of antibiotics and the publication of the first article describing resistance (or a new class of β-lactamases conferring resistance) to that class of antibiotics in a previously susceptible organism. (Source: Rice, 2012).

2.4.5. Clinical resistance of bacteria to drugs

Bacteria are said to be resistant to a host of classes of antibacterial agents; however, this is based on the inability of the stated therapeutic dose of these antibacterial agents to kill or control the growth of the bacteria. This is buttressed by the fact that different concentrations of a particular drug may be required to eradicate different bacteria. For instance, in Britain, the Minimum Inhibitory Concentration (MIC) of benzylpenicillin against *Streptococcus pneumonia* was 0.01mg/L while 32-64 mg/L was required to inhibit *Escherichia coli* (Hawkey, 1998). Such concentration (32-64mg/L) cannot be tolerated or attained in humans. This behoves the concept of clinical resistance – concept in which the specificity of the bacteria/organism, its metabolic state, its location in the body, the distribution of the antibiotic in the body and its concentration at the site of infection, and the immune status of the patient all interact (Hawkey, 1998) for possible initiation or onset of resistance. To elaborate, a susceptible bacteria residing deeply in an abscess may not be accessible to an antibacterial agent. A known susceptible bacterium may also become resistant if present in a biofilm attached to a foreign body. On the contrary, known resistant species to specific antibiotics (e.g. *Pseudomonas aeruginosa* and tetracycline) can be treated successfully if the infection is in the lower urinary tract where the concentration of the antibiotic is high and proportion of bacteria is low. Hence, laboratory methods ought not to be the sole criteria for definition of resistance.

2.4.6. Global epidemiology of bacterial resistance

Most times resistance of micro-organisms to drugs is acquired in the hospital; however, it is increasingly acquired in the community as well. Table 2.3 depicts a number of resistant organisms acquired in the hospital and community.

Table 2.3: The common clinically resistant micro-organisms acquired in the community and Hospital

Community-acquired	**Hospital-acquired**
Escherichia coli	*Bacteroides* species
Haemophilus influenzae	*Candida* species
Hepatitis B virus	Coagulase-negative staphylococci
Herpes simplex virus	*Enterobacter* species
Human immunodeficiency virus	*Klebsiella* species
Methicillin-resistant *Staphylococcus aureus*	Methicillin-resistant S. *aureus*
Multidrug-resistant tuberculosis	Multidrug-resistant tuberculosis
Neisseria gonorrhoeae	*Proteus* species
N. meningitidis	*Pseudomonas aeruginosa*
Salmonella species	*Serratia* species
Shigella species	*Stenotrophomonas maltophilia*
Streptococcus pneumoniae	Vancomycin-resistant enterococci
Viridans streptococci	

(Source: Virk and Steckelberg, 2000)

The epidemiology of resistance may be local, national or international. Resistance may be localized in a hospital or a community. The prevalence of resistance is highest where the use of antibacterial agents is immense and the most vulnerable patients are concentrated. Hence, it was observed that the prevalence of bacterial resistance such as MRSA are more in ICU than general wards (Livermore, 2003). It may be national and as such prevalence may be high in certain areas than others. For instance, in Europe, increase in resistance is experienced as one moves south – it is lowest in Scandinavia and immense in the Mediterranean countries. In addition, for North America, resistance is more prevalent in United States than Canada (Livermore, 2003). It was noticed that some of the worst resistance is experienced in the newly prosperous countries of East Asia and South America. To buttress this: methicillin-resistant strains comprise 30%–45% of all *S. aureus* from bacteraemia in Spain, Portugal, Italy, France, and the United Kingdom and 10%–15% in Germany and Austria, but less than 1% in the Netherlands and Scandinavia (Livermore, 2003). In addition, 70%–80% of *S. pneumoniae* were resistant or intermediately resistant to penicillin in countries such as Korea, Japan, Taiwan, and Vietnam as compared to 30%–40% in France and Spain, 5%–10% in the United Kingdom, and 1%–2% in Scandinavia (Livermore, 2003).

International stage of resistance is enhanced by transferable determinants prevalent worldwide and so resistance strains spread amongst countries and continents. Multidrug-resistant pneumococci of serotype 6B was imported from Spain to Iceland through children of holidaymakers. Furthermore, from Spain to the Far East, the Americas, and South Africa was propagated other penicillin-resistant pneumococci of serotype 23F. The first record of PER-1 ESBL collected from *P. aeruginosa* isolate was in France and subsequently, was found in numerous *P. aeruginosa, Salmonella, and Acinetobacter spp*. isolates from several cities in Turkey. Further investigation revealed that the discovery in France was from a Turkish patient visiting for treatment (Livermore, 2003). Indeed movement of people across nations will

continue to enhance the distribution of resistant strains even in countries with low bacterial resistance. Due to movement, there was multi-importations of plasmid carrying and AmpC β-lactamase producing Enterobacteriaceae from the Indian subcontinent into one Yorkshire hospital, UK over a 4 month period (Hawkey, 2008).

2.4.6.1. Epidemiology of bacterial resistance in Nigeria

Based on the literature review, study by Vlieghe *et al*. (2009) indicated that the worldwide trends of bacterial resistance is experienced in Sub-Saharan Africa. In Nigeria, it was discovered that penicillinase-producing *Neisseria gonorrhoeae* (PPNG) had a dramatic incidence within two years (1979 – 1981) from 2.7% to 51.7% (Osoba *et al*., 1987). While it became multi-resistant, PPNG was reported to be susceptible to spectinomycin though expensive. A study on *Shigella* isolates collected in Lagos between March 1999 and February 2000 revealed that over 70% of the isolates were resistant to two or more drugs including tetracycline and ampicillin (Iwalokun *et al.*, 2011) A study on *Salmonella* typhi isolated from patients admitted in some hospitals in Lagos between May 2000 and July 2001 revealed that 25 0f 41 strains (61.0%) were resistant to 4 or more antibacterial agents tested and these include ampicillin, chloramphenicol, cotrimoxazole and tetracycline (Akinyemi *et al.*, 2005). In addition, a study of *Enterobacter* species in two hospitals in Lagos, indicated high percentages of resistance for a host of antibacterial agents except imipenem employed in the study. The study was undertaken to assess the occurrence of the extended spectrum β-lactamases (ESBLs) in *Enterobacter* species in Nigeria (Aibinu *et al.*, 2003). The percent resistance of *Enterobacter* isolates to various antibacterial agents is shown in Table 2.4.

Table 2.4: Percent resistance of extended spectrum β-lactamase and non-extended spectrum β-lactamase producing *Enterobacter* spp.

Antibacterial agents	% resistance	
	ESBL isolates	**Non-ESBL isolates**
Ampicillin	100	100
Amoxicillin	100	100
Ticarcillin	100	100
Piperacillin	100	79.0
Ampicillin-sulbactam	87.5	53.9
Ticarcillin-clavulanic acid	87.5	53.9
Amoxicillin-clavulanic acid	100	82.5
Cefotaxime	62.5	10.3
Ceftazidime	100	20.5
Cefepime	37.5	10.3
Imipenem	0.0	0.0
Aztreonam	87.3	17.9
Kanamycin	87.5	51.3
Gentamicin	100	59.0
Amikacin	62.5	10.3
Nalidixic acid	75.0	12.8
Ofloxacin	75.0	15.4
Ciprofloxacin	75.0	23.1
Tetracycline	87.5	69.0
Chloramphenicol	82.1	61.5
Trimethoprim-sulfamethoxazole	100	82.1
Cephalothin	100	10.0
Cefoxitin	100	100
Ceftriaxone	73.0	40.0
Streptomycin	89.0	20.0

(Source: Aibinu *et al.*, 2003)

Studies undertaken in Lagos and Ibadan, Nigeria exposed that over 70% of Enterobacteriaceae strains which include *E. coli, Klebsiella and Proteus spp* were resistant to host of commonly available antibiotics. This led to use of expensive agents (Okesola and Oni, 2009). A study undertaken in University College Hospital Ibadan explored the susceptibility of micro-organisms causing infections such as urinary tract infections, otitis media, pneumonia, bacterial conjunctivitis, wound sepsis. It was found that resistance rates were prevalent in both Gram-positive cocci and Gram-negative bacilli isolates (Okesola and Oni, 2009). In *Klebsiella* species 100 (71.4%), *E. coli* 8 (80%) and *P. mirabilis* 15 (75%) were resistant to amoxicillin/clavulanate respectively. In addition, 110 (73.3%) of *P. aeruginosa*, 6 (60%) of *E. coli*, 10 (50%) of *Proteus* and 80 (57.1%) of *Klebsiella species* were resistant to cefotaxime. A published review study in 2005 of neonatal infections in Africa explained the growing concern of antimicrobial resistance (Zaidi *et al.*, 2005). Three major pathogens amongst the bloodstream isolates were *Klebsiella spp, Escherichia coli* and *Staphylococcus aureus* and the rate of resistance is shown in Table 2.5.

Table 2.5: Resistance in organisms causing neonatal infections in hospitals in Africa

	Antibiotic	**Africa**
Klebsiella spp.	Ampicillin	48/50 (96%)
	Gentamicin	52/88 (59%)
	Cefotaxime	24/81 (30%)
	Amikacin	7/50 (14%)
E. coli	Ampicillin	14/16 (88%)
	Gentamicin	1/16 (6%)
	Cefotaxime	2/17 (12%)
	Amikacin	0/10 (0%)
S. aureus	Methicillin	18/73 (25%)
	Co-trimoxazole	13/21 (62%)

(Source: Zaidi *et al.*, 2005)

A study of over 5 years (2002-2007) of 15 bacteria and their susceptibility to commonly prescribed antibacterial agents found that all bacteria were resistant to ampicillin, chloramphenicol and tetracycline (100%) (Okonko *et al.*, 2009). All isolates of Gram-negative bacteria were resistant to cotrimoxazole (100.0%), gentamicin (92.3%), streptomycin (92.3%) and nitrofurantoin (69.2%) while the isolates of Gram-positive bacteria were resistant also to gentamicin (100.0%), Streptomycin (100.0%), Cotrimoxazole (50.0%) and nitrofurantoin (50.0%).

Furthermore, a study undertaken on the prevalence of MRSA in Nigeria indicated that the rate of resistance was as high as 29.6% as of 1997 (Kesah *et al.*, 2003). It was envisaged that the rate of resistance of MRSA will continually increase to a higher level. However a study published in 2009 of two hospitals in Ibadan showed MRSA prevalence of 20.23% (Ghebremedhin *et al.*, 2009).

Pseudomonas aeruginosa, an organism known for its ability to cause a wide range of infections poses a challenge due to its resistance to a host of antibacterial agents. Indeed its resistance to a number of antibacterial agents has been studied in several centres in Nigeria including Jos University Teaching Hospital (JUTH). The study in JUTH showed that all isolates (100%) of *Pseudomonas aeruginosa* were resistant to penicillin, ampicillin, cloxacillin, tetracycline, nalidixic acid and nitrofurantoin (Jombo *et al.*, 2010).

An extensive profiling of antimicrobial resistance of 220 bacteria isolated from 1,006 episodes of blood stream infections (BSI) between January 2004 and December 2005 in a University Teaching Hospital, Southwestern Nigeria elaborated on the high antimicrobial resistance among clinical bacterial isolates to commonly prescribed antibiotics such as penicillin G, ampicillin, methicillin, cotrimoxazole, tetracycline and gentamicin (Taiwo *et al.*, 2008). The study observed cumulative resistance of all the bacteria isolates to ampicillin (79%), gentamicin (51%), ceftazidime (11%) and ciprofloxacin 6%. In addition, the researchers

observed that about 85% of the Gram positive bacteria were resistant to penicillin G, 79% to methicillin and 37% to erythromycin while 74% of the Gram negative bacteria were resistant to cotrimoxazole, 69% to tetracycline and 38% to chloramphenicol (Taiwo *et al.*, 2008).

The resistance of clinical bacteria to commonly and readily available antibacterial agents precipitates the need for use of expensive antibiotics most of which the low-income patients may not afford. Some patients would not buy enough to complete the dosage regimen as prescribed leading to abuse of antibiotics and subsequent development of resistance of bacteria to the expensive antibacterial agents. Other conditions promoting resistance include lack of functional antibiotic regulatory institutions, widespread availability of antibiotics and sub-standard formulations, use of broad-spectrum antibiotics for prophylactic therapy and general inadequacy of health care services exists in our environment (Taiwo *et al.*, 2008).

2.5. General characteristics of Staphylococci

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

The Family, *Staphylococcaceae* is comprised of the genera *Gemella, Macrococcus, Salinicoccus and Staphylococcus* (Whiley, 2003). *Staphylococcaceae* currently comprise of 41 species and their classification is in constant evolution (Vanni *et al.*, 2009). Table 2.6 shows most of *Staphylococcal* species and sub-species and sources of isolation. *Staphylococci* are Gram-positive aerobic or facultative anaerobic bacteria that occur in microscopic clusters appearing like a bunch of grapes. They are nutritionally undemanding and catalase-positive. While some species producing coagulase (such as *Staphylococcus aureus*, *Staphylococcus*

hyicus, *Staphylococcus intermedius* and *Staphylococcus schleiferi* sub sp. *coagulans*) are potentially pathogenic, *Staphylococci* are mainly coagulase-negative and harmless commensals. However, although, the view has been that coagulase-negative *Staphylococci* are non-pathogenic, this is changing as there is increasing evidence that they can be pathogenic for humans through enterotoxin production or may cause human diseases and nosocomial infections. Some species play double roles of being beneficial and pathogenic depending on where they are found. For instance, *Staphylococcus saprophyticus* contributes to sausage aroma formation, prevents off-flavour of sausage during ripening and also involved in acute urinary tract infections in young adult women (Irlinger, 2008). Due to the widespread of *Staphylococci* as the sources shown in Table 2.6 depict, it has become vital to identify them both at genus and specie level. Identifying the species is pivotal in human and veterinary medicine, for assessing the source of infection, characterizing the pathology and conducting epidemiological studies. Prompt and accurate identification and speciation of *Staphylococci* is vital, not only for medical, veterinary and societal applications but also for public health (Irlinger, 2008).

Table 2.6: *Staphylococcal* species and sub-species with standing in bacterial nomenclature as of May 2009

***Staphylococcus* spp.**	**Source**
arlettae	Poultry
aureus subsp. *aureus*	Primates, domestic animals, rodents, poultry
aureus subsp. *anaerobius*	Domestic sheep
auricularis	Primates, human external auditory meatus
capitis subsp. *capitis*	Human skin
capitis subsp *urealyticus*	Primates
caprae	Goats, humans
carnosus subsp. *carnosus*	Domestic artiodactyls
carnosus subsp. *utilis*	Domestic artiodactyls
chromogenes	Artiodactyls, horses
cohnii subsp. *cohnii*	Humans
cohnii subsp. *urealyticus*	Primates, tree shrews
condimenti	Soy sauce mash
delphini	Dolphins
epidermidis	Human skin
equorum subsp. *equorum*	Domestic horses, cattle
equorum subsp. *linens*	Surface-ripened cheese
felis	Cats
flueretti	Goat milk cheese
gallinarum	Poultry
hemolyticus	Human skin, primates, horses, Artiodactyls
hominis subsp *hominis*	Human skin
hominis subsp novobiosepticus	Human clinical specimens
hyicus	Artiodactyls (swine, cattle), poultry
intermidius	Carnivores, domestic horses, poultry
kloosii	Domestic artiodactyls, rodents
lentus	Artiodactyls, perissodactyls, cetaceans
lugdunensis	Human clinical specimens
lutrae	Otters
muscae	Flies

nepalensis	Goats
pasteuri	Humans, animals, food, artiodactyls, horses
pettenkoferi	Human clinical specimens
piscifermentans	Fermented fish
pseudintermidius	Domestic animals, humans
pseudolugdunensis	Human blood cultures
pulvereri	Human and animal specimens
saccharolyticus	Humans
saprophyticus subsp. *saprophyticus*	Human skin, primates, tree shews, rodents
saprophyticus subsp. *bovis*	Bovine nostrils
schleiferi subsp *schleiferi*	Human clinical specimens, carnivores
schleiferi subsp *coagulans*	Dogs, external auditory meatus
sciuri subsp. *carnaticus*	Artiodactyls, cetaceans
sciuri subsp. *rodentum*	Rodents, cetaceans
simiae	S. American squirrel monkeys
simulans	Human skin, carnivores, artiodactyls
succinus subsp. *succinus*	Dominican amber
succinus subsp. *casei*	Surface-ripened cheese
vitulinus	Food, artiodactyls, perissodactyls, cetaceans
warneri	Human skin, artiodactyls, horses
xylosus	Human skin, rodents, mammals, birds

(Source: Crossley *et al.*, 2009)

Species are differentiated from one another based on various phenotypic characters, cell wall composition, electrophoretic patterns of EcoRI restriction fragments labelled by hybridization with an rRNA operon from *Escherichia coli*, macro-restriction patterns of SmaI and NotI chromosome fragments, DNA-DNA liquid hybridization, DNA base composition and 16s rRNA sequences (Kloos *et al.*, 1998). These methods have also been used to differentiate *Staphylococcus* from a related specie, *macrococcus.*

Indeed, *Staphylococcus* is one of the most important bacteria species isolated and studied. The common identification methods for *Staphylococcus* species employed by a number of researchers include biochemical systems and GLC analysis of cellular fatty acids (Mendoza *et al.*, 1998). However, these methods are fraught with inaccuracy which lead to incorrect or non-identification of the isolates which necessitate the use of molecular methods such as ribotyping analysis, DNA PFGE, total genomic DNA-DNA hybridization, and genomic DNA hybridization to labelled probes specific to hypervariable sequences (Mendoza *et al.*, 1998). While these methods are fairly laborious, may be difficult to interprete, inter-laboratory reproducibility and standardization a challenge, Mendoza *et al.* (1998) proposed the use of 16s-23s rDNA intergenic spacer PCR analysis as a tool to identify *Staphylococcus* isolates at specie level. Other methods have also been proposed and utilized. These include highly conserved gene encoding the bacterial 60 kDaheat-shock protein (Hsp60) (Kwok and Chow, 2003), the *tuf*, *sod*A and *rpo*B genes as targets for both identification and phylogenetic studies (Irlinger, 2008). However, species-specific PCR may need the use of several primer sets, which is time-consuming, expensive and fairly laborious. Although some studies have undertaken multiplex PCR protocols that can determine the *Staphylococcus* genus and its main constituent species from food, food environment or a clinical origin, primers are not applicable for all species of CNS (Irlinger, 2008).

Recently, however, an oligonucleotide array targeting the manganese-dependent superoxide dismutase (*sodA*) gene was developed to circumvent the setbacks of the conventional methods of identification (Giammarinaro *et al.*, 2005). The oligonucleotide array tool called "staph array" permits rapid and accurate identification of staphylococcal strains from clinical and food origin at specie level. Furthermore, the tool is able to differentiate, in one shot, the validated staphylococcal species and also discriminates the two subspecies of *S. cohnii.*

2.6 The organism: *Staphylococcus aureus*

2.6.1 Morphology

Staphylococcus aureus is differentiated from other staphylococci species by the gold pigmentation of the colonies, positive results of the coagulase, mannitol-fermentation and deoxyribonuclease tests (Lowy, 1998). Various tests can be used to identify *S. aureus*, including production of protein A, cell-bound clumping factor, extracellular coagulase and heat-stable nuclease. Molecular methods have also recently been developed. (Chambers, 2005).

Figure 2.4 shows the grape-like morphology of *Staphylococcus aureus*. In 1884, Rosenbach described the two pigmented colony types of Staphylococci and proposed the appropriate nomenclature: *S. aureus* (yellow) and *S. albus* (white). The latter species is now *S. epidermitis* (Holt, 1994).

Staphylococci are perfectly spherical cells about one micrometre in diameter. They grow in clusters because staphylococci divide in two planes. The configuration of the cocci helps to distinguish staphylococci from streptococci which are slightly oblong cells that usually grow in chains. The catalase test is important in distinguishing streptococci (catalase-negative) from staphylococci which are vigorous catalase producers (Madigan and Martinko, 2005).

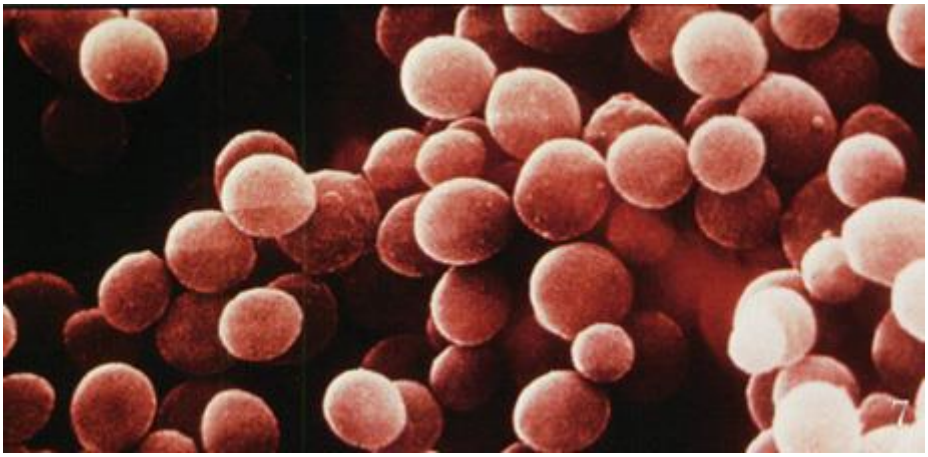


Plate 2.1: Scanning electron micrograph of *Staphylococcus aureus* (Source: Todar, 2012).

Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields lactic acid. *S. aureus* can grow at a temperature range of 15^{o} to 45^{o}C and at sodium chloride concentration as high as 15%. Nearly all strains of *S. aureus* produce the enzyme coagulase (Ryan, 2004).

2.6.2. Structure of *Staphylococcus aureus*

2.6.2.1. Genome of *Staphylococcus aureus*

The genome of *S. aureus*, fundamentally similar to other prokaryotic genomes consists of one circular chromosome as well as genetic elements which include prophages, plasmids, transposons (Lowy, 1998; Młynarczyk *et al.*, 1998). The genes found on the chromosome as well as the extrachromosomal elements govern the virulence and resistance to antibiotics. The transference of the genes between staphylococci strains, species and other Gram-positive bacterial species is undertaken through the extrachromosomal elements.

2.6.2.2. Cell wall, synthesis and surface proteins

The cell wall is the element that maintains shape in a bacterium and hence its integrity is critical to the bacterium's viability. The major amino acids in the cell wall of *S. aureus* are alanine, glutamic acid, lysine, glycine, glucosamic acid and muramic acid with no distinct appearance

of sugars (Cummins and Harris, 1956; Strominger *et al.*, 1959). It is also ascertained in another study that the cell wall of *S. aureus* like other gram-postive organisms contains lipoteichoic acid (LTA) and peptidoglycan (PepG) which are known to generate inflammatory response. LTA and PepG act in synergy to effect a systemic release of tumour necrosis factor (TNF-α) and interferon γ (INF-γ), induction of nitric oxide synthase (NOS), circulatory failure, multiple organ dysfunction syndrome (MODS), and death (De Kimpe *et al.*, 1995). LTA is a glycerol phosphate polymer attached to a glycolipid terminus established in the cytoplasmic membrane (Lowy, 1998). In addition, advances in research such as the discovery of an actin-like cytoskeleton involved in bacterial cell shape determination and the application of fluorescence microscopy have enhanced the knowledge of cell wall structure, characteristics and other cell components (Scheffers and Pinho, 2005).

The cell wall is 50% PepG which is made up of polysaccharide sub-units of *N*-acetylglucosamine and *N*-acetylmuramic acid with 1,4-*β* linkages in an alternating fashion (Lowy, 1998). PepG is synthesized in three stages in the cytoplasm, the membrane and the cell wall envelope (Perry *et al.*, 2002). The first stage is in the cytoplasm where nucleotide sugar-linked precursors UDP-*N*-acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) and UDP-*N*-acetylglucosamine (UDP-GlcNAc) are synthesized. In the second stage, precursor lipid intermediates are synthesized in the membrane. The phospho-MurNAc-pentapeptide moiety of UDP-MurNAc-pentapeptide is transported to the membrane acceptor bactoprenol, yielding lipid I [MurNAc-(pentapeptide)- pyrophosphoryl-undecaprenol]. Thereafter, GlcNAc from UDP-GlcNAc is added to lipid I, yielding lipid II [GlcNAc-β-(1,4)-MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol], which is the substrate for the polymerization reactions in *S. aureus* that have directly cross-linked PG (Scheffers and Pinho, 2005). The last stage which takes place in the cell wall envelope is the polymerization of the synthesized disaccharide-peptide units and subsequent incorporation into the growing PepG. This last stage

is accomplished by the actions of penicillin-binding proteins, which catalyse the transglycosylation and transpeptidation reactions in charge of the formation of the glycosidic and peptide bonds of the PG, respectively (Scheffers and Pinho, 2005). *S. aureus* has only four penicillin-binding proteins and cell wall synthesis occurs mainly in the septum. The PepG stimulates the release of cytokines by macrophages, activates complement and aggregates platelets.

Covalently linked to the cell wall envelope are polypeptides, called surface proteins (Figure 2.5). Surface proteins are mechanistic tools by which gram-positive bacteria adhere to specific receptors on the host tissues in order to inflict harm (infections) or by which they escape the host's immune response (Mazmanian *et al.*, 1999; Perry et al., 2002). They are linked by a cleavage of a conserved Leu-Pro-X-Thr-Gly (LPXTG) motif that occurs during assembly of the peptidoglycan cell wall. In the case of *S. aureus* protein A, protein A (Figure 2.5) is cleaved by a transpeptidase, sortase (protein specified by srtA gene) between the threonine and the glycine of a conserved LPXTG motif (Mazmanian *et al.*, 1999). Protein A possesses antiphagocytic properties which are based on its ability to bind the Fc portion of immunoglobulin (Lowy, 1998). The rare-limiting step in the anchoring of surface proteins to the cell wall is the nucleophilic attack of the peptidoglycan substrate that regenerates the active site of sulfhydryl of sortase (Perry *et al.*, 2002). Two experimental approaches undertaken suggested that sortase uses a peptidoglycan precursor as a substrate for surface protein anchoring. This precursor performs the nucleophilic attack.

2.6.2.3. Capsule

Some *S. aureus* strains produce microcapsules (uronic acid-containing extracellular polysaccharides) (Figure 2.5) which are not visible by negative staining but are detected by serological methods (Albus et al., 1991). The capsules enhance microbial virulence by causing the bacteria to be resistant to phagocytosis (O'Riordan and Lee, 2004). Microcapsules from at

least 18 strains have been described and of the stereotypes identified, stereotypes 5 and 8 are responsible for 75% of human infections (Lowy, 1998; O'Riordan and Lee, 2004).

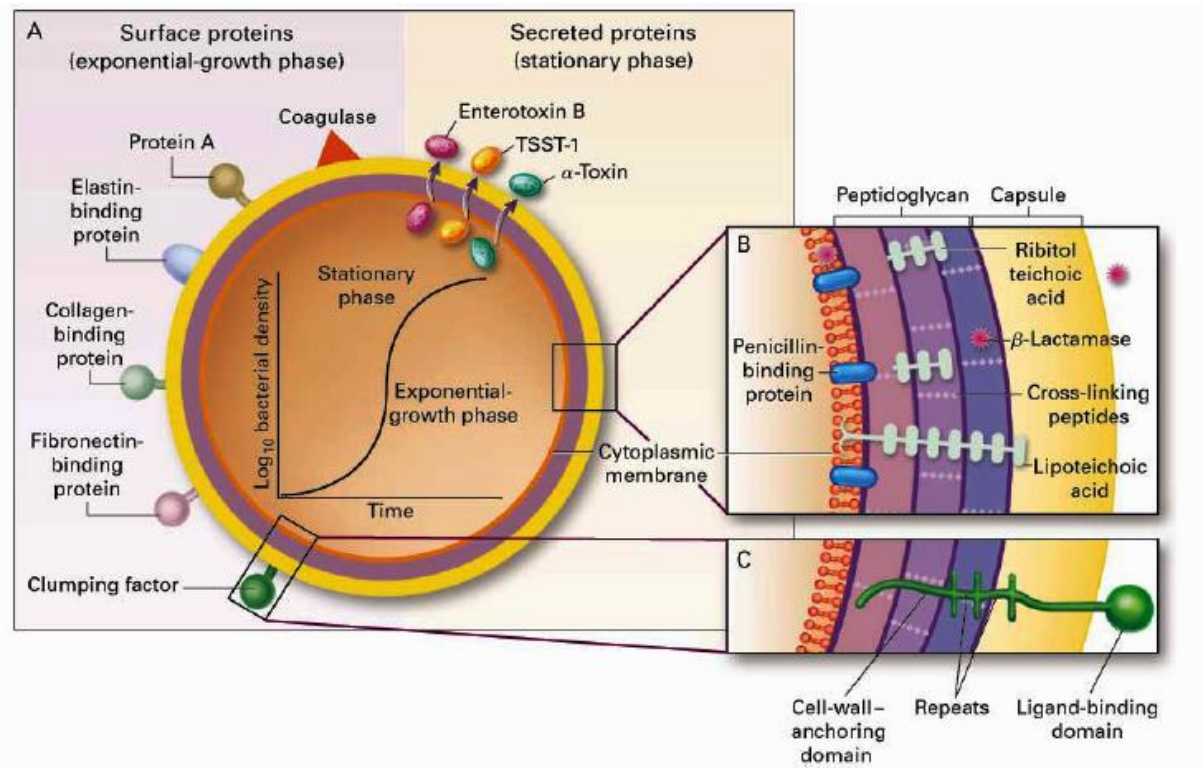


Fig 2.4: The structure of *S. aureus* depicting the surface and secreted proteins and cross-section of cell envelope. (Source: Lowy, 1998)

2.7 Laboratory Identification of *Staphylococcus aureus and MRSA*

To identify *Staphylococcus aureus,* the traditional Gram-staining test is undertaken. Gram positive bacterial organisms in clusters are characteristic of Staphylococcus. Then, to identify *Staphylococcus aureus* from other staphylococci and organisms, some specific tests are undertaken.

2.7.1. Coagulase test

This is a conventional method that has been employed over the years to identify *Staph. aureus.* Coagulase test is used to differentiate *Staph. aureus,* a coagulase-positive organism from coagulase-negative staphylococci. However, it has been observed over time that some other *Staphylococcus* species such as *S. hyicus, S. schleiferi* subspecies *coagulans, S. pseudintermedius or S. intermedius and S. delphini* may also be coagulase positive. The presence or absence of coagulase was used to divide staphylococci into coagulase positive and coagulase negative. However, it has been observed there are isolates of *Staph. aureus* defective in coagulase (Foster, 1996).

Aliquots of Pooled EDTA-anticoagulated human plasma diluted with sterile normal saline are mixed with nutrient broth cultures of the isolates and incubated at 37^{o}C for 4 – 6 hours. The tubes are examined at 30 minutes intervals for clot formation. Firm and complete clot that stays in place when tilted or inverted is considered positive for *Staph. aureus* (Bennett and Lancette, 1998).

2.7.2. Latex agglutination test

In order to combat the false negatives obtained from the coagulase test, latex particles (coated with immunoglobulin G and fibrinogen which bind protein A and the clumping factor, respectively, on the bacterial cell surface) are used to confirm *Staph. aureus* by testing colonies

for agglutination (Foster, 1996). The latest latex test (Pastaurex) minimizes false negatives by incorporating monoclonal antibodies to serotype 5 and 8 capsular polysaccharide.

2.7.3. Catalase Test

This test is employed to differentiate catalase-negative streptococci from catalase-positive staphylococci (Foster, 1996). Catalase-positive culture bubbles immediately when an agar slant or broth culture is flooded with several drops of 3% hydrogen peroxide.

2.7.4. Anaerobic utilization of glucose

Carbohydrate fermentation medium containing glucose is inoculated and the surface of agar is covered with a layer of sterile paraffin oil at least 25 mm thick. This is incubated for 5 days at 35-37^{o}C. The change of the indicator to yellow implies that acid is produced anaerobically and confirms the presence of *Staph. aureus.*

2.7.5. Anaerobic utilization of mannitol

The method described in section 2.7.4 is undertaken using mannitol instead of glucose as carbohydrate in medium. Although *Staph. aureus* is usually positive, some strains are negative.

2.7.6. Lysostaphin sensitivity

An isolated colony from agar plate is transferred into about 0.2mL phosphate-saline buffer and emulsified. Half of the suspended cells is transferred into another tube and mixed with 0.1mL phosphate-saline buffer as control. Then, 0.1mL lysostaphin dissolved in 0.02 M phosphate-saline buffer containing 1% NaCl is added into the first tube for concentration of 25µg lysostaphin/mL. The tubes are incubated at 35-37^{o}C for 2 hours max. Presence of *Staph. aureus* is confirmed if turbidity clears in test mixture (Bennett and Lancette, 1998).

2.7.7. Thermostable nuclease production

Thermostable nuclease production is a supportive test to coagulase test and not a substitute especially for 2+ coagulase reactions. The test is undertaken by spreading 3 mL toluidine blue-deoxyribonucleic acid agar on the surfaces of microscope slides. On solidification of agar, 2mm diameter wells are cut in agar and agar plugs are removed by aspiration. Then about 0.01 mL of heated sample of broth cultures used for coagulase test is added to each well. The slides are incubated in a moist chamber at 35-37^{o}C for 4 hours. The reaction is said to be positive for *Staph. aureus* if bright pink halo develops and extends at least 1 mm from the periphery of well (Bennett and Lancette, 1998).

2.7.8. E-test method

E-test method is another approach in determination of minimum inhibitory concentration and has an advantage over other MIC methods due to the convenience of its set up as a disc diffusion test (Brown *et al.*, 2005). The test conditions recommended include Mueller-Hinton (MH) agar with 2% NaCl, an inoculum density equivalent to 0.5-1.0 MaFarland standards, application of inoculum with a swab and incubation at 35-37^{o}C for 24 hours (Brown *et al.*, 2005).

2.7.9. The breakpoint test

Breakpoint test is similar to dilution MIC tests except that in breakpoint test, only the breakpoint concentrations (2 mg/L oxacillin, 4 mg/L methicillin) are tested. Both agar and broth methods are used for the breakpoint test.

2.7.10. Agar screening method

Agar screening method is used to screen colonies isolated on routine media and for confirmation of possible resistance observed in disc diffusion tests (Brown *et al.*, 2005). First, the test organism is suspended to the density of 0.5 McFarland standard and MH agar

containing 4% NaCl and 6 mg/L oxacillin is inoculated with a spot or streak of the organism. Thereafter, the plates are incubated at 35^{o}C for 24 hours. Any growth other than a single colony confirms resistance.

2.7.11. Cefoxitin disc diffusion test

Cefoxitin is the suggested alternative to oxacillin. Tests with cefoxitin have been found to be more reliable than those with oxacillin (Brown *et al.*, 2005). While hyper-production of penicillinase affect tests with oxacillin, it appears test with cefoxitin are not affected. Consequently, true methicillin- or oxacillin-resistant isolates would give no zone of inhibition.

2.7.12. Automated methods

Automated systems such as Vitek/Vitek2 (bioMérieux), Phoenix (Becton Dickinson) and Microscan (Dade Behring) encompass tests for methicillin/oxacillin susceptibility. Reliability of the systems for *Staph. aureus* has been confirmed; however, some incorrect results especially false resistance has been reported (Wallet *et al.*, 1996; Grisold *et al.*, 2001).

2.7.13. Quenching fluorescence method

Crystal MRSA ID test (Becton Dickinson) functions based on growth of MRSA resulting in a decrease of oxygen in a medium containing 4mg/L oxacillin and 2% NaCl. The decrease in oxygen in the medium lead to emission of fluorescence. The Crystal MRSA ID system detects staphylococci growth using an oxygen-sensitive fluorescence indicator which detects oxygen consumption due to bacterial metabolism (Zambardi *et al.*, 1996).

2.7.14. Molecular methods

The presence of mecA gene have been reportedly responsible for high-level of resistance. Consequently, polymerase chain reaction based methods have been used to detect *mecA* gene. *mecA* gene is used as a marker to identify MRSA. Resistance not mediated by *mecA* gene will not be detected. Commercial kits are available to identify *mecA* gene; however, they work only

with purified cultures or enriched screening broths (Brown *et al.*, 2005). Multiplexed PCR primers are used to detect genes which identify strains as *S. aureus* (*nuc* and *fem* are frequently used) and *mecA.* It is worthy to note that false positives have been reported and approaches to reduce false positives include adding oxacillin to enriched broths and the use of assays to detect *nuc* gene in broth pre-enrichment samples before using a specific *mecA* primer for those samples that are nuc positive. Broth enrichment systems have failed in specimens containing both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant CoNS (MRCoNS). Furthermore, to improve the speed of diagnosis, real-time PCR has recently been used for identification of MRSA. However, the best production time was still a challenge as incubation was overnight and so cannot be said to be rapid. To further reduce production time, (Brown *et al.*, 2005). The captured DNA is then released and utilized in a quantitative branch chain assay. The study indicated that the assay appears to offer good sensitivity and the production time has improved (6 h). Apart from decreased production time, additional benefit of using direct PCR tests targeted at *mecA* detection in an *S. aureus* is that they can detect markedly heteroresistant strains, which may be challenging to detect in phenotypic tests (Brown *et al.*, 2005).

In addition to multiplex PCR, isothermal signal amplification has been developed and applied for detection of MRSA. The commercially available amplification assay to detect mecA in S. aureus is the IDI-MRSA™ assay (Infectio diagnostic, Ste-Foy, Quebec), which amplifies only part of the SCC*mec* element from *S. aureus* using primers to all the different right-hand flanking sequences in SCC*mec* and a single primer in *orfX*. The assay is based on a real-time PCR format and can be completed in 2 h from sample acquisition (Brown *et al.*, 2005). This production time can be considered to be rapid.

2.8. Pathogenicity of *Staphylococcus aureus*

Staphylococcus aureus has a wide host range, diverse cellular and environmental lifestyles, and the capability to evolve to highly drug-resistant forms that can cause diseases with

epidermic potential (Crossley *et al.*, 2009). It causes both hospital-acquired and community-acquired infections. *Staphylococcus aureus* exhibits two distinct lifestyles – a commensal asymptomatic state where it is harboured by 20-50% of the general populations at the anterior nares; and the acute state at which it attacks specific tissues and pathogenicity follows consequently. Generally, *Staphylococcus aureus* cause infections by self- inoculation (a process whereby the organism breaches the natural barrier) or by direct or indirect contact (Crossley *et al.*, 2009). The spread or containment of an infection depends on a complex interplay between *Staphylococcus aureus* virulence determinants and the host defense mechanism (Lowy, 1998).

S. aureus which is commonly found on the skin and in the eyes, nose, and throat of animals and humans is one of the most common causes of infections worldwide. Though not a problem for healthy adults, it is potentially virulent and can cause serious infections of the skin, eyes, brain, blood, respiratory and digestive tracts as well as bone and connective tissues. Some infections such as bacteraemia can have a death rate as high as 40% (Ryan *et al*., 2004). It is a versatile pathogen causing a wide variety of infections which include cutaneous infections (e.g. furunculosis, impetigo and abscesses), organ infections (e.g. osteomyelitis, endocarditis and arthritis), and toxinoses (such as food poisoning, septic shock, scalded skin syndrome and toxic shock syndrome). *S. aureus* colonises mainly the nasal passages, but it may be found regularly in most other anatomical locales. It is not typically an invasive pathogen but it invokes symptoms by the production of a variety of extracellular proteins (virulence factors), some cell-bound, others secreted, which participate in the initiation and establishment of an infection (Holt, 1994; Novick *et al*., 2001).

Among the secreted factors are the pyrogenic toxin superantigens which include enterotoxins A, B, C, D, E, G, H, I, K, L and M, exfoliative toxins A and B and toxic shock syndrome toxin (TSST-1). These have the ability to activate large populations (10-50%) of T-lymphocytes,

bypassing the standard antigen presentation mechanism, and in a manner specific to the variable region of the B-chain of the T-cell receptor. The ensuing cytokine release results in the symptoms of toxic shock syndrome (TSS), including fever, hypotension, rash, vomiting, diarrhoea, multiple organ failure, disseminated intravascular coagulation, and desquamation. The enterotoxins are associated with both TSS and food poisoning and have proven emetic activities that appear to be separable from their superantigenic activity. (Novick *et al*., 2001; Yarwood *et al*., 2002).

Most, if not all, staphylococcal superantigens as well as other extracellular virulence factors are accessory proteins and so are not required for growth and multiplication. They are often encoded by accessory genetic elements such as plasmids, transposons, prophages and pathogenicity islands. It is thought that the ability to produce superantigens offers selective advantage, since they enable bacteria to disable the host immune system. Carriage by accessory genetic elements implies spread of virulence genes among staphylococcal strains as well as exchange of genetic information between staphylococci and other bacterial species by horizontal gene transfer (Novick *et al*., 2001; Yarwood *et al*., 2002).

2.9. Epidemiology/carriage of *Staphylococcus aureus*

Although the body's defences must be weakened or breached before *S. aureus* causes disease, many people are potential victims of *S. aureus* infections. It enters the body through wounds such as burns, deep cuts or surgical incisions (Krause, 1998).

Humans are natural reservoirs for S. aureus, and asymptomatic colonization is far more common than infection. Shortly after birth, colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur and may recur any time thereafter. Transmission occurs by direct contact with a colonized carrier. About 25 – 50% of the population are carriers although this may be higher in injection drug users, insulin-

dependent diabetic patients, patients with dermatologic conditions, patients with long-term indwelling catheters, and healthcare workers (Chambers, 2001). To elaborate on carriage; about 20% (10-35%) of the general population persistently carry *Staph aureus* especially in the anterior nares and then the skin, perineum and throat. Then 30% (20-75%) of the general population are intermittent carriers and the rest of the population – 50% (5-50%) appear not to be susceptible for unknown reasons (Ammerlaan et al., 2009; Kluytmans and Wertheim, 2005). Genotyping results indicate that persistent carriers usually harbour only one identical *Staph aureus* strain over time while intermittent carriers carry different stains over time. However greater risk of infection and dispersal occur with persistent carriers as the load of *Staph aureus* is higher in persistent carriers than intermittent carriers. In addition, children are found to be more of persistent carriers than adults and quite a number of people shift from persistent to intermittent carriage to non-carriage between the ages of 10 and 20 years. For untreated individuals, the half-life of MRSA carriage has been reported to be as long as 40 months. Nasal carriage is regarded as a well-defined risk factor for subsequent infection in basically all categories of hospitalized patients (Kluytmans and Wertheim, 2005). However, the studies undertaken to eradicate nasal carriage using mupirocin nasal ointment have been inconclusive in most groups and prolonged treatment in these groups have a potential to facilitate development of resistance. In recent times, prophylaxis is a growing concern as it facilitates development of resistance. Presently, there are increasing number of antibiotics to which *Staph aureus* has become resistant to leading to the advocacy of development of new antibiotics. However, lack of resistance to the new drugs is uncertain. Also uncertain of its applicability in ensuring treatment drugs are not used for prophylaxis and vice versa in order to prolong the use of certain drugs before resistance develops. This is crucial due to the period it takes for drug discovery and development and the resources involved.

Cases of community outbreak of infections were often traced to close contact with a person who had been hospitalized or other risk factors such as previous antimicrobial therapy. Epidemic strains are often resistant to many antibiotics (Chambers, 2001; Grisold *et al*., 2002). In a study in Northern Ireland, data were gathered on all MRSA cases identified in laboratories in Northern Ireland (the North) and in the Republic of Ireland (the South) over a two-week period. The prevalence rate per 100000 populations was 11.4 in the North and 14.0 in the South, with a marked variation across geographical regions. MRSA cases were located throughout hospitals and the community, were slightly more common in males than females, and occurred in all age groups, especially in the elderly. The majority of cases was inpatients in acute hospitals and was distributed across all types of wards. Most cases were colonized with MRSA but 5% of cases in the North and 10% in the South had invasive infection. Invasive infection was associated with intravascular lines and invasive procedures/surgery. Continuous surveillance is recommended to monitor the epidemiology of MRSA and the effectiveness of control measures (McDonald *et al.*, 2003).

2.10. Drug resistance in *Staphylococcus aureus*

The ability of *Staphylococcus aureus* to express various virulent factors is medically relevant when encountered in clinical specimens. It is even or more concern as it is able to develop and expand resistance to include a broad array of antimicrobial classes. Consequently, it is a leading pathogen encountered in both inpatient and outpatient clinical settings and has continued to significantly outrun other gram-positive organisms in this regard (Styers *et al.*, 2006). This behoves the need for analysis of antimicrobial resistance of *Staphylococcus aureus.*

2.10.1. Penicillin-resistant *Staphylococcus aureus*

With the introduction of penicillin in the early 1940s, the prognosis of staphylococcal infections improved dramatically. Shortly after in 1942, *S. aureus* resistance to penicillin was

reported and within 6 years, 25% of hospital strains were resistant. Less than two decades later that proportion had also been reached in the community. Hence, by 1960s, more than 80% of hospital-acquired and community-acquired *Staphylococcus aureus* isolates were resistant to penicillin (Lowy, 2003). This rapidly decreased the usefulness of penicillin in the treatment of serious staphylococcal infection especially among hospitalized patients in whom resistant strains were frequently found before they spread to the community. Resistance was linked to the production of the enzyme penicillinase. Irrespective of clinical settings, more than 90% of *Staphylococcus aureus* isolates now produce penicillinase (Lowy, 2003). On a large plasmid is located a transposable element of which the gene for β-lactamase is part of; often with additional microbial resistant genes. The gene, *blaZ* that encodes β-lactamase mediates the resistance of *Staphylococcus aureus* to penicillin (Figure 2.6a). β-lactamase is an extracellular enzyme synthesized when *Staphylococci is* exposed to β-lactam antibiotics. The enzyme hydrolyses the β-lactam ring thereby inactivating the antibiotic. Two adjacent regulatory genes, the antirepressor *blaR1* and the repressor *blaI* control *blaZ.* It has been demonstrated that the signalling pathway responsible for β-lactamase synthesis needs the sequential cleavage of the regulatory genes, *blaR1* and *blaI.* As *Staphylococci are* exposed to β-lactam, *blaR1*, a transmembrane sensor-transducer, cleaves itself (Lowy, 2003). Furthermore, it was hypothesized by Zhang *et al*. (2001) that cleaved *blaR1*, acts as a protease that cleaves *blaI* directly or indirectly and permits *blaZ* to synthesize β-lactamase.

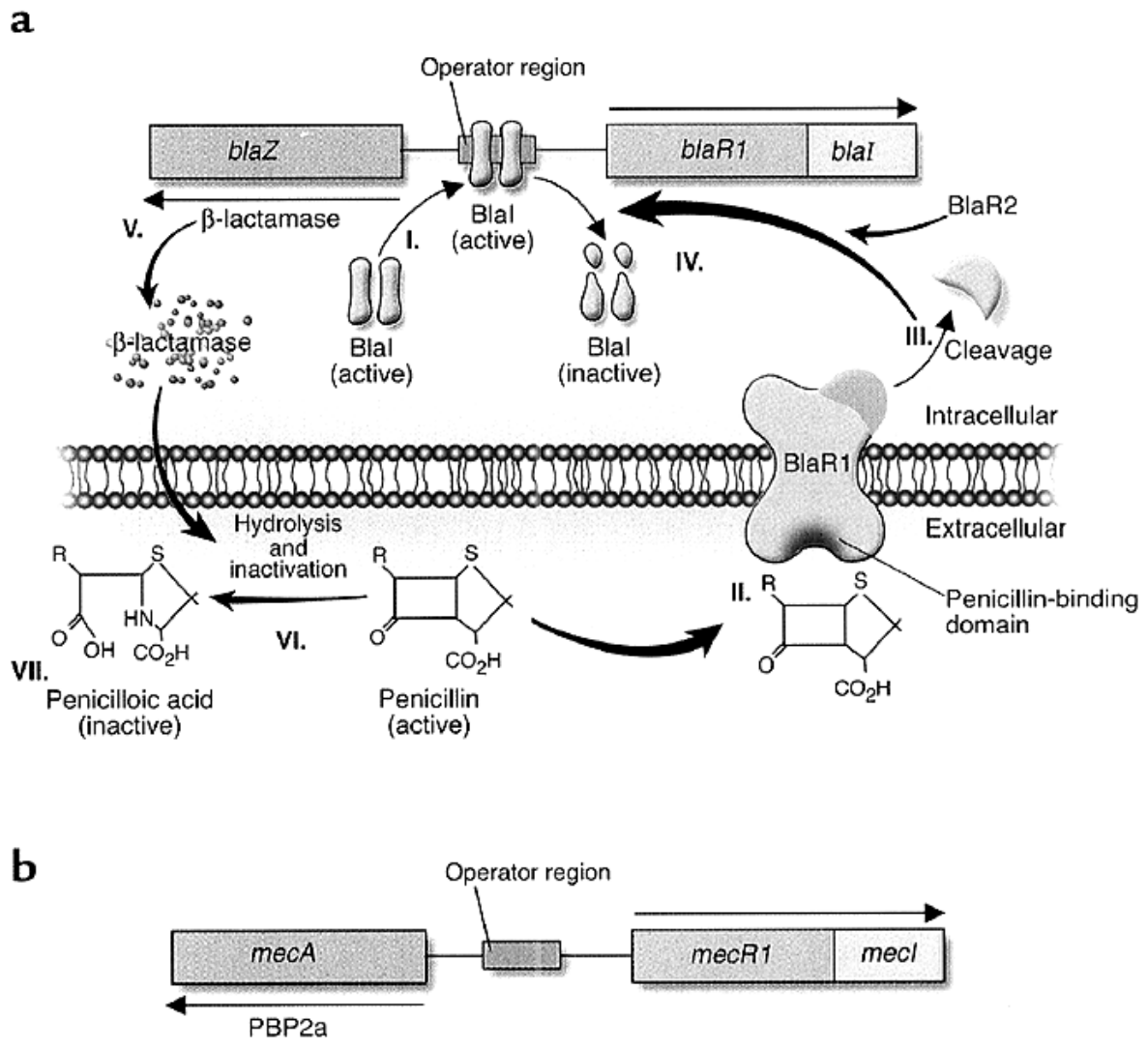


Fig 2.5: a) Induction of Staphylococcal β-lactamase synthesis in the presence of penicillin:

I. *blaI* binds to the operator region, thus repressing RNA transcription from both *blaZ* and *blaR1- blaI.* In the absence of penicillin, β-lactamase is expressed at low levels.

II. Binding of penicillin to the transmembrane sensor-transducer *blaR1* stimulates *blaR1* autocatalytic activation.

III-IV. Active *blaR1* either directly or indirectly (through a second protein *blaR2*) cleaves *blaI* into inactive fragments allowing transcription of both *blaZ* and *blaR1- blaI* to commence.

V-VII. β-lactamase, extracellular enzyme encoded by *blaZ* (V), hydrolyses the β-lactam of penicillin (VI), thereby rendering it inactive (VII), and;

b) Mechanism of *Staphylococcus aureus* resistance to methicillin.

Synthesis of PBP2a proceeds in a fashion similar to that described for the β-lactamase. Exposure of *MecR1* to a β-lactam antibiotic induces *MecR1* synthesis. *MecR1* inactivates *MecI*, allowing synthesis of PBP2a. *MecI* and *blaI* have co-regulatory effects on the expression of PBP2a and β-lactamase. (Source: Lowry, 2003).

Initially, the community acquired isolates were often then resistant to only penicillin, whereas nosocomial strains were resistant to multiple antibiotics including methicillin (Chambers, 2001). However, the community strains have also become resistant to multiple antibiotics.

2.10.2. Methicillin-resistant *Staphylococcus aureus*

Methicillin, a semi-synthetic penicillinase-resistant penicillin was introduced in 1961 (Lowy, 2003). Again, like penicillin, its introduction was speedily ensued by methicillin resistant isolates. It was first reported in a British hospital but within a short time was disseminated across international borders. Methicillin-resistant *Staphylococcus aureus* (MRSA) soon became responsible for a high percentage of nosocomial infections. Furthermore, like the penicillinase-resistant strains, MRSA isolates rapidly transferred resistant genes to other antimicrobial agents.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a challenge in hospitalized patients since the 1960s as approximately 20% of bloodstream infections were caused by *Staphylococcus aureus.* By 2003, in US intensive care unit, the percentage of hospital-onset of *Staphylococcus aureus* infections that were methicillin-resistant had reached 64.4% (Klevens *et al.*, 2007). MRSA became associated with longer duration in hospitals, higher mortality and increased cost. Subsequently, community-acquired MRSA emerged. The administration of β-lactams for treatment of soft tissue infections in many parts of the world has been compromised by the emergence and widespread of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) (Klevens *et al.*, 2007). Studies over the past two decades indicated that the isolates inflicting the community associated and healthcare associated MRSA infections were distinct (Naimi *et al.*, 2003). Isolates obtained from the community were susceptible to most non–β-lactam antimicrobial agents, carried staphylococcal cassette chromosome type IV, and frequently encoded the dermonecrotic cytotoxin known as Panton-Valentine leukocidin (Lina *et al.*, 1999; Ma *et al.*, 2002; Naimi *et al.*, 2003).

Methicillin resistance is due to the acquisition of a large DNA element, termed staphylococcal cassette chromosome *mec* (SCC*mec*) which integrates site- and specifically into the *S. aureus* chromosome. It is considered to be a novel type of mobile element, and has been termed a 'resistance island'. The prerequisite for methicillin resistance located on SCC*mec* is *mecA* (Figure 2.6b) which encodes for a low-affinity penicillin binding protein, PBP2' (synonym PBP2a) (Rohrer *et al.,* 2003). A characteristic of methicillin resistance is its usually heterogeneous expression, which means that growth in the presence of B-lactams selects highly resistant subclones from an MRSA population with low methicillin MICs. The frequency at which highly resistant clones arise is reproducible, strain specific and usually lies clearly above the rate of spontaneous mutation. With few exceptions, once high level resistance has been selected, it remains high. The degree of heterogeneity is variable but usually only 1CFU of 10^4 to 10^7 CFU are phenotypically resistant. Modification of growth conditions, which includes reduction in incubation temperature from 37^o to 40^o or increase in the osmolality of the growth medium, can increase the proportion of CFU that express resistance to various degrees and may even approach 100% (Hartman and Tomasz 1986; Tomasz *et al.*, 1991).

The term MRSA is somewhat deceptive in name because its spectrum of resistance reaches beyond methicillin to encompass all beta-lactam antibiotics and penicillinase-resistant penicillins, including methicillin, nafcillin, oxacillin, cloxacillin, and dicloxacillin (Wenzel *et al*., 1991). MRSA antibiotic resistance follows either of two mechanisms: beta-lactamase resistance or "intrinsic" resistance (Mulligan *et al*., 1993). Beta-lactamases are microbial enzymes, often coded for by extrachromosomal plasmids that destroy antimicrobial agents with beta-lactam rings. Intrinsic resistance occurs when MRSA develops a penicillin-binding protein with an abnormally low drug affinity (penicillin-binding protein-2a), thus reducing the penicillin's ability to reach its active site (Malouin and Bryan 1986). Of the two resistance

mechanisms, intrinsic resistance is the mechanism responsible for most resistance to anti-staphylococcal penicillins (Iwalokun *et al*., 2011).

2.10.3. Quinolone-resistant *Staphylococcus aureus*

The fluoroquinolones was introduced in the 1980s for the treatment of Gram negative bacteria; however, due to its Gram positive bacterial spectrum it was used to treatment infections caused by *pneumococci* and *staphylococci*. The quinolones such as ciprofloxacin, ofloxacin and norfloxacin were used to treat infections caused by MRSA. As quinolones were utilized for staphylococci infections, quinolone-resistance emerged rapidly among *Staphylococcus aureus.* The resistance was observed to be more prominent among methicillin-resistant strains (Harnett et al., 1991; Schaefler, 1989). This led to the reduction of the use of quinolones in the treatment of staphylococci infections. One contributing factor for the disparity in the rates of quinolone resistance between methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) may be antibiotic selective pressure especially in the hospital settings. This may have resulted in the selection and spread of more antibacterial resistant MRSA (Lowy, 2003).

Quinolone resistance emerges due to spontaneous chromosomal mutations targeting the antibacterial agent, topoisomerase IV or DNA gyrase or by inducing a multi-drug efflux pump. When quinolone is used to treatment bacterial pathogenic infections, subjects usually have *Staphylococcus aureus* colonize on their skins and mucosal surfaces. The exposure of *Staphylococcus aureus* colonies to sub-therapeutic concentrations will lead to colony of resistant mutants. These resistant mutants which are resident on these subjects become reservoir s for future infections.

The assemblage of high bacterial density, possible pre-existence of resistant subpopulation and sub-lethal concentration of quinolones at sites of *Staphylococcal* infections provides an

environment that promotes the selection of resistant mutants (Lowy, 2003). The mechanism of action of the quinolones is their activity on the DNA gyrase which relieves DNA supercoiling and topoisomerase IV which separates linked DNA strands. If there are amino acid changes at the critical points of the DNA-enzyme complex (quinolone resistance-determining region – QRDR), the affinity for quinolones will be reduced for both of its targets. The most common sites of resistant mutation are the parC subunit (Gr1A in *Staphylococcus aureus*) of topoisomerase IV and GyrA subunit in gyrase. Topoisomerase IV are the primary drug targets in *Staphylococci* and so its mutations are critical. Single amino acid mutations can be adequate to allow clinical resistance; however, additional mutations may be required for the more active quinolones. As resistance mutations concentrate at the QRDR sites, levels of resistance are increased. Additional mechanism of resistance observed in *Staphylococcus aureus* is the induction of NorA multi-drug resistance efflux pump. Low-level quinolone resistance can result from the increase expression of efflux pump in *Staphylococcus aureus.* Interestingly, in the linkage of virulence to antimicrobial resistance, a study revealed that exposure of a quinolone-resistant isolate to a quinolone, enhanced the organism's expression of fibronectin-binding protein which is a surface protein that mediates adhesion to tissue surfaces.

2.10.4. Vancomycin-resistant *Staphylococcus aureus*

Vancomycin was introduced and subsequently used to treat infections caused by MRSA, *Clostridum difficile, enterococci. Staphylococcal* resistant to vancomycin emerged and was first reported in a strain of *Staphylococci haemolyticus.* The first report of vancomycin intermediate – resistant *Staphylococcus aureus* (VISA) was from Japan and subsequently other cases were reported from other countries (Lowy, 2003). The VISA isolates were all MRSA and the patients had vancomycin therapy and had MRSA infections. Of great concern were the two reports of vancomycin-resistant *Staphylococcus aureus* (VRSA) because the reports exhibited

complete resistance and a different mechanism of dissemination. While the mechanism of resistance for VISA is chromosomally mediated, that of VRSA is by conjugal transfer of the *vanA* operon from an *Enterococcus faecalis* (Lowy, 2003).

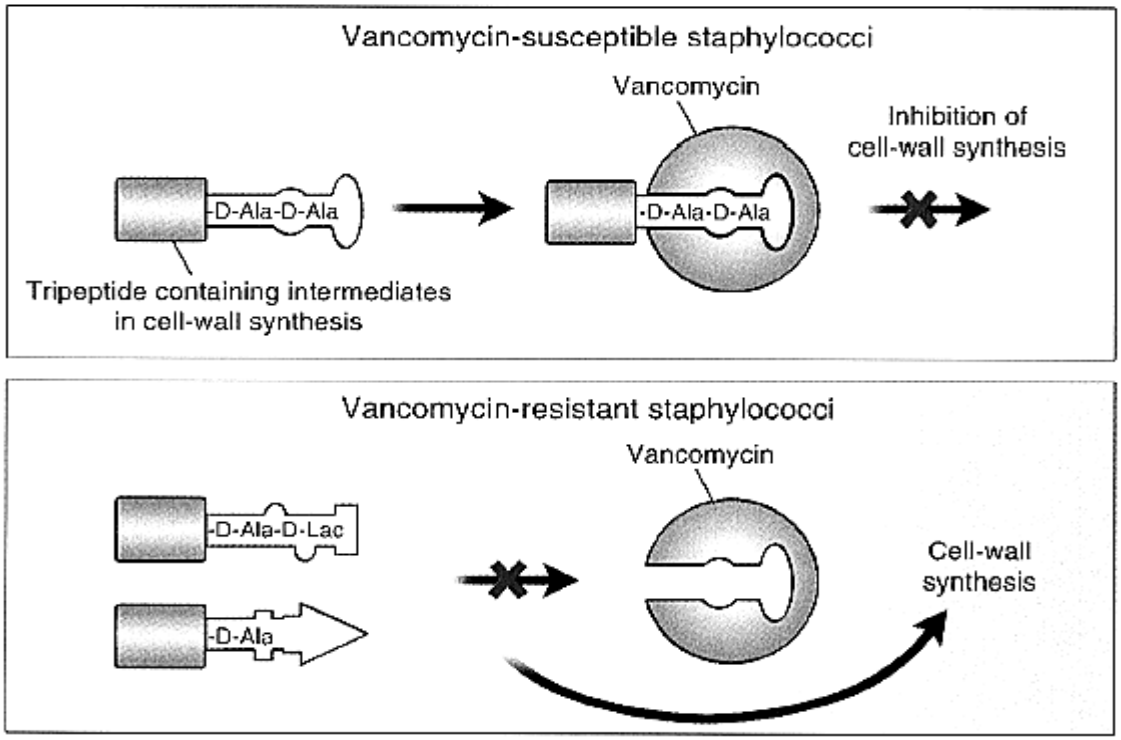


Fig 2.6: Mechanism of *Staphylococcus aureus* resistance to vancomycin: VRSA strains

VRSA strains are resistant to vancomycin because of the acquisition of *vanA* operon from an enterococcus that allows synthesis of a cell wall precursor that ends in D-Ala-D-Lac dipeptide rather than D-Ala-D-Ala. The new dipeptide has dramatically reduced affinity for vancomycin. In the presence of vancomycin, the novel cell wall precursor is synthesized allowing continued peptidoglycan assembly. (Source: Lowy, 2003).

There are two forms of *Staphylococcus aureus* resistance to vancomycin – one has been recognized in VISA strains which has MICs to vancomycin of 8-16 µg/mL and a pre-VISA stage of resistance, which is heterogeneously resistant. The hetereo-resistant strain is susceptible to vancomycin but harbours resistant subpopulation. The hypothesis is that on exposure to vancomycin, the VISA isolates are selected from the vancomycin-resistant subpopulation (Lowy, 2003). The decreased susceptibility to vancomycin may be due to changes in peptidoglycan biosynthesis (Plate 2.2). The VISA strains are known for added quantities of synthesized peptidoglycan which yield irregularly shaped thick cell walls. More D-Ala-D-Ala residues are exposed as a result of decreased cross-linking of peptidoglycan. The reduced crosslinking emanates from reduced amount of L-glutamine available for the amidation of D-glutamate in the pentapeptide bridge. Consequently, there are more D-Ala-D-Ala residues to bind and trap vancomycin (Plate 2.2). The bound and trapped vancomycin subsequently acts as a barrier to drug molecules reaching their target on the cytoplasmic membrane.

The second method of resistant is possibly due to conjugal transfer of *vanA* operon from a vancomycin-resistant *Enterococcus faecalis.* In a study by Showsh *et al*., (2001) the enterococcal plasmid harbouring *vanA* also encodes a sex pheromone that is synthesized by *Staphylococcus aureus* which suggests a plausible facilitator of conjugal transfer. Complete vancomycin resistance is observed with VRSA isolates exhibiting MICs ≥ 128µg/mL. In this case, resistance is achieved by change in the terminal peptide from D-Ala-D-Ala to D-Ala-D-Lac. Exposure to low concentrations of vancomycin precipitates the synthesis of D-Ala-D-Lac. Consequently, the added biosynthetic demands are restricted and the VRSA strain is ecologically fit (Lowy, 2003). Finally, Table 2.7 summarizes the mechanisms of *Staphylococcus aureus* resistance to a number of antimicrobial agents.

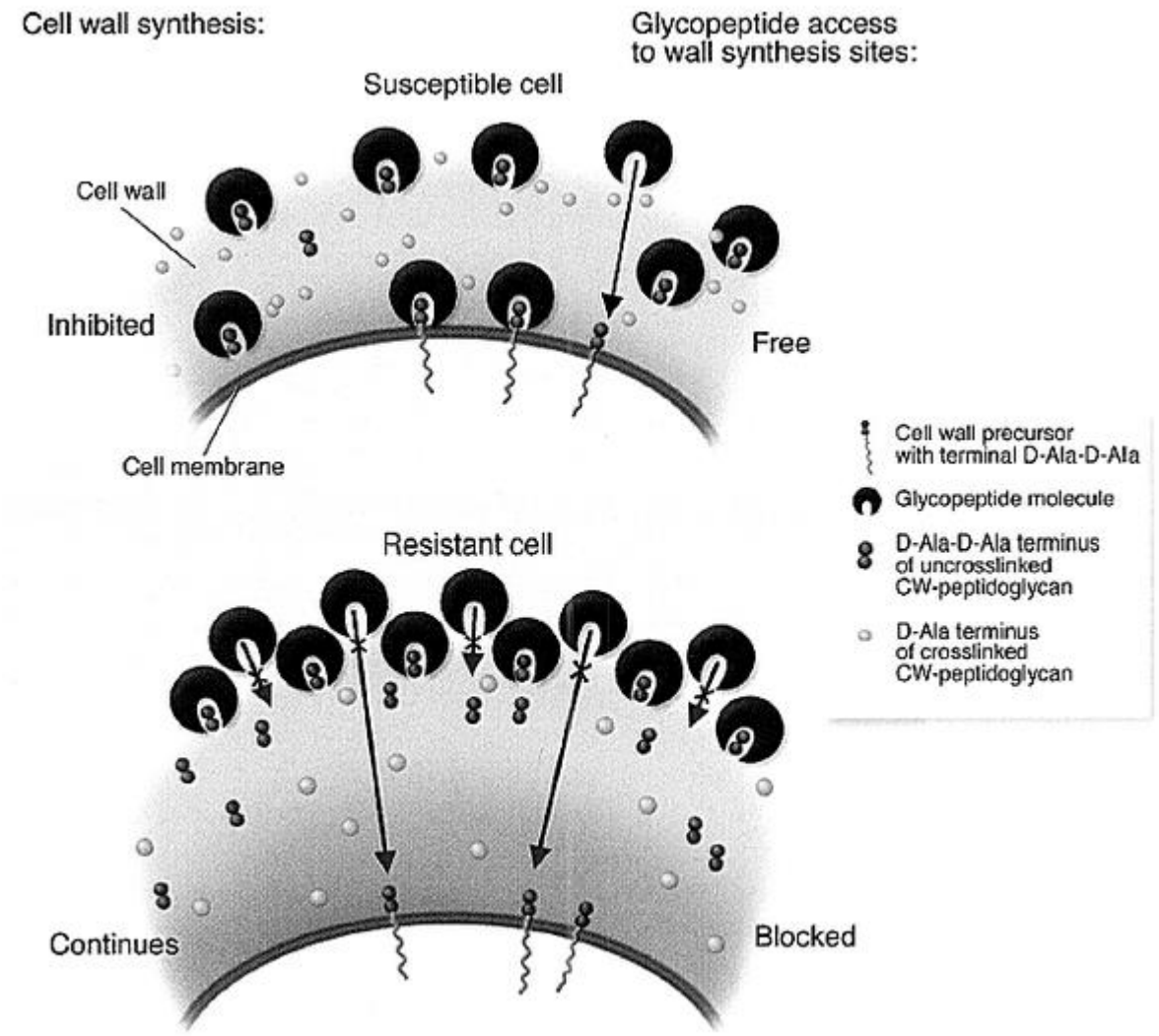


Plate 2.2: Mechanism of *Staphylococcus aureus* resistance to vancomycin: VISA strains.

VISA strains appear to be selected form isolates that are heterogeneously resistant to vancomycin. The VISA strains synthesize additional peptidoglycan with an increased number of D-Ala-D-Ala residues that bind vancomycin, preventing the molecule from getting to its bacterial target site. (Sources: Lowy, 2003; Sieradzki *et al.*, 1999)

Table 2.7: Mechanisms of *Staphylococcus aureus* to selected antimicrobial agents

Antibiotic	Resistance gene(s)	Gene product(s)	Mechanism(s) of resistance	Location(s)*
β-lactams	1) *blaZ* 2) *mecA*	1) β-lactamase 2) PBP2a	1) Enzymatic hydrolysis of β-lactam nucleus 2) Reduced affinity for PBP	1) PI:Tn 2) C:SCCmec
Glycopeptides	1) Unknown (VISA) 2) VRSA	1) Altered peptidoglycan 2) D-Ala-D-Lac	1) Trapping of vancomycin in the cell wall 2) Synthesis of dipeptide with reduced affinity for vancomycin	1) C 2) PI:Tn
Quinolones	1) *ParC* *2) gyrA or gyrB*	1) *ParC* (or grIA) component of topoisomerase IV 2) GyrA or GyrB component of gyrase	1,2) Mutations in the QRDR region, reducing affinity for enzyme-DNA complex for quinolones	1) C 2) C
Aminoglycosides (e.g. Gentamicin)	Aminoglycoside modifying enzymes (*aac, aph*)	Acetyltransferase, phosphotransferase	Acetylating and/or phosphorylating enzymes modify aminoglycosides	PI, PI:Tn
Trimethoprim-sulphamethoxazole (TMP-SMZ)	1) Sulfonamide: *sulA* 2) TMP: *dfrB*	1) Dihydropteroate synthase 2) Dihydrofolate reductase (DHFR)	1) Overproduction of ρ-aminobenzoic acid by enzyme 2) Reduced affinity for	1) C 2) C
Oxazolidinones	*rrn*	23S rRNA	Mutation in doman V of 23S rRNA component of the 50S ribosome. Interferes with ribosomal binding	C
Quinupristin-dalfopristin	1) Q: *ermA, ermB, ermC* 2) D: *vat, vatB*	1) Ribosomal methylases 2) Acetyltransferases	1) Reduce binding to the 2S3 ribosomal subunit 2) Enzymatic modification of dalfopristin	1) PI, C 2) PI

*PI, plasmid; C, chromosome; Tn, transposon; QRDR, quinolone resistant-determining region. (Source: Lowy, 2003).

2.11. Hospital Associated MRSA

Hospital associated MRSA (HA-MRSA) is referred to as that which develops in the hospital and was not incubating at the time of patient's admission. Hospital associated MRSA poses a threat to patients globally. Microbiological data from 300 laboratories in USA documented in 2005 MRSA rates were 59%, 55% and 48% for strains from non-intensive care unit (ICU) inpatients, ICU and outpatients respectively (Tacconelli *et al.*, 2008). The risk factors to hospital associated MRSA include prior hospitalization, use of antibiotics, underlying diseases, surgery, duration of hospitalization, enteral feeding, central venous catheterization and endotracheal intubation, admission to ICU, nursing staff workload and compliance to hand disinfection procedures (Tacconelli et al., 2008). Data has shown that HA-MRSA is mainly responsible for invasive MRSA infections (Stefani *et al.*, 2012).

2.12. Community associated MRSA

Community associated MRSA refers to that present or incubating at the time of admission and was not due to previous hospital admission. The rate and extent of the spread of community associated MRSA (CA-MRSA) has been striking. CA-MRSA has been reported in various countries. Globally, CA MRSA strains have displayed measurable diversity in the number of clones identified (Chambers and DeLeo, 2009). The sudden increase of community associated MRSA infections documented from different countries was linked to the detection of a unique *SCCmec* Type IV (Lowy, 2003). *SCCmec*, Type IV which is smaller than others seems more genetically mobile and does not at present carry additional resistance genes. It is been suggested that it has been heterologously transferred more readily from other staphylococcal species as it appears to occur in a more diverse range of MSSA genetic backgrounds. CA-MRSA strains are virulent and are implicated in such tissue-destructive infections as necrotizing fascilitis and fulminant necrotizing pneumonia rarely seen before emergence of CA-MRSA strains (Chambers and DeLeo, 2009). The risk factors known for patients with HA-MRSA are often

absent in patients with CA-MRSA infections. CA-MRSA has been observed to comprise of more diverse clonal groups than HA_MRSA (Huang et al., 2006). It is noteworthy that CA-MRSA is migrating to healthcare settings (Stefani *et al.*, 2012).

2.13. Transmission of MRSA

MRSA was first identified in Europe in the 1960s shortly after the introduction of methicillin. However, naturally-resistant strains were isolated in some countries before the use of methicillin or related agents. These strains may have spread at first from one or more ancestral genetic clones in natural populations of *Staph. aureus* by horizontal transfer and recombination (Ayliffe, 1997). These original strains increased in numbers and diversity as a result of selection by exposure to antibiotics and by cross infection. The main mode of spread is person-to-person within a unit or hospital. However, other modes of spread can occur such as through the air.

2.14. Molecular typing of MRSA

Typing is the identification of different types of organisms within specie. Conventional typing methods based on phenotype such as serotype, biotype, phage-type and antibiogram have been utilized. However, methods that identify and relate isolates at molecular level have transformed the ability to differentiate among bacterial types and subtypes (Sabat *et al.*, 2013). The selection of an appropriate molecular typing method (or methods) is based mainly on the problem to solve and the epidemiological context in which the method is to be utilized, as well as the time and geographical scale of its use (Sabat *et al.*, 2013). In an epidemic, a typing method should be able to differentiate both unrelated and closely related isolates in order to develop strategy to prevent the spread. In addition, it should be rapid, inexpensive, reproducible and easy to perform and interpret. When typing method is used for continuous surveillance, the method should yield stable results over time to permit implementation of efficient infection control

measures. Also, that which will be used for international networks should produce portable data which can be accessed through open source database via internet. It is worthy to note that none of the molecular typing methods developed is optimal for all forms of investigation. Consequently, it is paramount that the pros and cons of the typing methods are considered before a method is selected. Molecular typing methods for bacterial investigations include pulsed-field gel electrophoresis, amplified fragment length polymorphism, random amplification of polymorphic DNA, arbitrarily primed polymerase chain reaction, repetitive-element polymerase chain reaction, variable-number tandem repeat typing, single locus sequence typing, multilocus sequence typing, comparative genomic hybridisation, optical mapping, whole genome sequencing, and *Staphylococcus aureus* protein A gene-typing (Sabat *et al.,* 2013). The most frequently used typing methods for *Staph. aureus* are *spa* sequencing typing, multilocus sequence typing (MLST), *SCCmec* typing, Macrorestriction pattern analysis (PFGE) and multilocus variable-number tandem repeat (VNTR) analysis (MLVA). However, the preferred methods are *spa* sequence typing and staphylococcal cassette chromosome mec (*SCCmec*) typing (Stefani *et al.*, 2012).

2.15. Control of Antibiotic Resistance among *S. aureus* and preventive measures

MRSA infections remains an evolving public health challenge and successful controls in some countries offers guide on identifying the preventive measures and best practices. In Europe, Hospital-acquired MRSA has been minimized by employing a multifaceted preventive interventions which included measures focussing on screening, mandatory reporting of all MRSA bacteraemia by the hospital chief executive officers, public benchmarking of MRSA incidence rates, the establishment of a national hand hygiene campaign and other contact precautions, decolonisation, antibiotic stewardship, update and strengthening of national MRSA guidelines, surveillance and extension of prospective surveillance and care bundles focussing on key clinical procedures that can increase the risk of infection if not performed

appropriately (Robin et al., 2010). The approaches employed in the prevention, control and treatment of HA MRSA are applicable to CA MRSA. Screening and decolonization have been effective in controlling some outbreaks of CA MRSA (Skov et al. 2012). The purpose of screening for MRSA in healthy community carriers include: to minimize transmission of MRSA, prevent development of infections in carriers and to prevent further transmission, especially of clones that tend to spread effortlessly (Skov et al., 2012). Minimizing transmission will require such activities as hand hygiene and environmental cleanliness. In addition, antibiotic stewardship is vital in minimizing the development of resistance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Bacteriological Media

These includes Nutrient Broth (NB), Mueller Hinton Agar (MHA), Mannitol Salt Agar (MSA), and Nutrient Agar. All were products of Oxoid Ltd, Basingstoke, UK.

3.1.2 Antibiotics

The following antibiotic discs were from Oxoid Ltd., Basingstoke, UK: penicillin (1.5ug), oxacillin (1ug), cefoxitin (30ug), cefotaxime (30ug), vancomycin (30ug), ofloxacin (5ug), ciprofloxacin (5ug), gentamycin (10ug), cotrimoxazole (25ug), erythromycin (15ug), and tetracycline (30ug); as well as E-strips of Oxacillin and Vancomycin. Oxacillin powder and Vancomycin powders were products of Hospira, Illinois, USA.

3.1.3 Reagents and Chemicals

Sodium Chloride, Iodine crystals and Potassium Iodide all of analar grades from BDH Chemicals Ltd. Poole, England, while Ethidium bromide was from Sigma, Dorset, England.

3.1.4 Equipment

Autoclave (portable) Adelphi Mfg Co. Ltd. UK, Incubator (National Appliane Co. Ltd, Oregon, USA: model 1630, 240V and 2340W), Balance (analytical), Incubators, Hot air oven (Baird and Tatlock (London) Ltd, Chadwell Health Essex, England), Refigerator (Haier Thermocool: Model No.HRF-688-FF/A), PCR Thermocyler (Techne TC-312), Gel electrophoresis machine (Max Fill Scie-plas. Model HU10 serial no5237), Binocular microscope, Centrifuge (Eppendorf entrifuge 5417R), Membrane filter apparatus, Micropipette (automatic) 1000uL,

Micropipette (automatic) 10-40uL, Water bath, Votexing machine (Touch plate Super Mixer, CAT No 1291 La-line instrument Inc. USA), magnetic stirrer (W and T Avery Ltd. England).

3.1.5 Plasma

Citrated human blood for coagulase test was collected from the University of Jos Teaching Hospital, Jos, Nigeria. The blood was centrifuged and the plasma collected aseptically and stored in the refrigerator for use within 48 hours.

3.2 SAMPLE COLLECTION

Urine samples were collected from healthy volunteers who were not on any antibiotics (or had not taken any antibiotics) three-four weeks before samples were collected. They included male and female sciene and arts students. Volunteers were given dry, sterile universal bottles and instructed how to collect the mid-stream urine. The women were instructed to clean the area around the urethral opening with water and towel dry before collecting the urine samples. Convenience sampling method was used targrting 200 to 250 volunteers. The samples so collected were taken to the laboratory and analysed within four hours (4hrs.). A total number of 217 urine samples were collected.

3.2.1 Informed consent

All the participants in the study gave informed consent following detailed explanation of study and assurance of anonymity.

3.3 EXPERIMENTAL METHODS

3.3.1 Preparation of Growth media

The media were reconstituted according to the manufacturer's directive and sterilized at 121^{o}C for 15 minutes. The media were freshly prepared as required.

For physiological saline, 9g of sodium chloride was weighed and dissolved in 1 litre of distilled water and sterilized in an autoclave at 121^{o}C for 15 minutes. This was used in diluting the bacterial culture as required.

3.3.2 Isolation of organism

The urine samples were inoculated into nutrient broth and incubated for 18-24 hours at 37^{o}C. The overnight cultures were then streaked on to dried mannitol salt agar and incubated at 37^{o}C for 24 - 48 hours. Characteristic colonies were re-streaked on mannitol salt agar and incubated. Isolated characteristic colonies were noted and inoculated into nutrient broth and nutrient agar slants, incubated at 37^{o}C overnight and kept for further characterization to establish their identity.

3.3.3 Characterisation and identification of *S. aureus* isolates

Staining

Smears of characteristic colonies were made on a clean slide, fixed, gram-stained and viewed under the microscope. Isolates that appeared gram-positive in characteristic clusters were subcultured in duplicates unto Nutrient agar slants and incubated overnight at 37^{o}C.

Catalase test

The test for the presence of the enzyme catalase was demonstrated by the addition of about 1 ml of 3% hydrogen peroxide on a 24-hour slant culture of the isolates. Positive cultures were characterized by rapid evolution of gas bubbles. The test differentiates Staphylococci from Streptococci species.

Coagulase test

The latex test method, employing the BactiStaph latex test kit, was used to differentiate *Staphylococcus aureus* (coagulase positive) from the coagulase negative *Staphylococcus* spp.

Following the manufacturer's instruction, a drop of the BactiStaph latex reagent was dropped in a circle on the slide provided and with the stirring stick, two-three colonies were picked and applied to a dry portion of the circle on the slide and slowly blended with the latex reagent and spread over the entire circle. The slide was rotated in a circular motion for not more than 60 seconds and observed for clumping/agglutination. The same procedure was followed for the positive and negative controls on the same slide but different circles. Large black clumps of agglutination with subsequent loss of black background is positive for coagulase.

Identification using Rapid Test Kits

Microgen™ STAPH-ID kit was used for the final identification of the isolates. Single colonies of 24 hour cultures of the 135 coagulase positive isolates were emulsified in the suspending medium of the Microgen test kit and mixed thoroughly. There were 12 wells and with the aid of sterile Pasteur pipette, 3 drops of the bacterial suspension were added to each well of the strips. Wells 10 and 11 were overlaid with 3 drops of mineral oil. The top of the microwell test strips were sealed with the adhesive tape and incubated at 37^{o}C. The test strips were read after 24 hours against a template. The readings were fed into a software and the isolates were identified to sub-specie level.



Plate 3.1: Digital image of the Rapid test reactions for identification of *S. aureus*

Test for B-lactamase production

A solution of Penicillin G (10,000 units/ml) in phosphate buffer solution (pH 7) was prepared. An emulsion of each isolate was made in a 0.5 ml aliquot of this solution with a sterile wire loop touched unto a few colonies of a 24-hour culture of the organisms. To this was added 2 drops of a freshly prepared 1% aqueous starch solution, shaken gently, and one drop of iodine solution. The solution was allowed to stand for 10 minutes at room temperature and any colour change noted. B-lactamase producers changed the colour from blue-black to colourless.

3.3.4 Tests for the susceptibility of *S. aureus* isolates to the antibiotics

3.3.4.1 Agar Diffusion test

The antibiotic susceptibility patterns of the 171 staphylococcal isolates were determined using the disc diffusion test method of the Clinical Laboratory Standards Institute (CLSI, 2015) formerly (NCCLS). Sterile 20ml aliquots of Mueller-Hinton agar were poured into sterile Petri dishes and allowed to set. Overnight cultures of the isolates were diluted in normal saline to

0.5 McFarland turbidity standard (ca. 10^8 cfu/ml) and used to flood the Mueller-Hinton agar plates. The excess inoculum was poured out and the plates over dried in the incubator for 10 minutes. The antibiotic discs were applied ensuring that every part of the discs touched the agar. The plates were left at room temperature for one hour before incubating in an inverted position at 37ºC for 18-24 hours. The inhibition zone diameters were measured to the nearest millimetres using a transparent plastic rule and classified accordingly as sensitive, intermediate or resistant based on the CLSI interpretive chart for zone sizes (CLSI, 2015). Three determinations were done for each isolate and the average of the readings calculated.

3.3.4.2 Epsilometer test (E-test)

The isolates that were resistant to methicillin (cefoxitin and oxacillin) were subjected to E-test. The same procedure for the diffusion test was followed except that the e-test strips for oxacillin and vancomycin were used. The plates were incubated at 37ºC for 24 hours. The contact point of the zone to the e-strip was taken as the MIC.

3.3.4.3 Breakpoint test

Agar plates containing 6µg/mL of oxacillin were prepared and standardized overnight culture of the isolates that were methicillin resitant by diffusion test were streaked on the plates and incubated overnight for 24 hours at 37ºC. Growth on the plates were noted. The same procedure was followed for vancomycin using a concentration of 2µg/mL.

3.3.5 Plasmid curing test

Serial dilutions of ethidium bromide was prepared in Mueller-Hinton broth. Standardized overnight culture of the resistant isolates were inoculated into the broths and incubated at 37ºC for 24 hours. The MIC were recorded. Subcultures were made from the dilutions just below the MIC and were re-tested for susceptibility to the test antibiotics by the diffusion method (Darini, 1996).

3.3.6 Extraction of DNA of *S. aureus* isolates

Sixteen out of the test isolates that have been shown to be phenotypically methicillin resistant (resistance to cefoxitin/oxacillin) by the diffusion test were used for all the molecular analysis. An overnight culture in tryptic soya broth (TSB) were centrifuged at 2000rpm for 10 minutes and the supernatant was discarded into a disinfectant jar. Then 480 microlitres of 50mmoL EDTA at pH 8 was added to the sedimented pellets and vortexed to re-suspend the cells. A master mix containing 30µl of lysostaphin, 15µl mutanolysin and 75µl lysozyme per reaction were vortexed to mix. Aliquots of 120µl from the master mix were made in 1.5 mL microcentrifuge tubes labelled to correspond with the isolates. This pre-treatment was done to weaken cells to allow for efficient cell lysis. The cell and EDTA mixture were poured into corresponding labelled 1.5 mL microcentrifuge tubes. The samples were incubated at 37^{o}C for 60 minutes and centrifuged for 3 minutes at 13500 rpm. The supernatant were decanted into a disinfectant jar. To the pellets, 600µl nuclei lysis solution was added and inverted severally to re-suspend. It was further incubated at 75^{o}C for 5 minutes in heatblock to lyse the cells. On cooling to room temperature, 2µl of RNA solution was again added to the cell lysate, inverted 2-5 times to mix and further incubated at 37^{o}C for 45-60 mins. Finally, 200µl of protein precipitation solution was added to the RNA treated cell lysate and vortex vigorously at high speed for 20 secs to mix. The protein precipitation solution/cell lysate were incubated on ice for 5 minutes and brought to room temperature. The sample was centrifuged at 13500 rpm for 3 minutes. The supernatant approx. 800µl containing the DNA was transferred into a clean 1.5 mL microcentrifuge tube containing 600µl isopropanol. They were gently mixed by inversion and further centrifuged for 3 minutes. The supernatant was decanted and the DNA pellets were washed with 600µl 70% ethanol kept at room temperature. The extracted DNA was stored at -20^{o}C till required for polymerase chain reaction.

3.3.7 Polymerase Chain Reaction tests

3.3.7.1 *S. aureus spa* typing

The Staphylococcal protein A (*spa*) sequence typing was done for the polymorphic region of protein A (repeat polymorphism of the X-region of the *spa* gene) and the protocol used was as described by Shopsin *et al,* (1999). A master mix was prepared based on the number of DNA templates. The reagents and the amount used were as presented in

The volume of all the reagents was 48µl for each reaction before the 2µl DNA templates was added. The number of isolates for *spa* typing was multiplied by the volume of each reaction to give the final volume of the master mix. Before the addition of the Taq polymerase as was the case in the multiplex PCR, the mastermix was vortexed to mix for a few seconds. On addition of the Taq polymerase, the master mix was inverted several times to mix and dispensed using auto-repeater into PCR tubes at a volume of 48µl. Finally, 2µl of each DNA template was added to the corresponding PCR tube. The PCR tubes were agitated and placed in the thermocycler. The following primers as designed by Shopsin *et al*, (1999) were used.

Table 3.1: Primers sequence for spa typing

gene	primer sequence	expected amplicon size
***spa* 2F**	GAACAACGTAACGGCTTCATCC	(250-637bp)
***spa* 1514R**	CAGCAGTAGTGCCGTTTGCCT	~425bp

The thermocycler was programmed according to the manufactures' instructions. Optimization of the conditions was necessary for maximum yield. Each *spa* PCR program started with an initial heat-activation step at 95^{o}C for 10 minutes to activate the Taq DNA polymerase. This was followed by 3-step cycling: denaturation for 30 seconds at a temperature of 95^{o}C; annealing for 30 seconds at a temperature of 60^{o}C; and extension step for 1 minute at 72^{o}C. The total number of cycles was 30 and the final extension time was 10 minutes at 72^{o}C.

Agarose gel was prepared as described earlier, loaded with the *spa* PCR product and electrophoresed. Following electrophoresis, it was viewed using the transilluminator (Bio-rad Gel Doc XR) and the picture of the bands was taken.

The *spa* products were purified before sequencing. The purification protocol as described by the manufacturer was followed. The Qiagen PCR purification kit (QIAquick) was used. To 1 volume of the *spa* PCR reaction, 5 volumes of buffer PB (binding buffer) were added and mixed. A QIAquick column was placed in a 2ml collection tube and the PCR DNA was applied to it and centrifuged for 1minute for the DNA to bind to it. The flow-through was discarded and the QIAquick column was placed back in the same tube. To wash the DNA, 750µl of buffer PE (washing buffer) was added to the QIAquick column and centrifuged for 1 minute. The flow-through was again discarded and the QIAquick was returned back in the same tube. This was centrifuged for another I minute to remove the entire residual wash buffer. After the centrifugation, each QIAquick column was placed in a clean 1.5ml microcentrifuge tube. To elute the DNA, 50µl buffer EB (eluting buffer) consisting of 10mM Tris-Cl with pH of 8.5 was added at the centre of the QIAquick membrane and centrifuged for 1 minute. This was allowed to stand for 1 minute and re-centrifuged. The flow-through was collected for quantification.

The concentration of each PCR sample was determined using Spectrophotometer. This was to determine the amount of each sample needed in each reaction to include 5ng/100bp of the amplified product. The amplified *spa* region was ~425bp in length so that 20ng of product was needed. The sequencing protocol required two wells (one for forward primer and the second for reverse primer) as the DNA core utilizes cycle sequencing. The molecular grade water added to each reaction was determined using the formula: Template + primer A + dH_2O = 11µl total volume. The dH_2O needed for each reaction was aliquoted into the respective well of 96 well plates. To each well, 1µl of the appropriate primer was added which were the same with forward and reverse used in the PCR. The amount of DNA determined earlier was then added

to the corresponding wells. After loading the wells, a tinfoil lid was placed on the plate and sealed and sent off to the DNA core laboratory where the sequencing was done.

spa results were analysed using the spa-RIDOM website and recorded. The repeats varied according to strains forming bases for its usefulness as a molecular tool.

3.3.7.2 Multilocus sequence typing (MLST) of novel *spa* types of *S. aureus*.

A multiplex PCR was carried out to detect the seven housekeeping genes: *arcC, aroE, glpF, gmk, pta, tpi, and yqiL*. The protocol used was as described by Enright *et al,* (2000). Seven master mixes were prepared one for each house keeping gene. The final volume of the master mix was determined by the number of reaction/the DNA templates. The mastermix was votexed to mix before the addition of the Taq polymerase which was inverted severally to mix and aliquoted in volumes of 48µl into PCR tubes. Finally, the template DNA was added, agitated to mix and then loaded in the thermocycler. The positive control used was MRSA USA300. Primers used were as shown in Table 3.2

Table 3.2: Primers used for multilocus sequence typing

gene	primer sequence	expected amplicon size
***arcC*-Forward**	TTG ATT CAC CAG CGC GTA TTG TC	456bp
***arcC*-Reverse**	AGG TAT CTG CTT CAA TCA GCG	
***AroE*-Forward**	ATC GGA AAT CCT ATT TCA CAT TC	456bp
***AroE*-Reverse**	GGT GTT GTA TTA ATA ACG ATA TC	
***glpF* Forward**	CTA GGA ACT GCA ATC TTA ATC	465bp
***glpF* Reverse**	TGG TAA AAT CGC ATG TCC AAT TC	
***gmk*,Forward**	ATC GTT TTA TCG GGA CCA TC	429bp
***gmk*,Reverse**	TCA TTA ACT ACA ACG TAA TCG TA	
***pta*,Forward**	GTT AAA ATC GTA TTA CCT GAA GG	474bp
***pta*,Reverse**	GAC CCT TTT GTT GAA AAG CTT AA	
***tpi* Forward**	TCG TTC ATT CTG AAC GTC GTG AA	402bp
***tpi* Reverse**	TTT GCA CCT TCT AAC AAT TGT AC	
***yqiL* Forward**	CAG CAT ACA GGA CAC CTA TTG GC	516bp
***yqiL* Reverse**	CGT TGA GGA ATC GAT ACT GGA AC	

Source: (Enright et al., 2000)

The optimization process involved initial heat activation of the Taq DNA polymerase at 94ºC for 5 minutes followed by 3-step cycling: denaturation for 30 seconds at a temperature of 94ºC; annealing for 30 seconds at a temperature of 55ºC; and extension step for 60 seconds at 72ºC. The total number of cycles was 30 and the final extension time was 5 minutes at 72ºC.

The amplified DNA product for each of the seven housekeeping genes was run on a large gel in which 3 grams of agarose was gradually heated to dissolve in 200ml of TAE buffer and 8µl of ethidium bromide was added to stain the DNA. The PCR products were loaded on the gel and run on a Bio-rad electrophoretic machine at 120V for 1.5hrs.

At the end, the gel bands were viewed using the Bio-rad Gel Doc XR transilluminator. The PCR products were purified and quantified as described in 3.4.7.1 and sent out for sequencing.

3.3.7.3 Staphylococcal cassette chromosome *mec* (SCC*mec*) typing

A multiplex PCR was carried out to detect *mecA* gene for methicillin resistance and to determine the type of staphylococcal cassette chromosome *mec* (SCC*mec*). Primers (forward and reverse) used were for type I to type V SCC*mec* according to a protocol previously described (Ghaznavi-Rad *et al*., 2010).

The master mix was prepared to include the *mecA* and the SCC*mec* types I-V primers according to the number of DNA templates. The mastermix was votexed to mix before the addition of the HotStarTaq which on addition was inverted severally to mix and aliquoted in volumes of 48µl into PCR tubes. Finally, the template DNA was added, agitated to mix and then loaded in the thermocycler. The positive control used was MRSA USA300. The primers used were as shown in Table 3.3.

Table 3.3: Primers used for *SCCmec* typing

Gene	**primer sequence**	**expected amplicon size**
Type I Forward	GCTTTAAAGAGTGTCGTTACAGG	
Type I Reverse	GTTCTCTCATAGTATGACGTCC	613bp
Type II Forward	GATTACTTCAGAACCAGGTCAT	
Type II Reverse	TAAACTGTGTCACACGATCCAT	287bp
Type III Forward	CATTTGTGAAACACAGTACG	
Type III Reverse	GTTATTGAGACTCCTAAAGC	243bp
Type IVa Forward	GCCTTATTCGAAGAAACCG	
Type IVa Reverse	CTACTCTTCTGAAAAGCGTCG	776bp
Type IVb Forward	AGTACATTTTATCTTTGCGA	
Type IVb Reverse	AGTCATCTTCAATATGGAGAAAGTA	1000bp
Type IVc Forward	TCTATTCAATCGTTCTCGTATT	
Type IVc Reverse	TCGTTGTCATTTAATTCTGAACT	677bp
Type IVd Forward	AATTCACCCGTACCTGAGAA	
Type IVd Reverse	AGAATGTGGTTATAAGATAGCTA	1242bp
Type IVh Forward	TTCCTCGTTTTTTCTGAACG	
Type IVh Reverse	CAAACACTGATATTGTGTCG	663bp
Type V Forward	GAACATTGTTACTTAATGAGCG	
Type V Reverse	TGAAAGTTGTACCCTTGACACC	325bp
mecA Forward	TCCAGATTACAACTTCACCAGG	
mecA Reverse	CCACTTCATATCTTGTAACG	162bp
Sa442 Forward	AATCTTTGTCGGTACACGATATTCTTCACG	
Sa442 Reverse	CGTAATGAGATTTCAGTAGATAATACAACA	108bp

Source: Ghaznavi-Rad *et al*., 2010

The optimization process involved initial heat activation of the Taq DNA polymerase at 95^{o}C for 15 minutes followed by 3-step cycling: denaturation for 30 seconds at a temperature of 94^{o}C; annealing for 90 seconds at a temperature of 57^{o}C; and extension step for 90 seconds at 72^{o}C. The total number of cycles was 30 and the final extension time was 10 minutes at 72^{o}C.

The amplified DNA product was run on a large gel in which 3 grams of agarose was gradually heated to dissolve in 200ml of TAE buffer and 8µl of ethidium bromide was added to stain the DNA. The PCR products were loaded on the gel and run on a Bio-rad electrophorectic machine at 120V for 1.5hrs. At the end, the bands were viewed using the Bio-rad Gel DocXR trans-illuminator. The SCCmec type was determined on the basis of the band pattern obtained.

CHAPTER FOUR

4.-0 RESULTS

4.1 Isolation and Characterisation

Of the total of 217 samples collected, 171 were positive for *Staphylococcus* by the cultural method giving a prevalence of 78.8%. All the 171 isolates grew on Mannitol Salt Agar producing yellow colonies with yellow zones in 24-48 hours. Staining showed Gram-positive cocci in clusters. Out of the isolates, 84 were catalase positive, 135 were coagulase positive and 98 were B-lactamase producers (Table 4.1). Of the culturally identified *Staphylococcus* isolates 135 were tested with the Microgen™ STAPH-ID kit and 73 (54.1%) of these were confirmed *S. aureus*. The different staphylococcal species identified are as shown in Table 4.2 below.

4.2 Antibiogram of the staphylococcal isolates

The zone diameters were interpreted using the Fluka zone interpretive chart (appendix A) which is based on CLSI standards. The results of the susceptibility of the 171 staphylococcal isolates to the various antibiotics are as shown in Table 4.3 below. All the isolates were susceptible to all the antibiotics at varying degrees of susceptibility. However, only 8.2% (14) and 19.3% (33) were susceptible to penicillin and cefotaxime respectively. Of the quinolones, ciprofloxacin was effective against most of the isolates - 88.3% (151). Interestingly, majority of the isolates – 79.5% (136) were resistant to ofloxacin which is intriguing as ofloxacin has same mechanism of action as ciprofloxacin. Susceptibility to co-trimoxazole was 63.2% (108) while vancomycin seems to demonstrate good effectiveness. Cefoxitin and oxacillin had 21.1% (36) and 39.2% (67) susceptibility respectively

Table 4.1: Proportion of samples positive for *Staphylococcus*

Parameters	Frequency or Number	%
Urine samples analysed	217	100
Staphylococci	171	78.8
Coagulase positive Staph.	135	62.2
β-lactamase positive Staph.	98	45.2
S. aureus	73	33.6

Table 4.2: Distribution of coagulase positive Staphylococcus

Staphylococus species (n=135)	Number	%
S. aureus	73	54.1
S. hominis	7	5.2
S. hyicus	7	5.2
S. intermedius	16	11.9
S. xylosus	11	8.1
S. schleiferi	5	3.7
S. cohnii	11	8.1
S. haemolytius	2	1.5

.

Table 4.3: Susceptibility of all the *Staphylococcus* isolates (n=171) to given antibiotics by the disc diffussion test.

Test Antibiotic	Susceptibility	
	Frequency	%
Tetracycline (10µg)	60	35.1
Erythromycin (15µg)	89	52.0
Cefoxitin (30µg)	36	21.1
Ciprofloxacin (30µg)	151	88.3
Ofloxacin (5µg)	35	20.5
Co-trimoxazole (25µg)	108	63.2
Vancomycin (30µg)	125	73.1
Gentamicin (10µg)	112	65.5
Oxacillin (1µg)	67	39.2
Penicillin (1.5)	14	8.2
Cefotaxime (30µg)	33	19.3

4.3 Susceptibility and Resistance Patterns of staphylococcus isolates

Fig. 4.1 depicts the multi-resistant pattern of the isolates to the antibiotics indicating the number of antibiotics to which a certain number of isolates were resistant. In addition, Tables 4.5a – 4.5c display the specificity of the antibiotics to which certain isolates are resistant. Most isolates were resistant to at least one antibiotic while minimum of 3 isolates were resistant to 11 antibiotics. The number and type of antibiotics to which an isolate was resitant differed in some instances from other iolates. The calculated multiantibiotic resistant index is shown in Table 4.4.

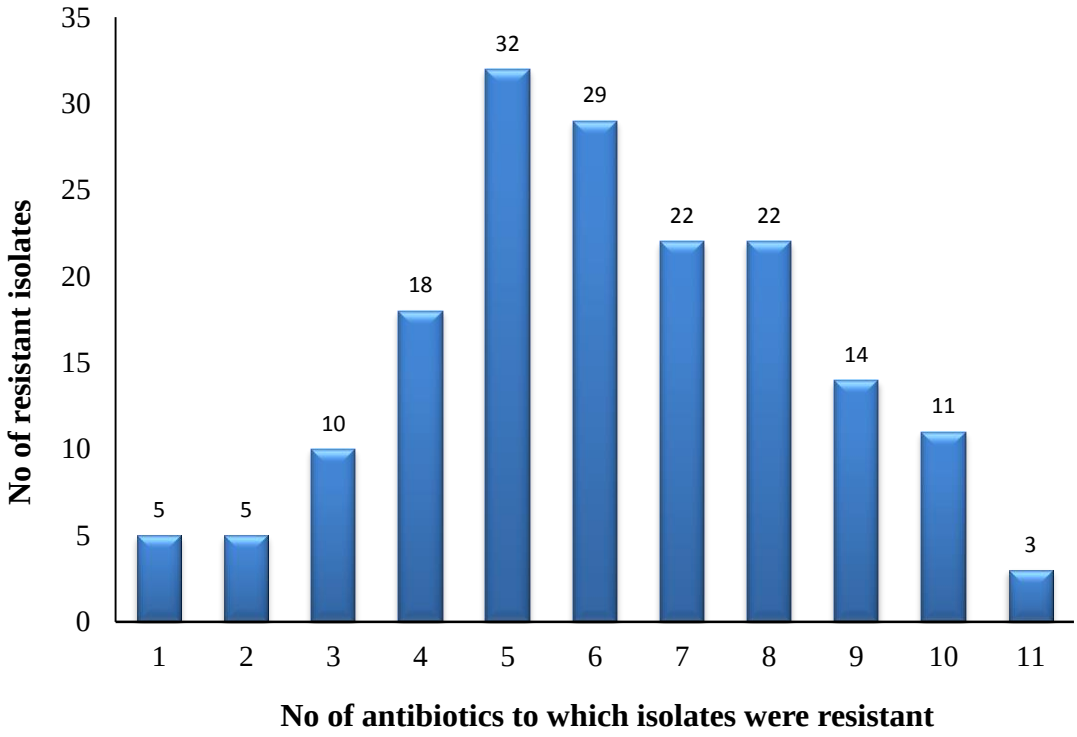


Fig 4.1: Multi-antibiotic resistant patterns of the staphylococcus isolates

Table 4.4: Multi Antibiotic Resitant Indices (MARI) of test isolates (n=171)

No. of Antibiotics to which Resistant	MARI	No. of Isolates	Cumulative % at the MARI
1	0.09	5	2.92
2	0.18	5	5.85
3	0.27	10	11.70
4	0.36	18	22.22
5	0.45	32	40.94
6	0.54	29	57.84
7	0.64	22	70.76
8	0.73	22	83.63
9	0.81	14	92.81
10	0.91	11	98.24
11	1.0	3	100.0

*Percentage of isolates with MARI ≥ index value.

Table 4.5a: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	**No of Isolates**
OX	104
OX, CN	42
OX, CN, VA	19
OX, CN, VA, STX	14
OX, CN, VA, STX, OFX	14
OX, CN, VA, STX, OFX, CIP	4
OX, CN, VA, STX, OFX, CIP, FOX	4
OX, CN, VA, STX, OFX, CIP, FOX, E	4
OX, CN, VA, STX, OFX, CIP, FOX, E, TE	4
OX, CN, VA, STX, OFX, CIP, FOX, E, TE, P	4
OX, CN, VA, STX, OFX, CIP, FOX, E, TE, P, CTX	3

Table 4.5b: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	**No of Isolates**
FOX	135
FOX, E	69
FOX, E, TE	54
FOX, E, TE, P	51
FOX, E, TE, P, CTX	47
FOX, E, TE, P, CTX, OX	43
FOX, E, TE, P, CTX, OX, CN	19
FOX, E, TE, P, CTX, OX, CN, VA	13
FOX, E, TE, P, CTX, OX, CN, VA, STX	10
FOX, E, TE, P, CTX, OX, CN, VA, STX, OFX	10
FOX, E, TE, P, CTX, OX, CN, VA, STX, OFX, CIP	3

Table 4.5c: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	**No of Isolates**
VA	46
VA, STX	21
VA, STX, OFX	19
VA, STX, OFX, CIP	4
VA, STX, OFX, CIP, FOX	4
VA, STX, OFX, CIP, FOX, E	4
VA, STX, OFX, CIP, FOX, E, TE	4
VA, STX, OFX, CIP, FOX, E, TE, P	4
VA, STX, OFX, CIP, FOX, E, TE, P, CTX	3
VA, STX, OFX, CIP, FOX, E, TE, P, CTX, OX	3
VA, STX, OFX, CIP, FOX, E, TE, P, CTX, OX, CN	3

4.4 E-Test

The MIC of 33 of the isolates that were resistant to cefoxitin/oxacillin and vancomycin by diffusion test were determined by E-test to confirm resistance of the isolates to vancomycin and oxacillin (Table 4.6). Two isolates out of 33 isolates had MIC values ≥4μg/ml and were resistant to oxacillin while none of the isolates (MIC <8μg/ml) were resistant to vancomycin (Table 4.6).

4.5 Breakpoint Test

For breakpoint test, absence of bacterial growth is indicative of susceptibility while growth indicates resistance. Seventy-one of seventy-three *S. aureus* isolates, which accounted for 97.3%, were resistant.

4.6 Curing Results:

The MIC against ethidium bromide was found to range from 3.125ug/ml to 100ug/ml. Most of the isolates were inhibited at MIC values of between 6.25-100ug/mL. Susceptibility of the cured isolates to the test antibiotics are as shown in Table 4.5. The ethidium bromide treated isolates became very susceptible to ciprofloxacin, cotrimoxazole erythromycin and vancomycin, but were still very resistant to cefoxitin, ofloxacin and penicillin.

Table 4.6: Susceptibility of isolates to oxacillin and vancomycin by E-Test (MIC values)

MIC (ug/ml)*	Frequency	
	Oxacillin	**Vancomycin**
0.015	1	0
0.03	0	0
0.06	0	1
0.12	1	1
0.25	1	1
0.5	9	2
1.0	13	5
2.0	5	13
4.0	1	10
8.0	0	0
16.0	1	0
32.0	0	0

*Resistance value: Oxacillin ≥ 4 µg/mL; Vancomycin ≥ 8µg/mL

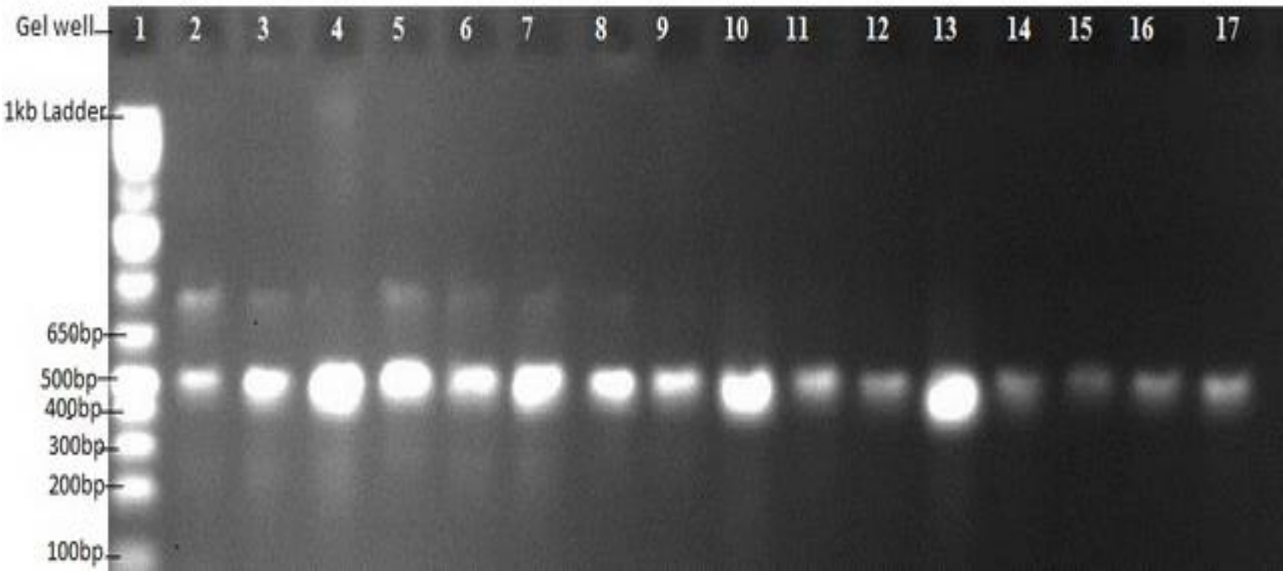
Table 4.7: Susceptibility of *S. aureus* isolates to given antibiotics after curing with Ethidium Bromide

Antibiotic	No. of isolates	% susceptile
Tetracycline (10µg)	26	42.3
Erythromycin (15µg)	31	80.6
Cefoxitin (30µg)	27	14.8
Ciprofloxacin (30µg)	35	94.3
Ofloxacin (5µg)	32	9.4
Co-trimoxazole (25µg)	27	88.9
Vancomycin (30µg)	33	90.9
Gentamicin (10µg)	34	58.8
Oxacillin (1µg)	22	54.5
Penicillin (1.5)	25	16.0
Cefotaxime (30µg)	25	40.0

*Total should be 35; however, there were no growth was observed with some isolates re-tested.

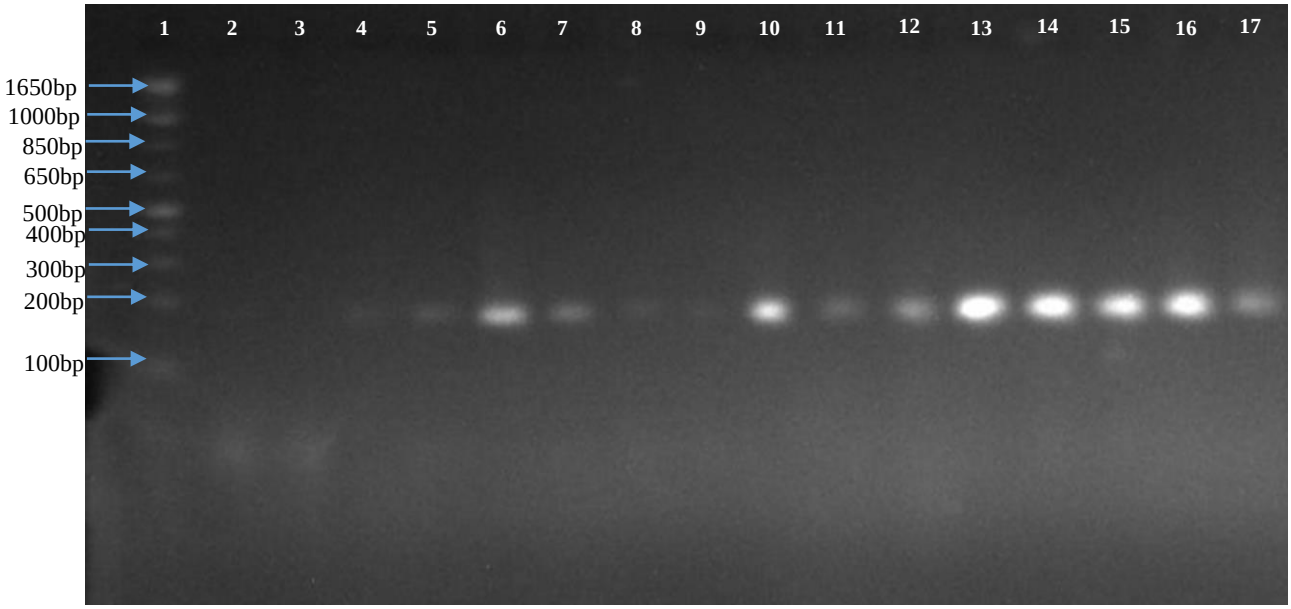
4.7 DNA Extraction and PCR analysis

As shown in Plate 4.1, all the 16 isolates contain *mecA* gene while only six of the tested isolates (6, 10, 13, 14, 15 and 16) indicated the presence of *mecB* gene (Plate 4.2). The chromatogram for the characteristic gene Saa442 showed bands below 100bp while that and *nuc* were absent (Plates 4.3 and 4.4 respectively) but Plate 4.5 showed the presence of the *spa* gene. Plates 4.6 and 4.7 are the confirmatory chromatograms indicating the presence of DNA. All the 16 isolates had *mecA* gene with bands at 500 base pair (bp) while Isolates 6, 10, 13-16 inidcated prominent bands for *mecB* 200bp. The bands for sa442 and *nuc* gene were below 100bp while those for *spa* were at 100pb.



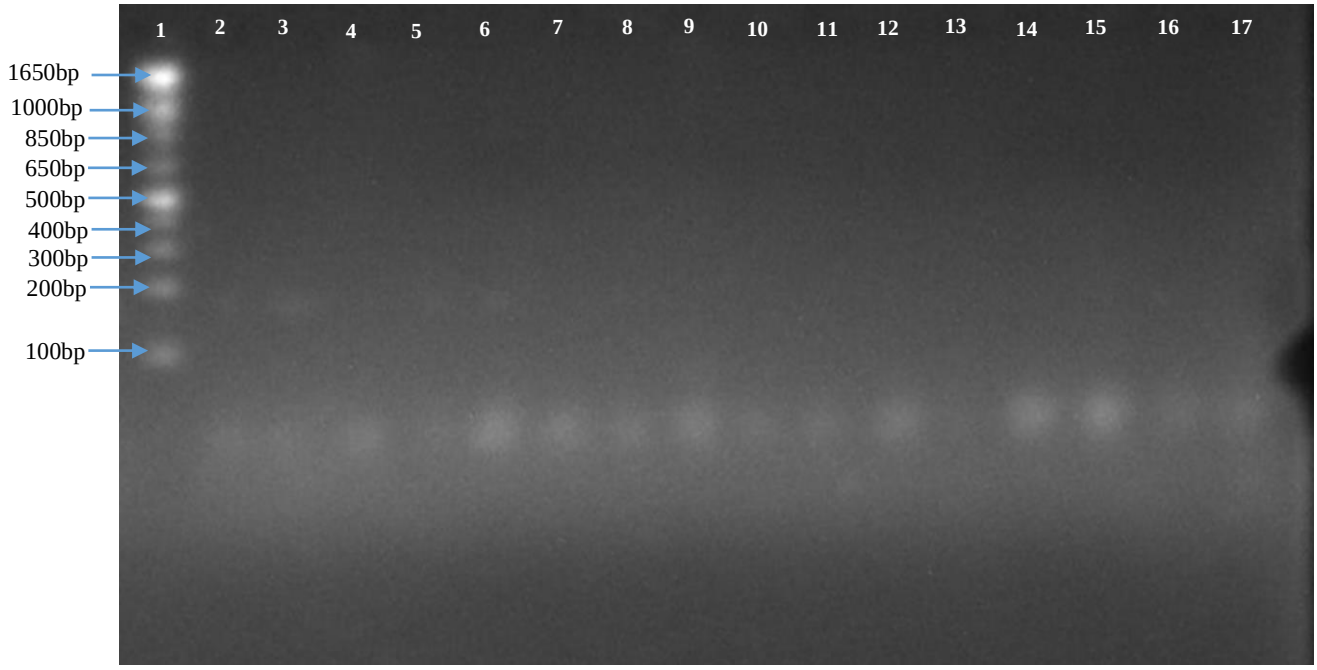
Key: Lane 1 is the ladder and lanes 2 – 17 are *S. aureus* isolates

Plate 4.1: Electrophoretogram of *MecA* gene (500bp) amplification products *S. aureus* isolates



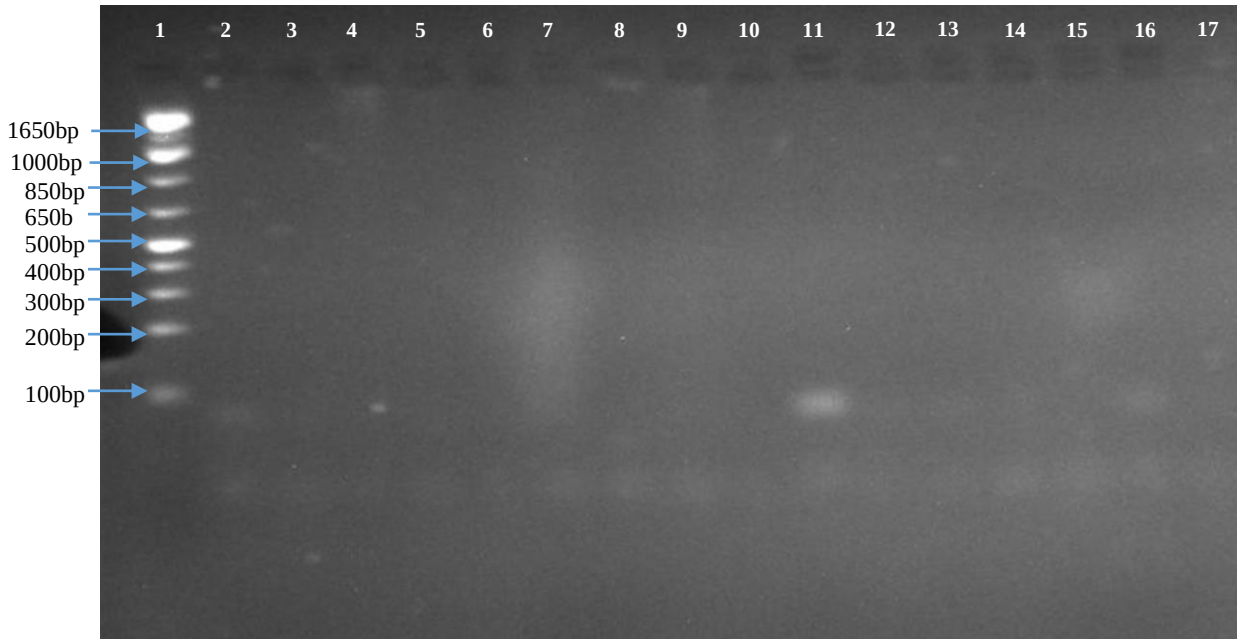
Key: Lane 1 is the ladder and lanes 2 – 17 are *S. aureus* isolates

Plate 4.2: Electrophoretogram of *MecB* gene (200bp) amplification products *S. aureus* isolates



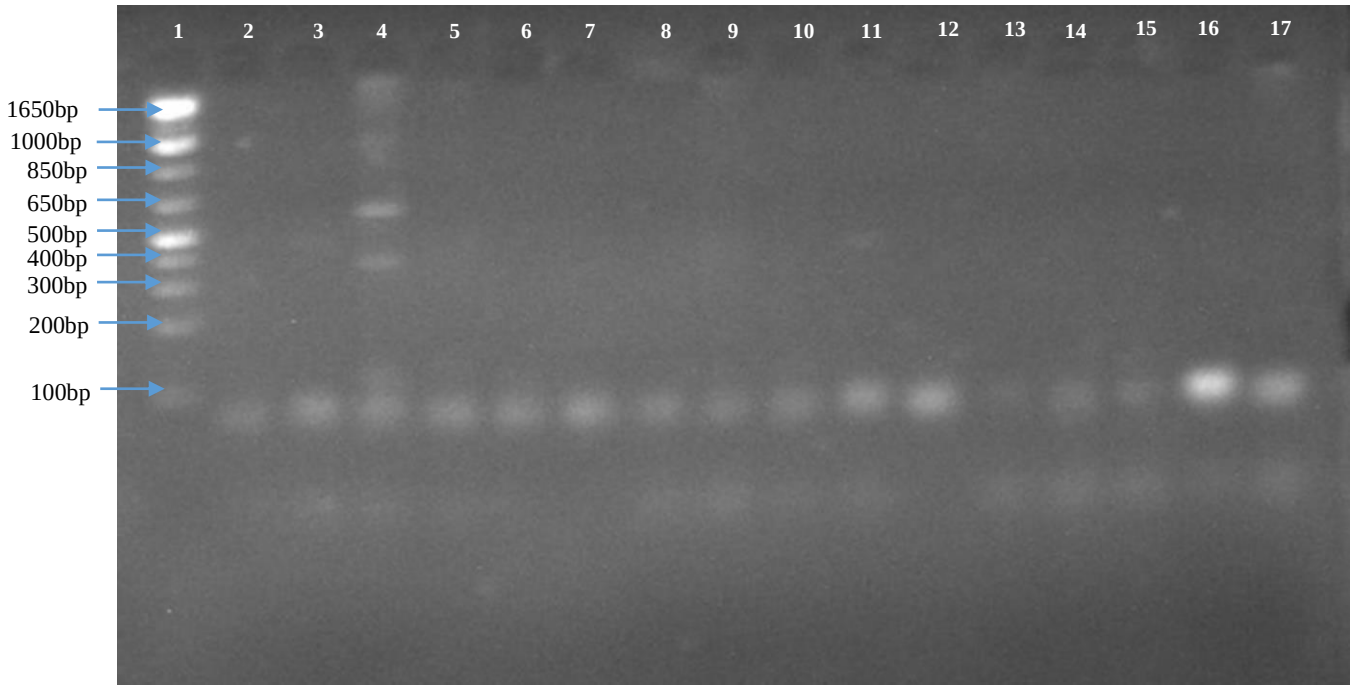
Key: Lane 1 is the ladder and lanes 2 – 17 are *S. aureus* isolates

Plate 4.3: Electrophoretogram of *Sa442* amplification products *S. aureus* isolates



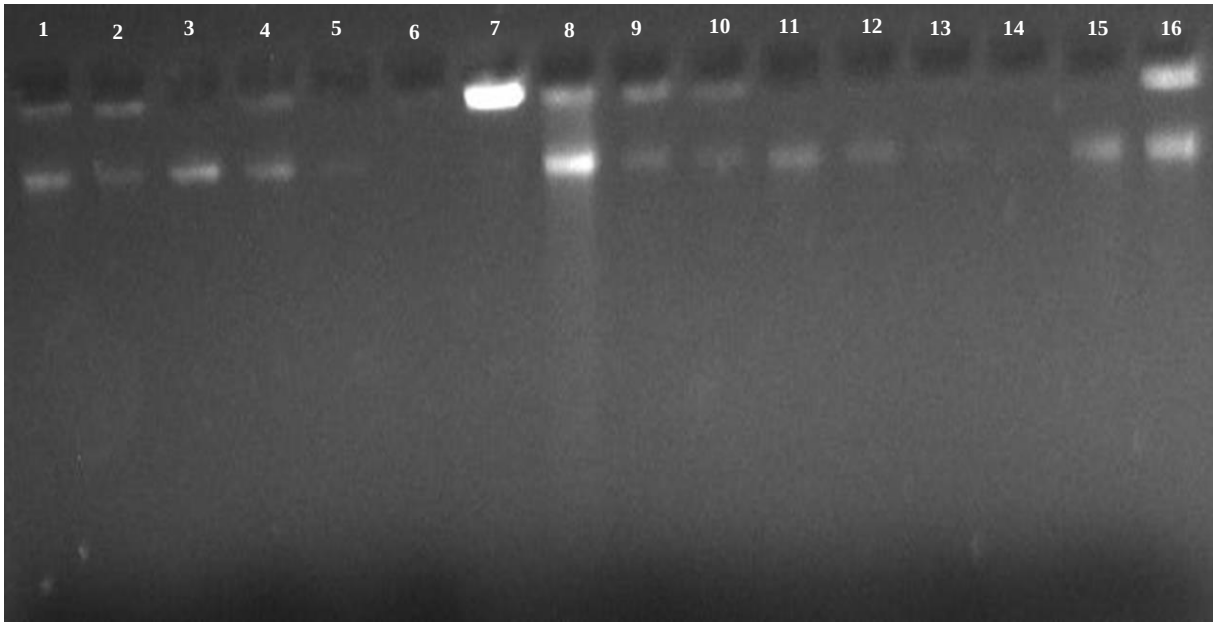
Key: Lane 1 is the ladder and lanes 2 – 17 are *S. aureus* isolates

Plate 4.4: Electrophoretogram of *nuc* gene amplification products *S. aureus* isolates



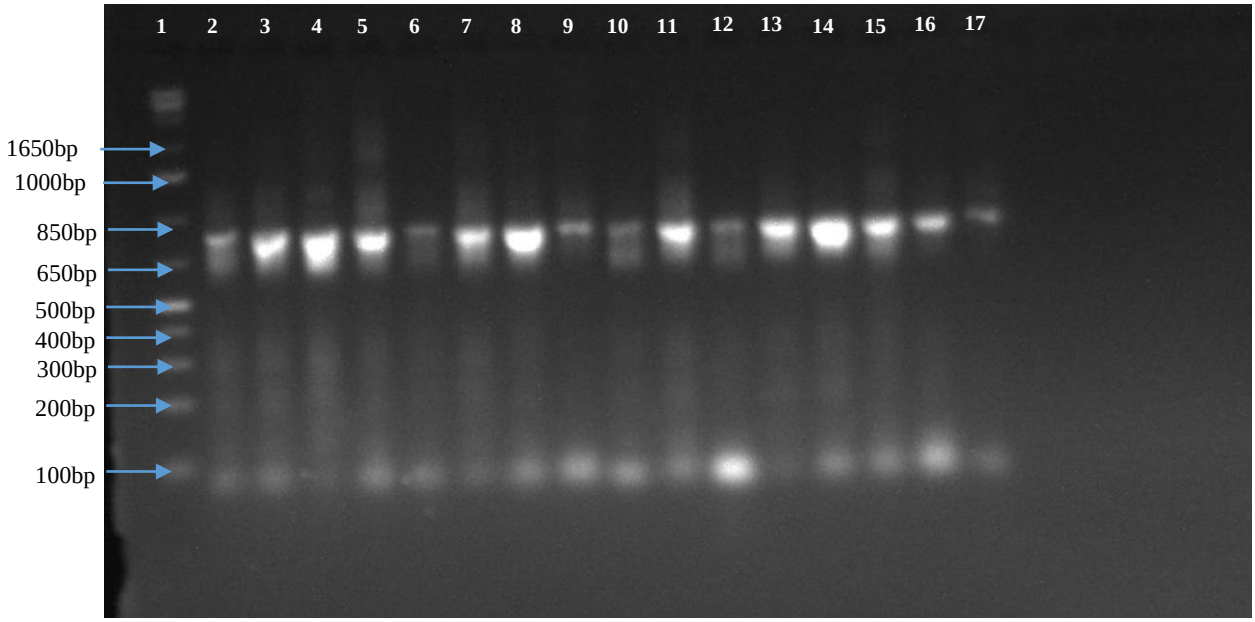
Key: Lane 1 is the ladder and lanes 2 – 17 are *S. aureus* isolates

Plate 4.5: Electrophoretogram of *spa* gene (250-637 bp) amplification products *S. aureus* isolates



Key: Lanes 1-16 are *S. aureus* isolates

Plate 4.6: Electrophoretogram of extracted DNA showing that the DNA had not degraded



Key: Lane 1 is the ladder and lanes 2 – 17 are *S. aureus* isolates

Plate 4.7: *S. aureus* electrophoretogram of *16SrRNA* gene (800bp)

4.8 Phylogenetic analysis

Phylogenetic tree was produced using BLAST pairwise alignments on NCBI site and the tree method was neighbor joining with 0.75 maximum sequence difference. Within the nucleotide collection and 16S ribosomal RNA (Bacteria and Archaea) databases of NCBI, there was no significant similarity found for isolate 5, 9 and 16. However, nucleotide collection database was employed for blasting the isolates as it was a larger database than that of 16S ribosomal RNA (Bacteria and Archaea). Only one similar Staph aureus was found for isolate 11 on nucleotide collection and no significant similarity found on 16S ribosomal RNA (Bacteria and Archaea) database. The phylogenetic relationships between the isolates and significantly similar *Staphylococcus aureus* are as shown in Figures 4.11 – 4.23. Isolates 1, 2, 4, 5, 8, 10, 11, 12, 13 and 15 are remotely related to other identical *Staphylococcus aureus.* Isolates 3 and 14 shared an immediate common lineage (ancestor) with *Staphylococcus aureus* 8 BWI while isolate 7 shares an immediate common lineage with *Staphylococcus aureus* S1123. The alignment view of blasts of isolates against other identical *Staph. aureus* strains are in **Appendix A**

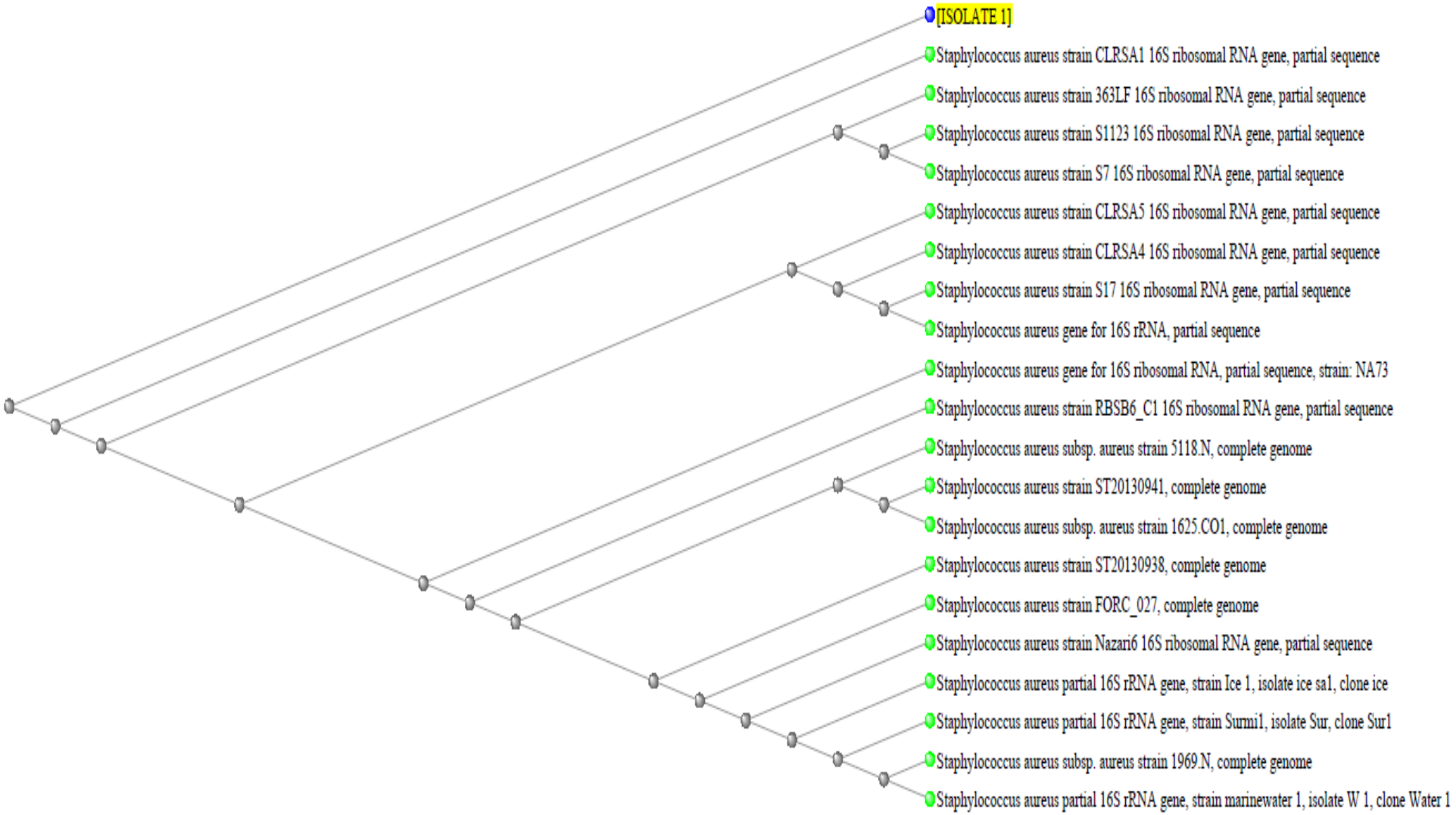


Fig 4.2: Phylogenetic tree showing the relationship between Isolate 1 and other identical *Staph. aureus* strains from GenBank database

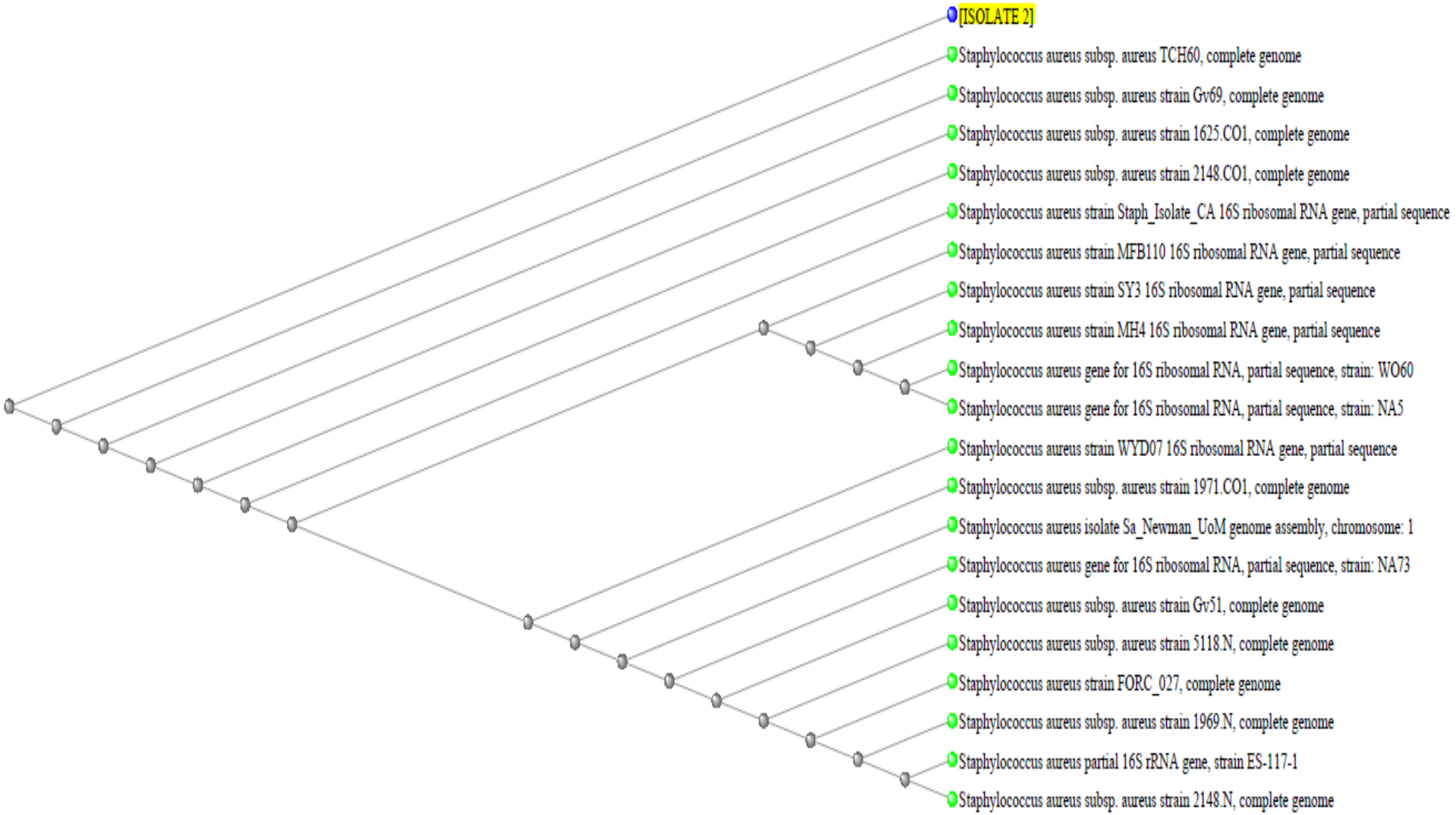


Fig 4.3: Phylogenetic tree showing the relationship between Isolate 2 and other identical *Staph. aureus* strains from GenBank database

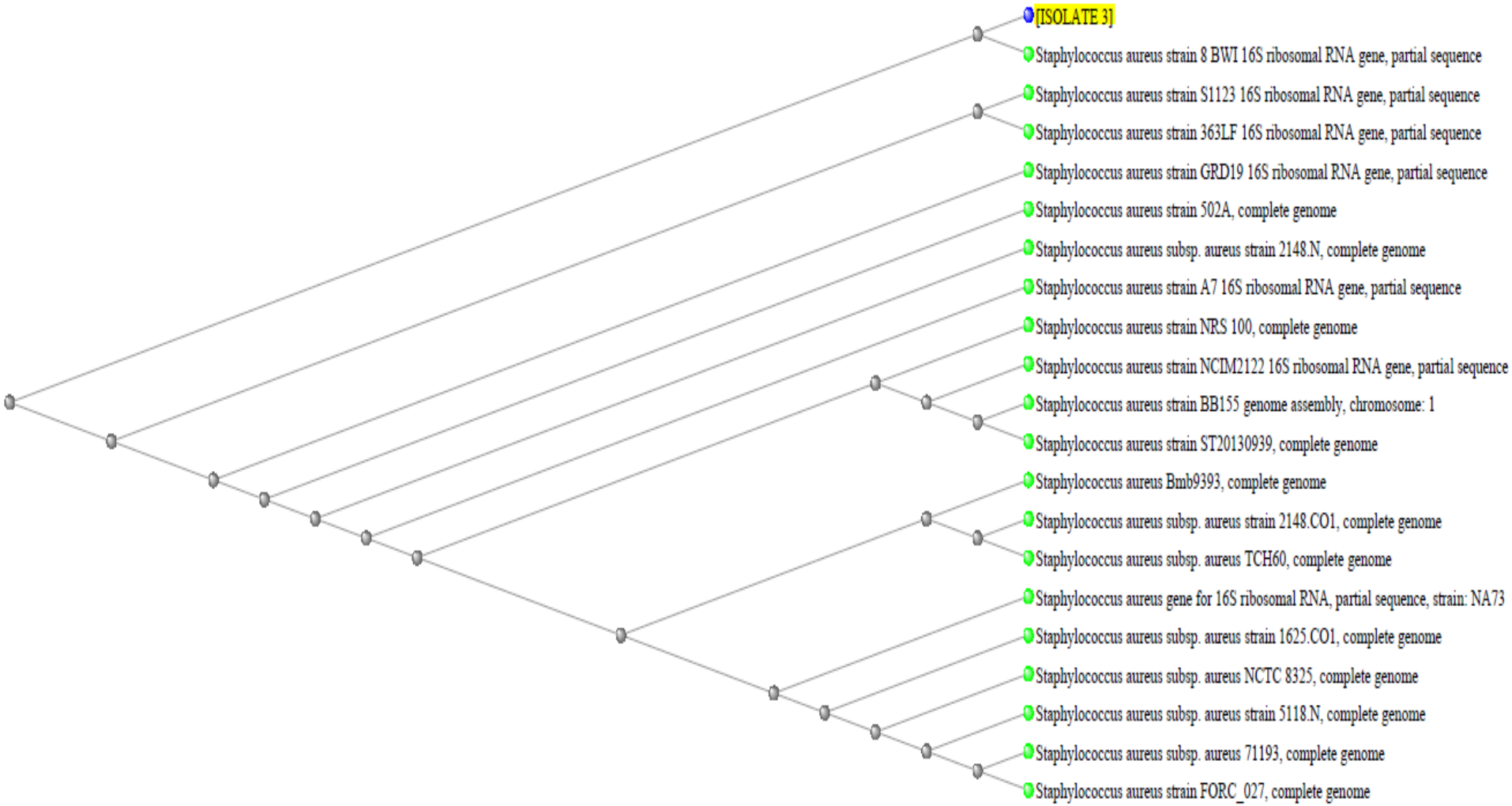


Fig 4.4: Phylogenetic tree showing the relationship between Isolate 3 and other identical *Staph. aureus* strains from GenBank database

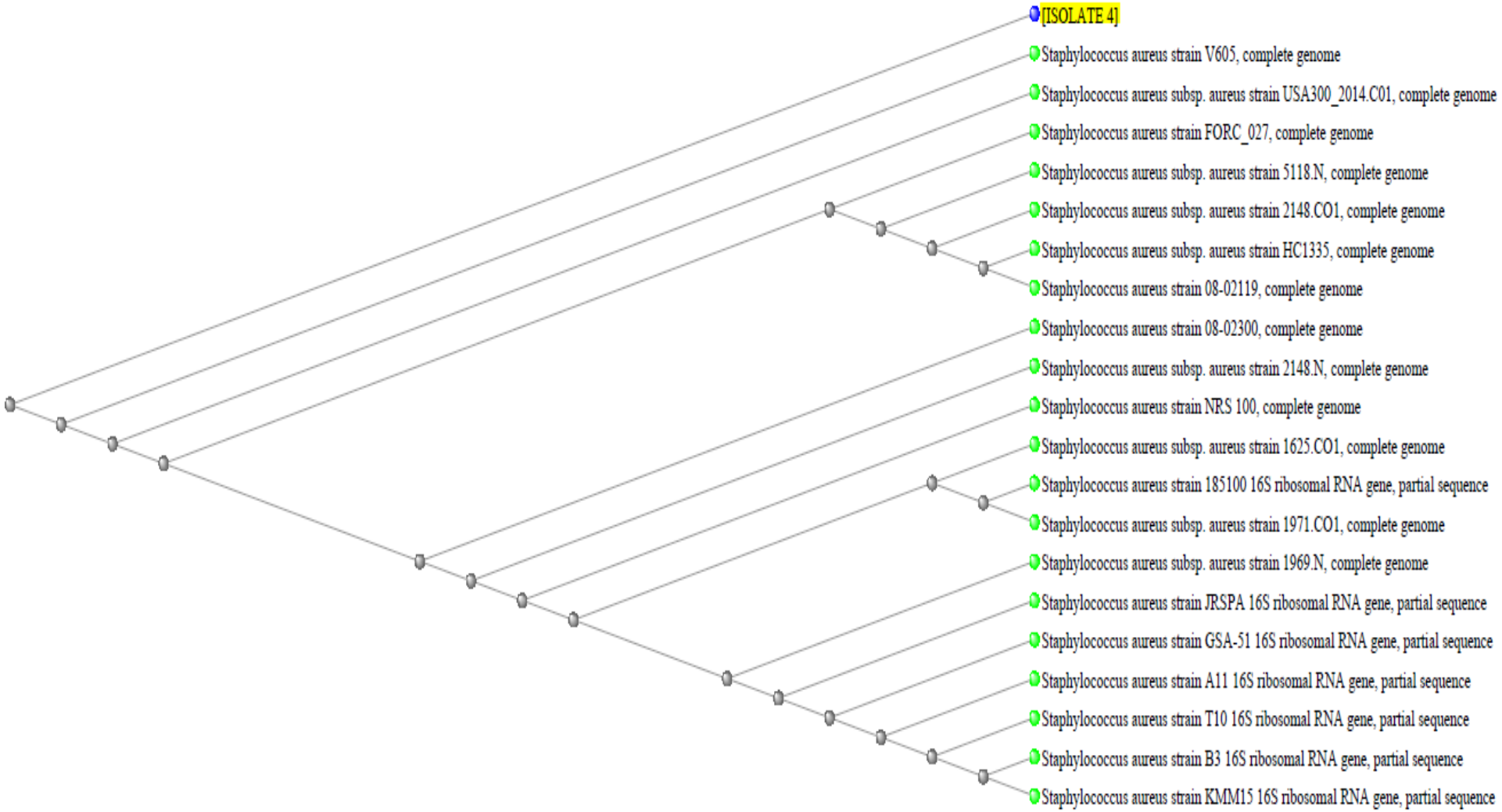


Fig 4.5: Phylogenetic tree showing the relationship between Isolate 4 and other identical *Staph. aureus* strains from GenBank database

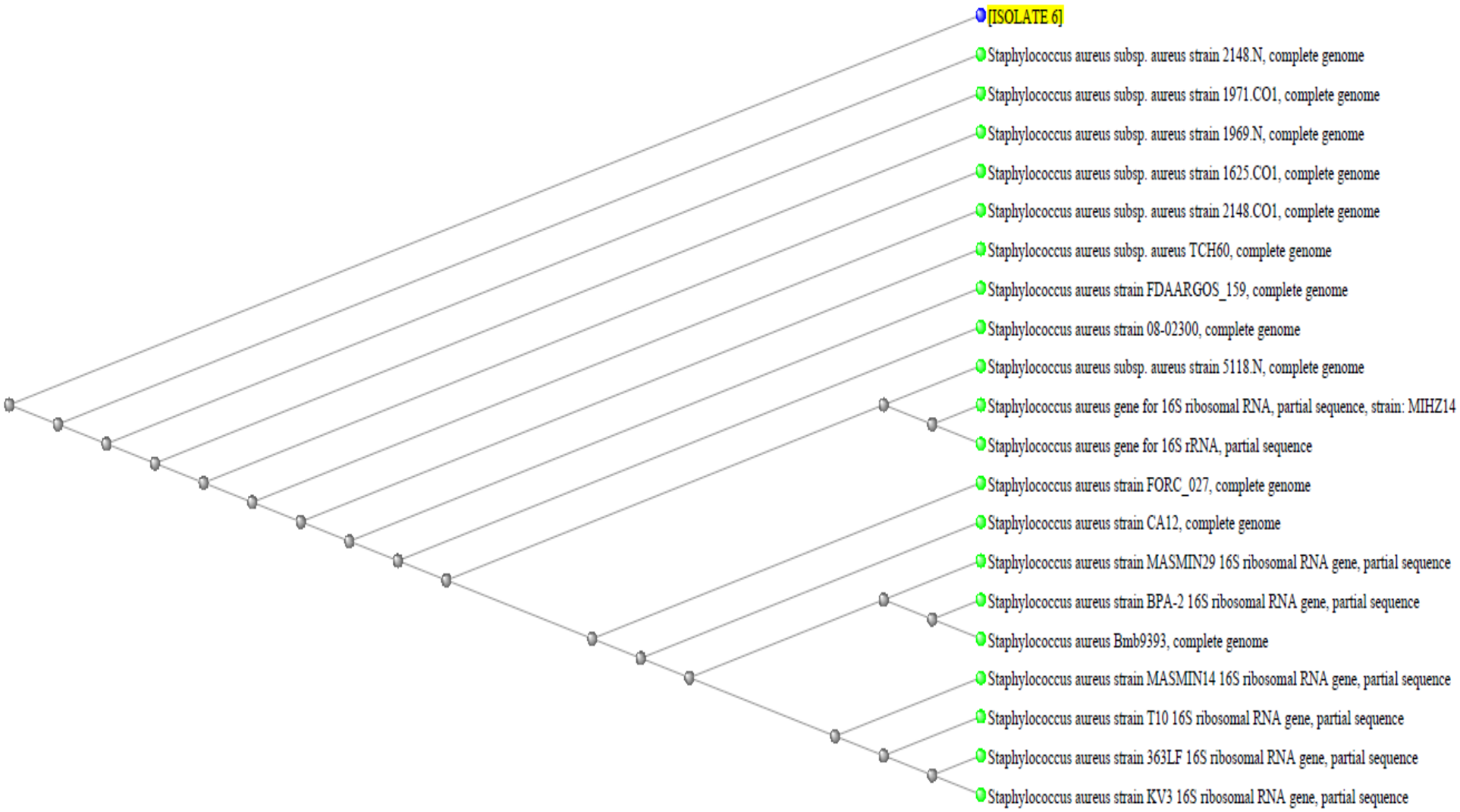


Fig 4.6: Phylogenetic tree showing the relationship between Isolate 6 and other identical *Staph. aureus* strains from GenBank database

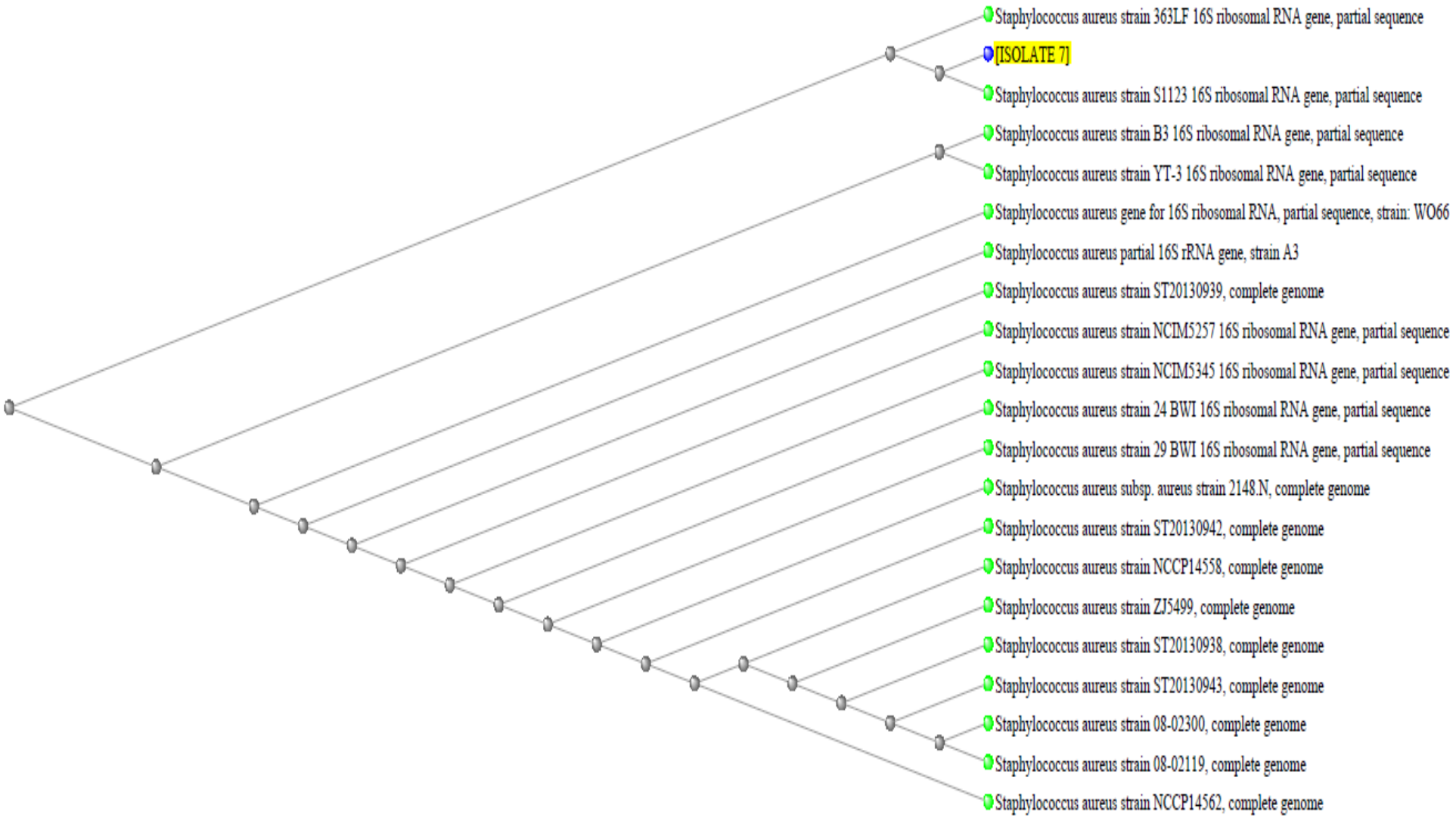


Fig 4.7: Phylogenetic tree showing the relationship between Isolate 7 and other identical *Staph. aureus* strains from GenBank database

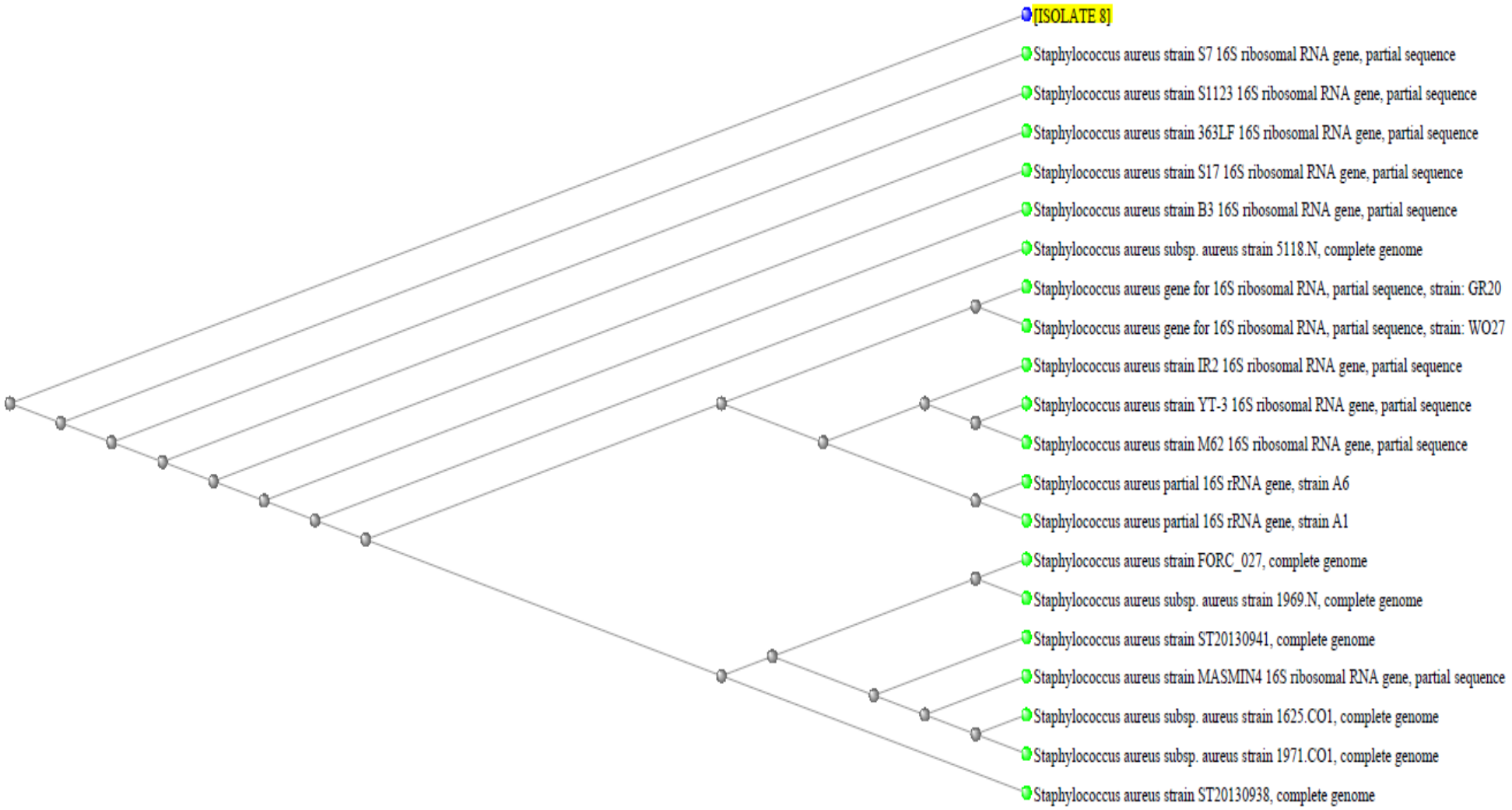


Fig 4.8: Phylogenetic tree showing the relationship between Isolate 8 and other identical *Staph. aureus* strains from GenBank database

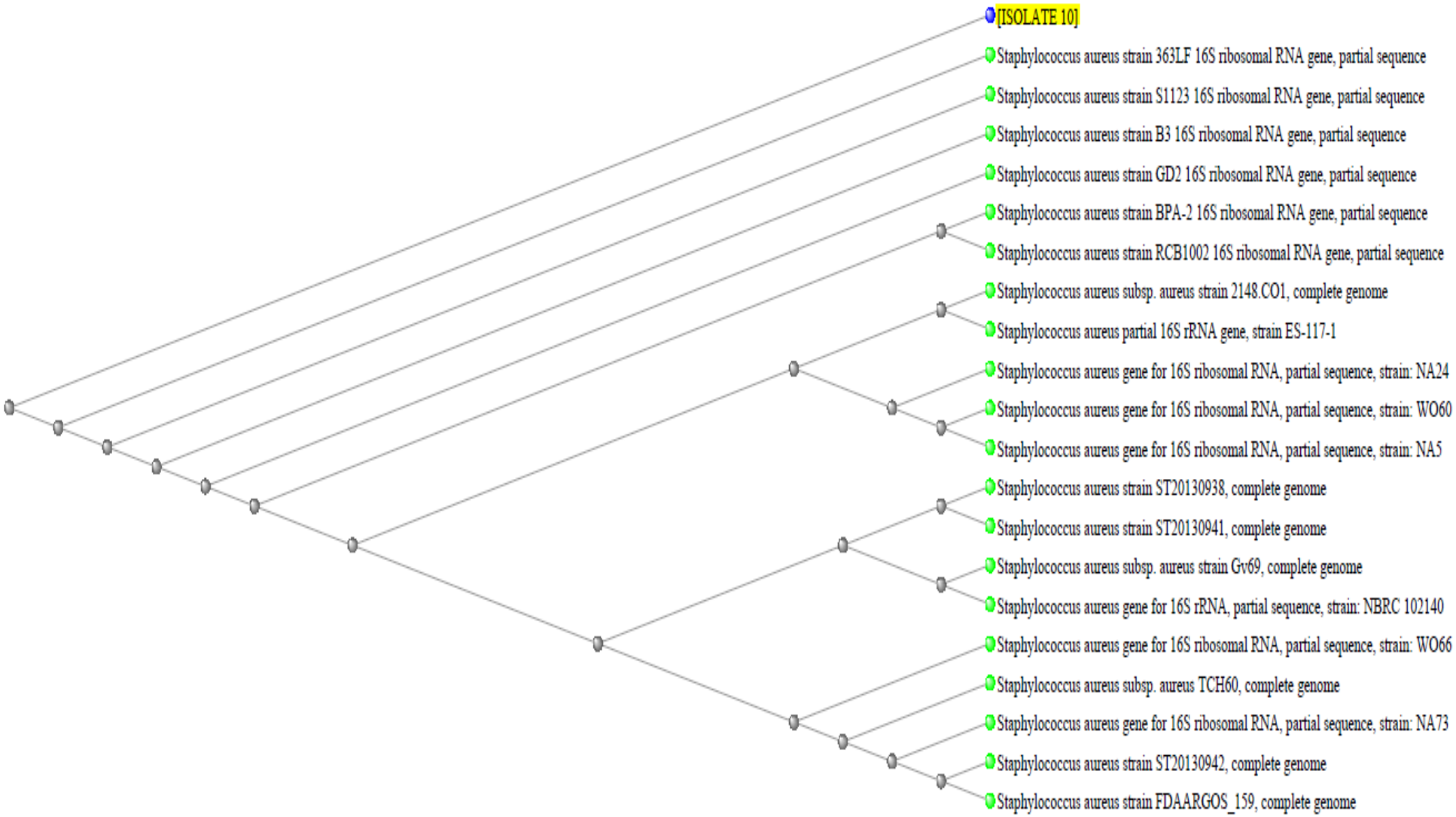


Fig 4.9: Phylogenetic tree showing the relationship between Isolate 10 and other identical *Staph. aureus* strains from GenBank database

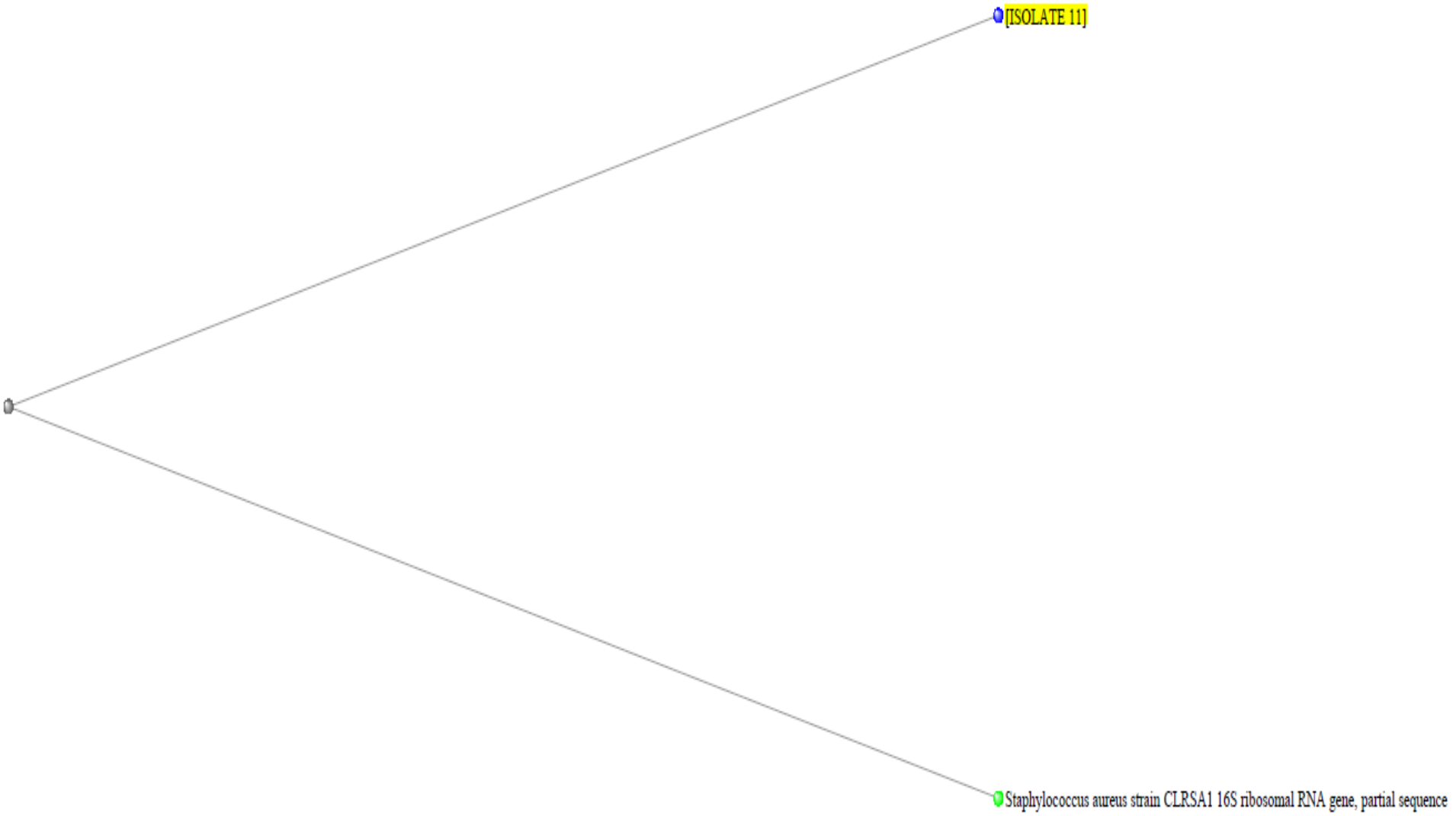


Fig 4.10: Phylogenetic tree showing the relationship between Isolate 11 and other identical *Staph. aureus* strains from GenBank database

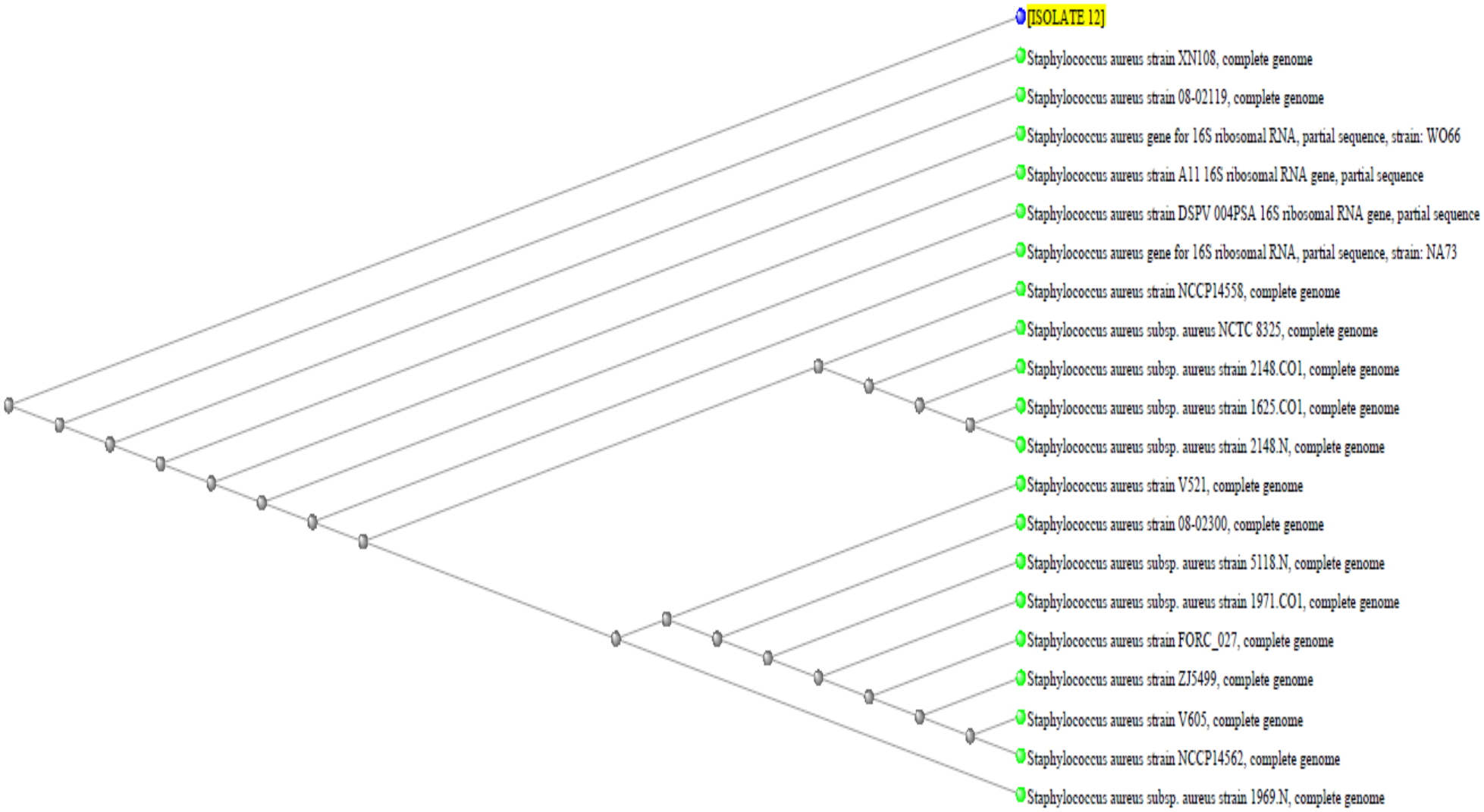


Fig 4.11: Phylogenetic tree showing the relationship between Isolate 12 and other identical *Staph. aureus* strains from GenBank database

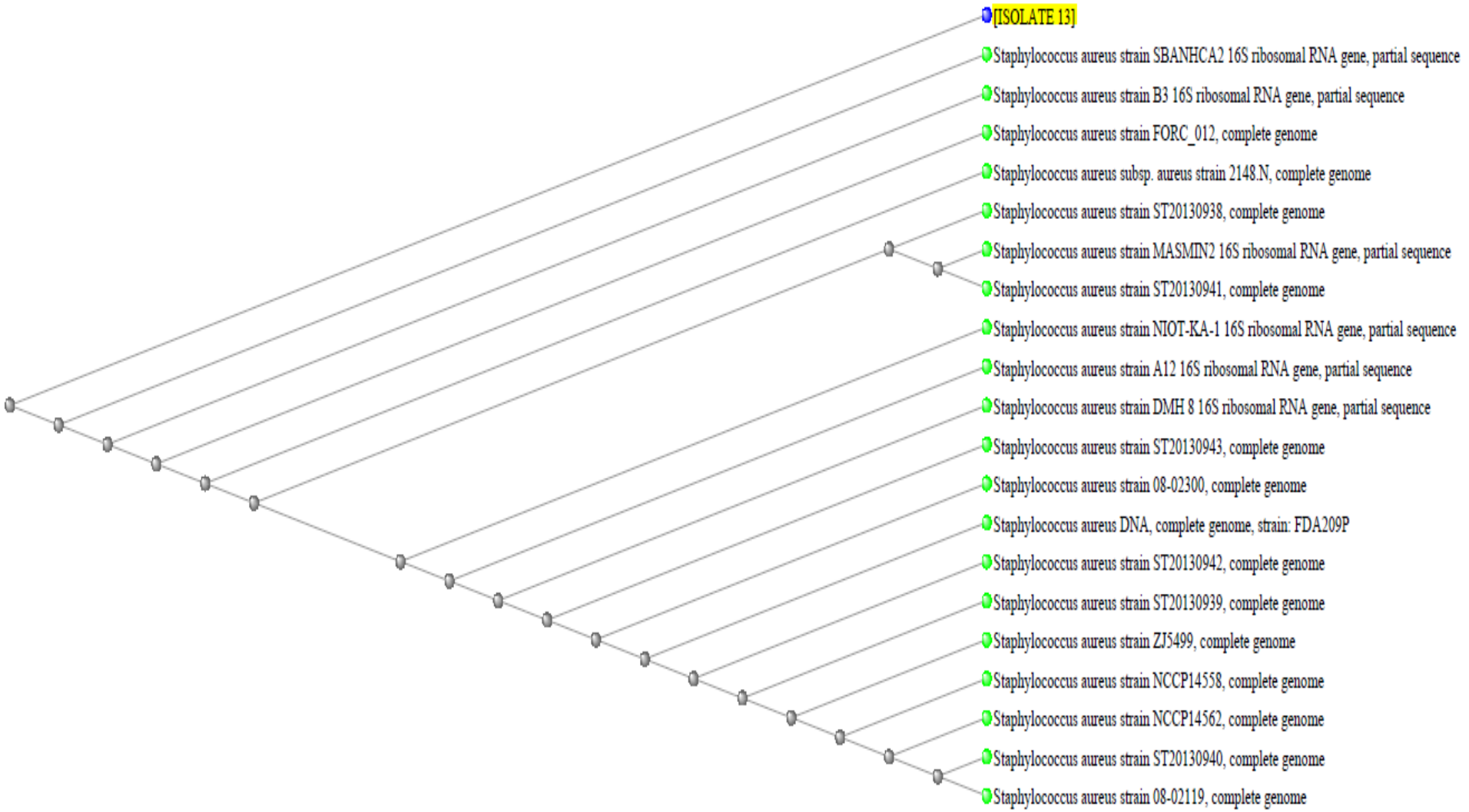


Fig 4.12: Phylogenetic tree showing the relationship between Isolate 13 and other identical *Staph. aureus* strains from GenBank database

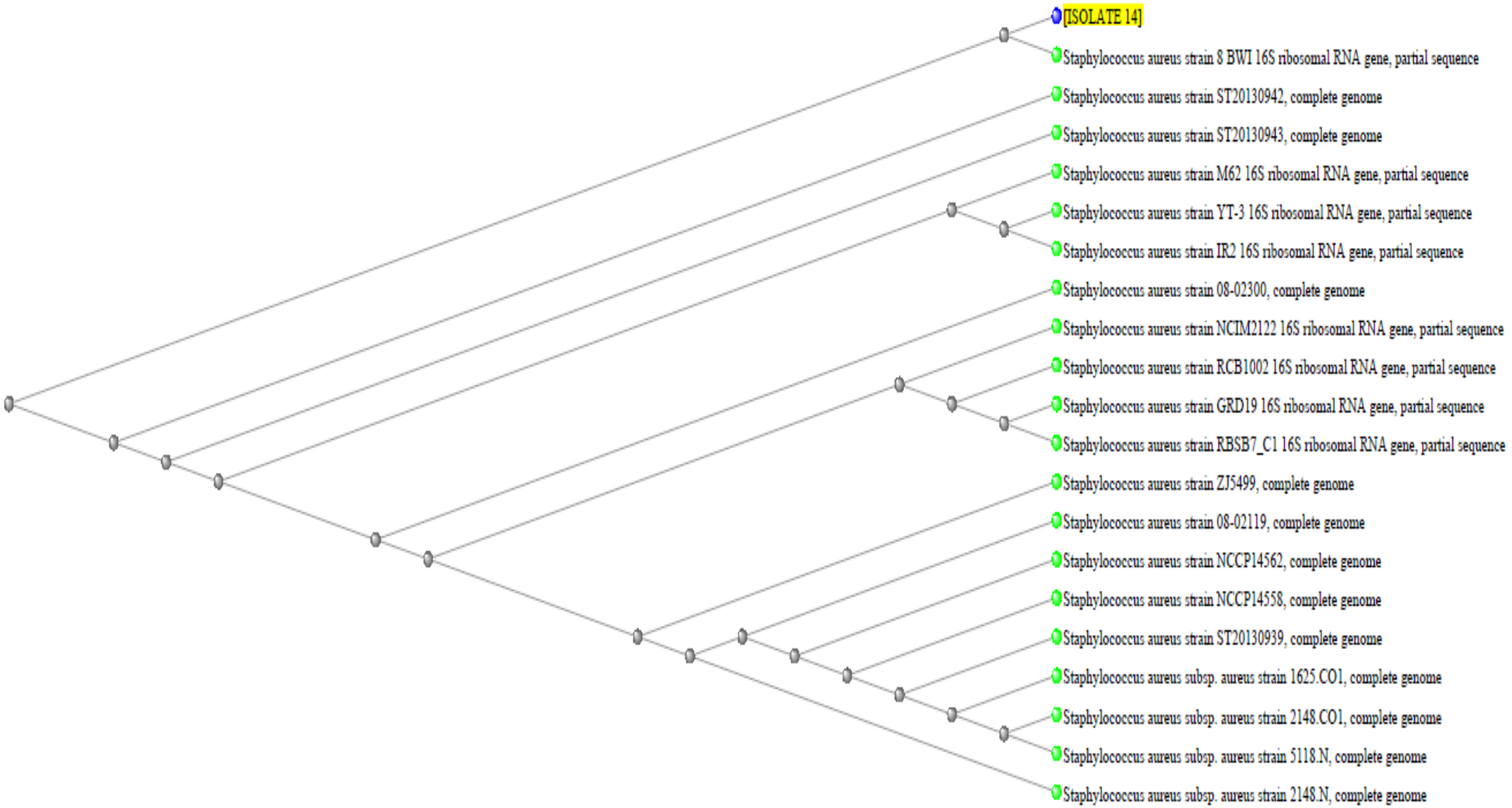


Fig 4.13: Phylogenetic tree showing the relationship between Isolate 14 and other identical *Staph. aureus* strains from GenBank database

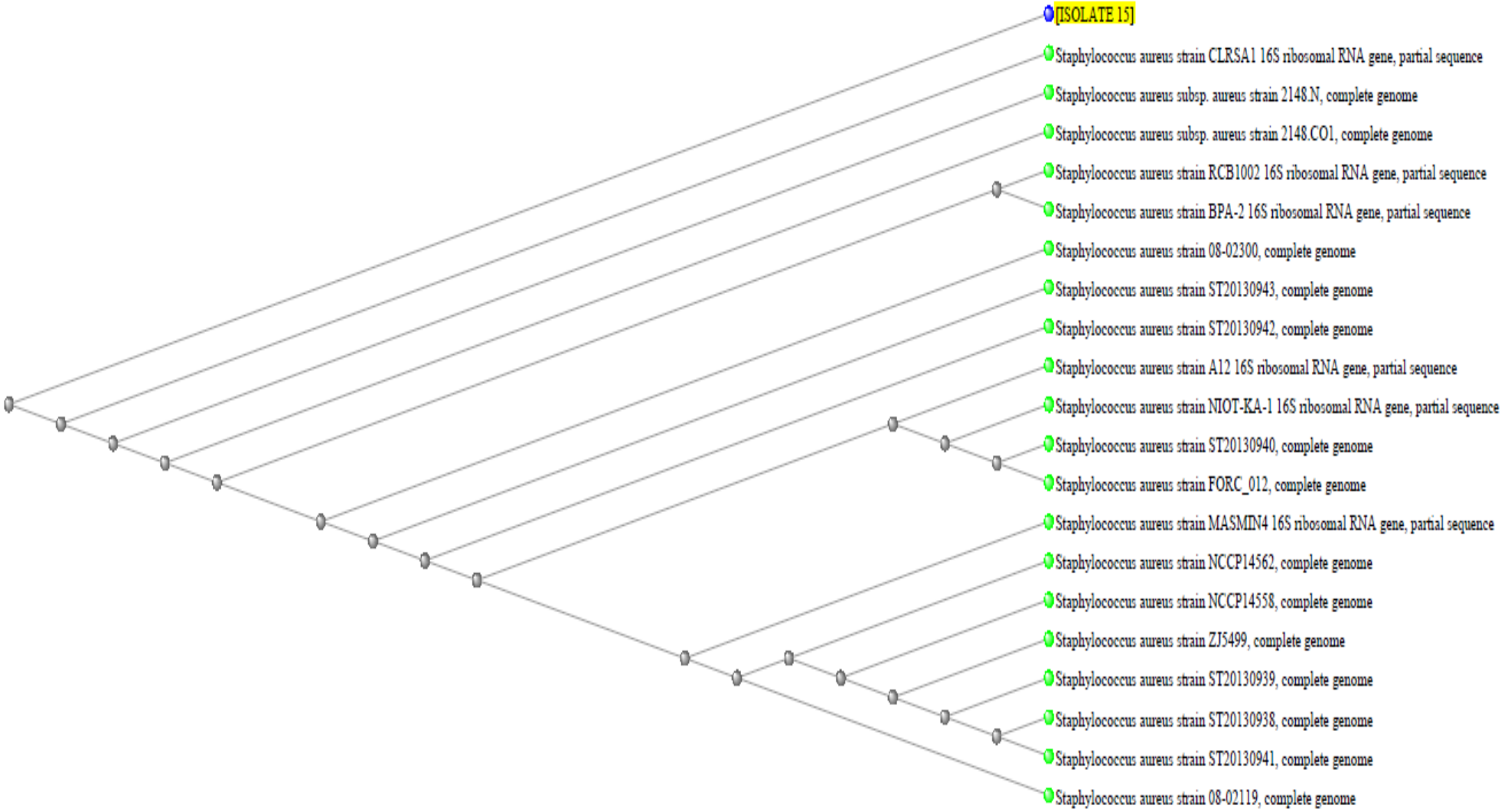


Fig 4.14: Phylogenetic tree showing the relationship between Isolate 15 and other identical *Staph. aureus* strains from GenBank database

4.8.1 Relationship between isolates and *Staph aureus* N315

The isolates were blasted with the first fully sequenced MRSA strain N315 to determine the degree of similarity and alignment. The percentage similarity identities of isolates in comparison to N315 are shown in Table 4.6. Isolate 7 was found to be the closest to N315 having sequence similarity value of 94% while isolate 4 had the least sequence similarity value of 77%. The phylogenetic relationship between N315 and the isolates is shown in Figure 4.24. From the phylogenetic tree, it was observed that isolate 7 and N315 share immediate lineage (common ancestor) while isolate 14 share the last lineage (ancestor) with N315. Figure 4.15 displays the outcome of nucleotide blasting of *Staph aureus* N315 against the isolates indicating the query numbers which appears on the alignment view (Table 4.7) instead of isolate numbers. Isolate 14 was not found in the alignment view. The individual alignment views of blasts of isolates against N315 is shown in **Appendix B.**

4.8.2 Relationship amongst isolates

The sequence similarity values among isolates are from 25.3 – 90.4 % and are shown in Table 4.8. Isolates 5 and 16 have the least sequence similarity with other isolates (25 – 48%). While between 2 and 7, 3 and 14, the sequence similarity values are 90.4 and 90.3% respectively.

Table 4.8: Blasting of some of the isolates against MRSA strain N315 (NCBI Reference Sequence: NC_002745.2)

Isolate No	% Query cover	% similarity identity
1	60	90
2	96	92
3	100	79
4	73	77
5	No significant similarity	No significant similarity
6	85	88
7	95	94
8	69	88
9	No significant similarity	No significant similarity
10	96	84
11	No significant similarity	No significant similarity
12	97	88
13	96	90
14	85	80
15	93	84
16	No significant similarity	No significant similarity

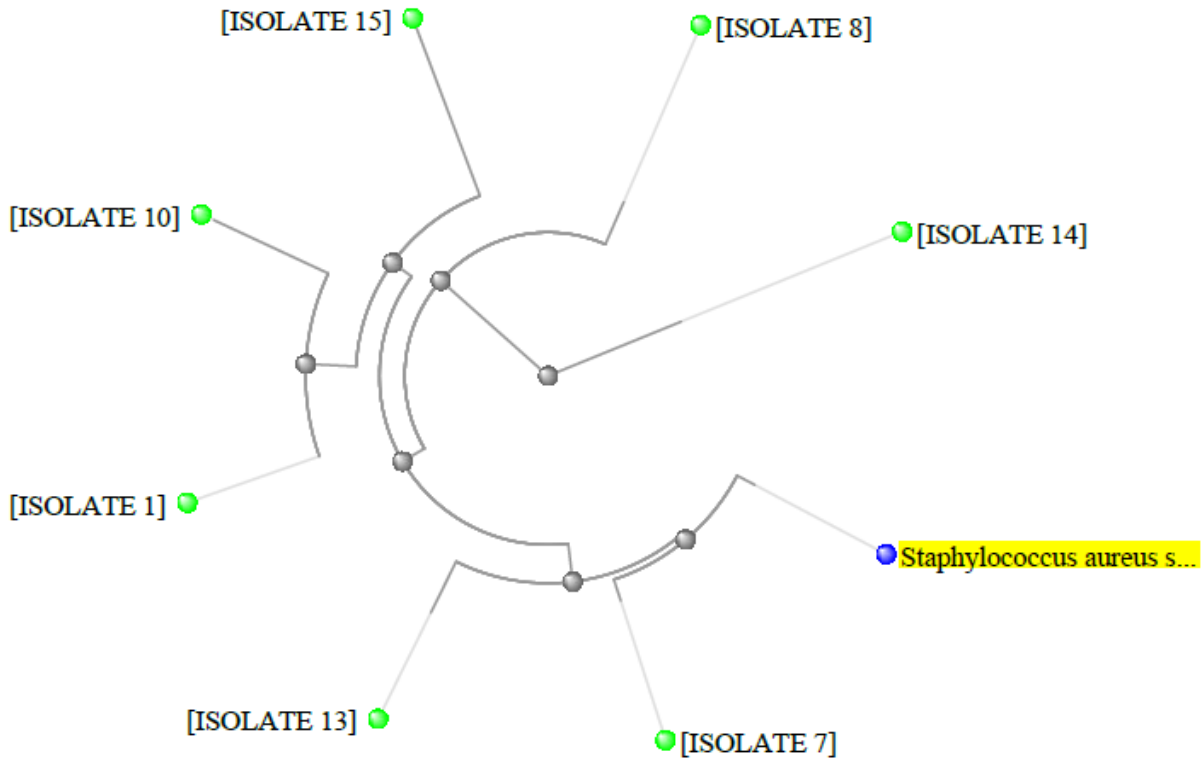


Fig 4.15: Circular layout of the Phylogenetic relationship between isolates and *Staph aureus* N315 (highlighted)

Sequences producing significant alignments:

Select: All None Selected:12

Alignments Download Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
[ISOLATE 7]	1234	6173	0%	0.0	94%	Query_126866
[ISOLATE 2]	1094	5460	0%	0.0	92%	Query_126862
[ISOLATE 13]	1046	5231	0%	0.0	90%	Query_126871
[ISOLATE 12]	941	4694	0%	0.0	88%	Query_126870
[ISOLATE 6]	833	4158	0%	0.0	88%	Query_126865
[ISOLATE 10]	773	3865	0%	0.0	84%	Query_126868
[ISOLATE 15]	712	3560	0%	0.0	84%	Query_126873
[ISOLATE 8]	658	3270	0%	0.0	88%	Query_126867
[ISOLATE 1]	577	2864	0%	5e-164	90%	Query_126861
[ISOLATE 14]	494	2470	0%	5e-139	80%	Query_126872
[ISOLATE 3]	477	2382	0%	5e-134	79%	Query_126863
[ISOLATE 4]	283	1412	0%	1e-75	77%	Query_126864

Fig 4.16: Outcome of nucleotide blasting showing the sequence similarity values and query numbers for each isolates

Table 4.9: Alignment view of blast of *Staph aureus* N315 against the isolates

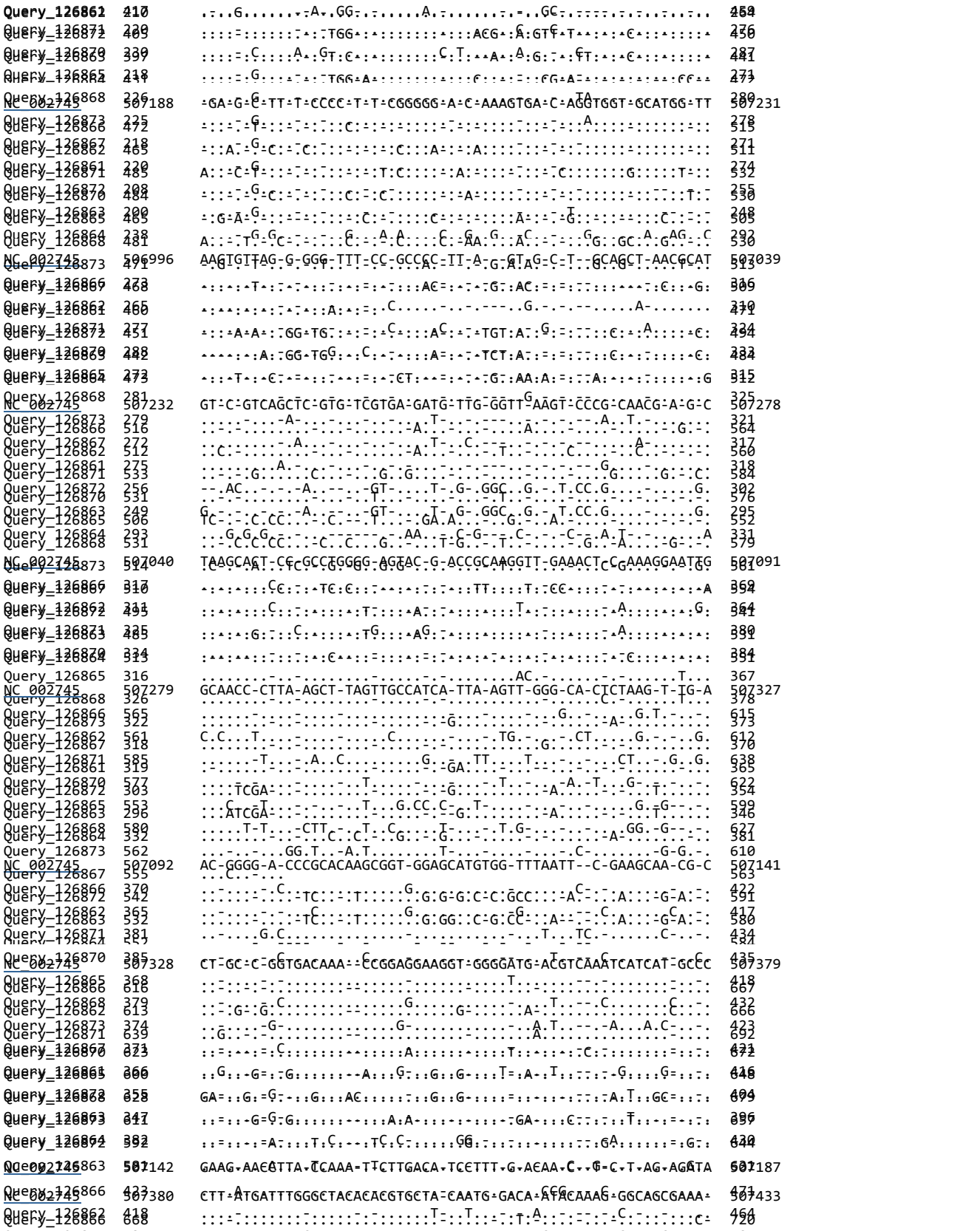
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NC_002745      506729   TTATT-GGG-CGT-AAAGCG-CGCGT-AGGCGGT-TT-TTT-AAGT-CT-GATGTG-AAA  506777
Query_126866   23             ...-..--......-.....--.........---.-C.-.....-.--..--..-...  59
Query_126862   16             ...-..--......-.....--.........---.-C.-.....-.--..--..-...  52
Query_126871   21             ...-..C-......-.....--....-..-..-C..-..TC-.C-..-.C.-...  61
Query_126870   19           ..C-...-..C-......-....C-........-C-..C-....-..C..-.C.-...  65
Query_126865   16             ...-..C-....G.-....C-........-..-.--....-..C..--..-...  57
Query_126868   26             ...-..--......-....C-........-.--C..-....-.--..--..-...  64
Query_126873   24           ..-...-..C-......-.....---C.T..--..-...-....-..C..-.C.-...  66
Query_126867   14        .....-...-GCA-.......-A...C-........-.---C.-..-.-..C..-.C.-...  59
Query_126861   20             ...-.TC-......-....C-........-.--C..-....-.--.-.-..-...  59
Query_126863   12             ...-..--......-.A..C-........-.---G.-....-.--..--..--..  48
Query_126864   11           ....C...T...C..G...T.....C....T..C..C...C.G..C.A-..G...C...  68

NC_002745      506778   -GCC-CACGGCTCAACCGTGGAGGGTCATTGGAAACT-G-GAAAACT-TGAGTG-CAG-A  506830
Query_126866   60       -...-.....-......-....-.............-.-.GG....-......-...-.  109
Query_126862   53       -...-.....-......-....-.............-.-.G.....-......-...-.  102
Query_126871   62       -...-......C.....-....-.............-.-.G.G...-......-...-.  112
Query_126870   66       -...-......C.....-....-....T.C......C-.-.G.....-......-...-.  116
Query_126865   58       -...-.....-.......G...-....-.........-.-.GG....-......-...-.  107
Query_126868   65       -...-............-....-.............-.-.G.G...-......-...-.  115
Query_126873   67       -...-......C.....-.-..-.............-.-.G.G...-......-...-.  116
Query_126867   60       -...-.....-.A.....G...-.............-.-.GGG...-......-...-.  110
Query_126861   60       -...-............-.-..-.....-........-.-.GGG...-......-...-.  108
Query_126863   49       -T..-.-...G.....T.-...AT.-.-..T......-.-.C..G..-A.....--.-C-T  95
Query_126864   69       G...T.................TA....C..T......C.T..GGG..C......C.G.A.  128

NC_002745      506831   AGAGGAAAGTGGAA-TTCCAT-GTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACC  506888
Query_126866   110      ......G.-.....-......-.......................-.............  165
Query_126862   103      ........-.....-......-.......-..............-.............  157
Query_126871   113      ........-...-.-......-...-...................-.............  166
Query_126870   117      ..............-......-.......................-.............  173
Query_126865   108      ......G.......-......C.......................-.............  165
Query_126868   116      ......G.......-......-.......................-.............  172
Query_126873   117      ..............-......-..................T-....-.............  172
Query_126867   111      ......G.-...-.-......-.......................-.............  165
Query_126861   109      ......G.......-......-.......................-.............  165
Query_126872   114                  .-.....G-..................-T....-.C........T...  156
Query_126863   96       .....-GG..A.-.-.....G-...-.............-T....-.C........T...  148
Query_126864   129      ...-..........T....CA-.G..............CT.A.AT..-...........  185

NC_002745      506889   AGTGGCGAAGGCGACTT-TC-T-GGTCTGTA-ACT-GACGCT-GATGTGCG-AAAGCGT-  506940
Query_126866   166      ............G..C-..-.-........-...-......-........-.......-  217
Query_126862   158      ..................-..-.-........-...-......-........-.....T.-  209
Query_126871   167      ..................-..-.-........-...-......-........-.......G  219
Query_126870   174      ..................-..C.C..C.....-...C......C....CC..-........-  229
Query_126865   166      ................C-..-.-........-...-.......-..G.A...-........-  217
Query_126868   173      ............G...C..-.-........-...-.......-..G.C...-........-  225
Query_126873   173      ..................-..-.-.......-.-...-...A..-..G.C...-.......G  224
Query_126867   166      ............G..C-..-.-........-...-......-..G.C...-......G-  217
Query_126861   166      ................C..-.-........-...-......-..G.C...A.......-  219
Query_126872   157      G............G.CC-C.-.-..A.-.A.G...-.......-C.G.....-......-.-  207
Query_126863   149      G............G.CC-C.-.-..A.-.A.G...-.......-C.G.....-......-.-  199
Query_126864   186      ..................-.T-.-..A.C.G.-...-..A..C-..A.CA..-......G-  237

NC_002745      506941   GGGG-ATCAAA-CA-GGATTAGATACCCTGGTAGTCC-ACG-CCGTAAACGATGAGTGCT  506995
Query_126866   218      ....-......-..-.....................-...-.................  272
```



```
Query_126878  620     T..A.A..TGG.-..AGG-.-..G....-.TG..T.TT.A.C-.-GC-.-.A..-A..TC  730
Query_126867  422     ....-.......-...GG-.-.......-.......-.-..CG-.--.-.-.-..-.....  467
Query_126873  658     ...-T...CC-.....-.....-.....-..GG--.....A..TC-.-...-  706
Query_126872  645     ...-.C..CCA.....-.....-.....-.CGC-.....-A.A.....CC-  698
Query_126863  632     ...-.C..G.A.....-.....-.-..T.....-.-A.A.....CC-  684

NC_002745     507434  CC-GTGAGGTCAAGCAAATCCCATAAAG-T-TGTTCTCAGTTCGGATTGTAGTCTGCAAC  507490
Query_126866  721     ..-.C.....-.....--.-.A.....-.....  774
Query_126862  722     ..C.C..-....T.....-.-.....-.....-.  776
Query_126871  744     ..-.C..A....T.....-.-.....-.....-..  798
Query_126870  725     ..C.C..-....T....T...-.-.C..-.--.....-.....-.....-.  775
Query_126865  701     ..  702
Query_126868  736     ..-.A....C.G.T.....T.....AA.-.....CG.A..G..G...G...CT.  793
Query_126873  707     ..-.C.G..GG.-..T....T.TA...C-C-C.....-.....G.-..G-..G...  759
Query_126872  699     T.-.C...AG.....GG.C.T.....-.-GCG...-.TCC.....G.....  754
Query_126863  685     T.-.C...AG.....GG.C.T.....C-.-.A...-..-.C.C.....G.A.....-.  738

NC_002745     507491  T-C-GACTACATGAAGCTGGAATCGCTAGT-AATCGTAGATCAGCATGCTACGGTGAATA  507547
Query_126866  775     .-.-.....-.....G.....-.....-...-..  828
Query_126862  777     .-.-.....-.....-.T.....  813
Query_126871  799     .-.-.....G.....-.....G.....-.....-...-..  852
Query_126870  776     .-.-..-...-...T..-.C.-...A.T.--....-....CA...-.....-...-..  823
Query_126868  794     .G.-...C..C.A....G.....T.....--....A.....A.....  839
Query_126873  760     .-.C.C....-.....-.....--....  789
Query_126872  755     .-.C....C.-.....-.C.....-.....-.G.....A-....C-.....-..  806
Query_126863  739     .C.-....C.C....T.-.C.....T..C.....A.....  780

NC_002745     507548  CGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCC  507607
Query_126866  829     ..  830
Query_126871  853     .  853
Query_126870  824     .  824
Query_126872  807     .  807
```

Table 4.10: Comparative DNA sequence identities of the staphylococcal isolates

	% identity with isolates															
Isolates	**Isolate 5**	**Isolate 16**	**Isolate 4**	**Isolate 11**	**Isolate 3**	**Isolate 14**	**Isolate 1**	**Isolate 8**	**Isolate 9**	**Isolate 15**	**Isolate 12**	**Isolate 13**	**Isolate 2**	**Isolate 7**	**Isolate 6**	**Isolate 10**
Isolate 5		25.344	28.077	30.479	26.245	25.791	29.961	29.151	29.192	30.675	30.854	29.812	28.791	29.772	30.627	29.211
Isolate 16	25.344		44.254	42.995	44.471	43.632	51.031	47.533	45.082	47.255	47.281	46.743	48.208	48.944	48.272	47.575
Isolate 4	28.077	44.254		50.000	50.114	50.686	54.843	55.257	53.333	59.091	54.525	54.817	54.525	56.378	59.368	56.236
Isolate 11	30.479	42.995	50.000		54.748	54.900	57.824	59.427	62.220	65.083	60.844	61.213	61.494	61.854	62.768	61.414
Isolate 3	26.245	44.471	50.114	54.748		90.401	56.438	59.977	62.750	63.498	64.005	69.029	70.848	74.148	65.643	65.473
Isolate 14	25.791	43.632	50.686	54.900	90.401		56.438	61.511	62.604	65.053	63.647	68.197	69.895	73.200	67.101	65.157
Isolate 1	29.961	51.031	54.843	57.824	56.438	56.438		65.249	59.784	65.614	64.897	64.988	65.836	67.310	67.883	67.895
Isolate 8	29.151	47.533	55.257	59.427	59.977	61.511	65.249		63.509	68.171	69.022	69.072	72.474	73.154	72.182	71.263
Isolate 9	29.192	45.082	53.333	62.220	62.750	62.604	59.784	63.509		70.130	71.412	72.926	74.009	76.533	69.267	69.873
Isolate 15	30.675	47.255	59.091	65.083	63.498	65.053	65.614	68.171	70.13		69.464	74.742	73.984	75.000	74.078	72.822
Isolate 12	30.854	47.281	54.525	60.844	64.005	63.647	64.897	69.022	71.412	69.464		77.423	78.291	79.395	73.585	74.338
Isolate 13	29.812	46.743	54.817	61.213	69.029	68.197	64.988	69.072	72.926	74.742	77.423		84.828	85.829	75.086	77.299
Isolate 2	28.791	48.208	54.525	61.494	70.848	69.895	65.836	72.474	74.009	73.984	78.291	84.828		90.387	75.726	80.116
Isolate 7	29.772	48.944	56.378	61.854	74.148	73.200	67.310	73.154	76.533	75.000	79.395	85.829	90.387		78.538	80.997
Isolate 6	30.627	48.272	59.368	62.768	65.643	67.101	67.883	72.182	69.267	74.078	73.585	75.086	75.726	78.538		76.163
Isolate 10	29.211	47.575	56.236	61.414	65.473	65.157	67.895	71.263	69.873	72.822	74.338	77.299	80.116	80.997	76.163	

CHAPTER FIVE

5.0 DISCUSSION

Nasal carriage of Staphylococci is the predominant mode of carriage in about 20-80% of the population (Brown *et al.*, 2014; Zanger *et al.*, 2011). However, in this study, of the 217 urine samples taken, Staphylococci isolates were recovered from 171 (78.8%). However, only 73 (33.6%) were actually identified to be *Staphylococcus aureus*. The prevalence found in this study corroborates with the findings of Brown *et a*l. (2014) and Zanger *et al. (*2011).

The conventional cultural method of *Staphylococcus aureus* identification gave a higher positive value for the prevalence. However, subjecting them to Microgen ID test kit, showed that some of the isolates identified as *Staphylococcus aureus* by the cultural method were actually other staphylococcal species such as *S. hominis, S. hyicus, S. intermedius, S. xylosus, S. schleiferi, S. cohnii and S. haemolyticus.* A substantial proportion of the isolates (45.9%) were inaccurately identified by the conventional method. These non-*Staph aureus* species are normally associated with animals such as pigs, cattle, dogs and birds except *S. hominis* and *S. haemolyticus* which are found in humans. It is surprising to find these otherwise animal associated Staphylococcus species in urine samples collected from healthy volunteers. Even more of a concern is that the volunteers are student population and not farmers. In addition, most of them live within the University environment and not near animal farms. While *S. aureus* and other Staphylococcus species were coagulase positive, the Microgen ID test kit was a more specific method of identifying *S. aureus* from other Staphylococcus species.

For susceptibility test, ciprofloxacin demonstrated the greatest activity against all the isolates (88.3%) while penicillin was least effective. The activity of co-trimoxazole was comparable to those of ciprofloxacin and vancomycin. However, its performance was better than that of cefotaxime, a 3rd generation cephalosporin. This may be due to the limited prescription and use

of co-trimoxazole especially with the advent of resistance. Co-trimoxazole may be a better alternative to vancomycin in the treatment of MRSA infections. However, Bishara et al (2003) observed progressive increase in susceptibility of MRSA to cotrimoxazole from 31% in 1988 to 81% in 1997 in a retrospective study undertaken in Israel using blood samples. On the contrary, a study by Japoni *et al* (2010) in Iran recorded low susceptibility of MRSA and Methicillin Susceptible *S. aureus* (MSSA) to co-trimoxazole, ciprofloxacin, tetracycline, erythromycin and gentamicin. The samples were obtained from multiple specimens such as blood, urine, wounds, nasal washing, abscess, and skin lesions. In Yenagoa, Nigeria, the study of *S. aureus* from urine samples indicated resistance of 80.4% to cotrimoxazole, 73.9% to gentamicin, 69.6% to Vancomycin 34.8% Ofloxacin and 32.6% to ciprofloxacin (Onanuga and Awhowho, 2012). However, the above mentioned studies obtained samples from patients and not healthy volunteers. Generally, a high level of resistance is expected from hospital isolates. In addition, selective pressure of antibiotic use is higher in hospitals.

A similar study undertaken in Ota, Nigeria with regards to participants who are healthy student volunteers indicated that 82.4% of MRSA isolates were resistant to co-trimoxazole and vancomycin (Owolabi and Olorioke, 2015). However, the samples were swabs from the nose and neck. This is in contrast with our findings where 63.2% and 73.1% of the isolates were susceptible to co-trimoxazole and vancomycin respectively. Although both studies were undertaken with student community, the contrast may be due to variation in prior antibiotic exposure, pattern of prescriptions and policies of health facilities in the various locations.

Oxacillin and preferably cefoxitin are used as markers for methicillin resistance. Therefore, 60.1% or 78.9% of the isolates were found to be MRSA using oxacillin or cefoxitin respectively.

Considering the fact that ciprofloxacin and ofloxacin are in the same class of fluoroquinolone, the unexpected significant disparity in the susceptibility of the isolates to both is surprising though not disturbing as studies have shown such disparities (El-Mahmud *et al*., 2009; Kang *et al*., 1994). While ofloxacin has been reported to be more effective than ciprofloxacin against *S. aureus* (Gönüllü *et al*., 2001; Obi and Nwanebu, 2008), some studies have shown similarity in susceptibility to the two antibiotic agents (Momoh *et al*., 2007; Shah *et al.*, 2004). There are also other studies that have demonstrated greater efficacy of ciprofloxacin over ofloxacin against MRSA (El-Mahmood *et al.*, 2009; Gönüllü *et al.*, 2001; Kang *et al*., 1994; Shalit *et al.*, 1989).

It was found that 81.8% of the isolates had MAR index ≥ 0.2 (Table 4.4). This indicates that the isolates were probably from high risk contamination source where antibiotics are heavily used or the isolates have had previous exposure to antibiotics. The latter may be the case in the study population as people have easy access to antibiotics in Nigeria without prescriptions.

It was observed that out of 11 antibiotics utilized in this study, 6 of them had over 50% of the isolates resistant to them while only ciprofloxacin had less than 20% of the isolates resistant to it. Most of the isolates demonstrated resistance to at least 4 classes of antibiotics and consequently can be said to be not just multi-antibiotic resistant but also multi-drug resistant. This is an indicator of an emerging challenge in SA and MRSA related infections. *S. aureus* is known to cause severe diseases, including necrotizing fascitis, sepsis, endocarditis, and pneumonia. Consequently, multidrug resistance is a threat to public health as selection of commercially available efficacious antibiotics for treatment of these diseases may be a herculean task. This is not just a situation of cross resistance amongst the same class of antibiotics: these isolates have also developed various mechanisms of resistance across different classes through different mechanism, thereby limiting the choice of antibiotics to be used in treating infections caused by them. This finding corroborates the study of an epidemic

clone of community-acquired meticillin-resistant *S. aureus* USA300 published by Diep and co-workers (Diep *et al.,* 2006). USA300 was found to be resistant to β-lactams, fluoroquinolones, tetracycline, macrolide, clindamycin, and mupirocin; an ability facilitated by its acquisition of multiple resistance genes. In addition, another study by Holden and co-workers (2010) found in a variant of MRSA (TW20) a display of an extended antibiotic resistance pattern. TW20 displayed multidrug resistance to penicillin, methicillin, erythromycin, ciprofloxacin, gentamicin, neomycin, trimethoprim, and tetracycline. The ability of TW20 to survive in clinical setting was attributed to its acquisition of drug and antiseptic resistance genes carried on mobile genetic elements (MGEs), large chromosomal insertions, and point mutations in housekeeping genes (Holden *et al.,* 2010).

Breaking point tests confirmed the existence of methicillin resistance among the isolates. These 71 isolates which were resistant may be said to be methicillin resistant *Staph aureus* phenotypically.

Many species of bacteria contain plasmid DNA and plasmid-mediated resistance is known to be reversible as some plasmids undergo spontaneous segregation and deletion. However, some plasmids are stable and so would require some procedures such as the use of curing agents to facilitate the ejection of plasmids. Curing was used to identify the genetic location of the resistance determinants. Those that became susceptible after curing with ethidium bromide indicated that the resistance was plasmid coded and those still resistant indicate that the resistance may be chromosomal mediated. It thus appeared that the ethidium bromide utilized facilitated the ejection of plasmids from some isolates making them susceptible to antibiotics. The isolates were initially resistant to the 11 antibiotics utilized. It was observed that cefoxitin, ofloxacin, and penicillin resistance was not eliminated by ethidium bromide. It is worthy to note that the degree of elimination of resistance achieved by ethidium bromide is reported to vary from 8 – 100% (Bouanchaud *et al.*, 1968; Sonstein and Baldwin, 1972)

The isolates were resistant to methicillin by the phenotypic tests which was confirmed by the detection of *mecA* gene in the 16 isolates subjected to PCR analysis (Figure 4). All the isolates showed bands at 500 bp (Figure 4). *MecA* gene is thought to be widely disseminated by horizontal transfers (Wielders *et al*., 2002). This may explain their detection in what are community isolates which have not been previously exposed to the antibiotics. *MecA* gene cassette codes for high level methicillin resistance and can also harbour genes coding for resistance to other non β-lactam antibiotics. This may explain the high level of resistance observed with tetracycline (64.9%), and ofloxacin (79.2%) and moderate level of resistance to erythromycin (34.1%), co-trimoxazole (36.8%) and gentamicin (34.5%).

Staphylococcal cassette chromosome (SCC), a vehicle and mobile element for the methicillin resistance genes (such as *mecA* gene) and other drug resistance genes is found in staphylococcal and micrococcal species. Although *mecA* was first identified in MRSA, it has been found in Staphylococcal species such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, and *Staphylococcus fleurettii* (Ito *et al*., 2012). The *mecA* genes identified in these species have >98% sequence identity with the *mecA* harboured by MRSA strain N315 which was the first completely sequenced prototype. In addition, scientific findings indicate that *mecA* is also harboured by non-staphylococcal species such as *Enterococcus, Proteus, Morganella*, (Kassem *et al.*, 2008), *Pseudomonas and Aeromonas* species (Seyedmonir *et al.*, 2015). Seyedmonir and co-workers (2015) reported that the phylogenesis of partial *mecA* sequences obtained from staphylococcal and non-staphylococcal isolates indicated sequence similarity of 8 %–100 % confirming the phylogenetic relationships between species. The detection of *mec*B is a further confirmation of the existence of MRSA among isolates in this study.

Based on their extent of homology to the earliest identified *mecA* gene, four groups of mecA homologues have been identified, *mecA1, mecA2, mecB, mecC*. The prototype strains depicting each *mec* gene are *S. sciuri* K11 for *mecA1*; *S. vitulinus* CSBO8 for *mecA2*; *M. caseolyticus* JCSC5402 for *mecB,* and *S. aureus* LGA251 for *mecC.* The recently identified fourth *mecA* gene homologue in *S. aureus* strain LGA251 shows 69% identity to *mecA* of N315 (Ito *et al.,* 2012). *MecB* is the third group of *mecA* gene homologues found on the chromosome and plasmids of *Micrococcus caseolyticus*. *M. caseolyticus* is a member of a genus that is phylogenetically closely related to Staphylococcus. The *mecB* carried by *M. caseolyticus* strains have 62% nucleotide sequence identity to *mecA* of N315 (Baba *et al*., 2009). While *mecC* has been found in staphylococcal species, *mecB* is yet to be reported in staphylococcal species. However, in this study**,** *mecB* gene seem to be prominent in urinary isolates No 6, 10, and 13 – 16 (Figure 4.5); while weak signals of *mecB* were observed with isolates No 5, 7, 11, 12. Isolates No 2, 3, 4, 8 and 9 did not indicate the presence of *mecB*. Isolates with no indication of *mecB* corroborates the findings of Hiramatsu *et al.* (2013, 2014) as they did not find *mecB* as well. It is suggested that the explanation of the absence of *mecB* in staphylococcal species may be due to it being carried by transposon while *mecA and mecC* are carried by SCC (Hiramatsu et al., 2013). The phenotypic tests including Microgen ID test kit confirmed the presence of *Staph aureus* in this study. While mutational activity or plasmid mediation may be possible postulations to the presence of *mecB* in the isolates, further investigation will be required to confirm the presence or absence of *mecB* in these isolates.

There are at least 11 types of SCC*mec* and more are envisaged to be discovered in future (Hiramatsu et al., 2013). SCC*mec* type comprises the type of cassette chromosome recombinase (*ccr*) gene complex and the class of *mec*-gene complex. Cassette chromosome recombinase (ccr) mediates integration into, and precise excision of SCC from chromosome (Katayama *et al*., 2000). The older SCC*mec* types, Types I-III are born by HA-MRSA,

relatively big sizes and carry multiple antibiotic resistance determinants while Types IV and V are new types of SCC*mec* basically carried by CA-MRSA, short and typically carrying no antibiotic resistance genes other than *mec*-gene complex. Types IV and V are largely disseminated among methicillin-resistant non-aureus staphylococci (MRNAS). Studies have shown the presence of these new SCC*mec* types in methicillin resistant *S. epidermis and S. haemolyticus* in the community (Hisata *et al.*, 2005; Ruppe *et al*., 2009). This suggests that MRNAS serves as the reservoir for horizontal gene transfer of new types of SCC*mec* to *S. aureus* (Hiramatsu et al., 2013). Study by Okuma and co-workers (2002) indicated that CA-MRSA strains carrying type-IV and type V SCC*mec* grow faster, are more virulent, resistant to fewer antibiotic classes (non-multi-resistant) and lower in the degree of beta-lactam resistance (heterogeneously methicillin resistant). In contrast to Okuma and co-workers report that CA-MRSA are generally non-multi-resistant, similar isolates in this study were multi-antibiotic resistant. While Okuma and co-workers had one strain as an exception, over 50% of the isolates in this study were exceptions. The increasing shift of CA-MRSA from its characteristic nature may be due to exposure to antibiotics. This is more likely the case in Nigeria as antibiotics are bought over the counter. In addition, the indiscriminate disposal of pharmaceuticals may lead to antibiotics finding their way to drinking waters; thus further aggravating the incidence of drug resistance.

The presence of *sa442* and *nuc* gene which are characteristic for *S. aureus* confirms the identity of the isolates. However, the bands for the *sa442* were faint and appeared below 100bp. Similarly, the bands for the *nuc* gene appeared faint except for isolates 1, 3, and 10 which have bands at 100bp. The spa typing also confirmed *S. aureus*. However, isolate 3 was distinct as it showed more bands than other isolates at 400 and 650bp.

As observed from the phylogenetic tree (Figure 22) of N315 and the isolates, not all the isolates were found on the tree despite the significant values of sequence similarity (Table 5). While isolates 1, 7, 8, 10, 13, 14 and 15 were on the tree, isolates 2, 3, 4 and 12 were not found despite the fact that they were all blasted together. This is an indication that although 2, 3, 4 and 12 are significantly similar to N315, they are not related to N315 and maybe of different lineage (s).

The fact that these isolates are from a community of students and not from hospital environment and yet show evidence of methicillin resistance gene (*mecA*) and largely multi-drug resistant, indicate that they have been formed from the onset by the horizontal transfer of *mecA* to resident methicillin susceptible lineages, as was observed in vivo when the gene was transferred from *S. epidermidis* to *S. aureus* during antibiotic treatment (Wielders *et al*., 2002). The report by Wielders and co-workers (2002) suggested that *mecA* gene is disseminated widely in *Staph aureus* population: they observed the repeated horizontal transfer of *mecA* to at least eight resident *S. aureus* lineages. They also suggested that coagulase-negative staphylococci (CoNS) serves as donors, facilitating the dissemination of *mecA* in *S. aureus* population. It is of a concern that with 70-75% of CoNS being a huge reservoir of resistance and with horizontal transfer of *mecA* gene, new MRSA may continue to emerge.

CHAPTR SIX

6.0 SUMMARRY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

This study has demonstrated Staphylococcal urine carriage of 78.8% of which 33.6% of these staphylococcal isolates were *S. aureus*. The other species are normally associated with animals. Their isolation in this human population is of much concern. The traditional method of characterising *S. aureus* was found to be grossly inadequate in identifying *S. aureus*. The isolates exhibited high susceptibility to ciprofloxacin, vancomycin, erythromycin, gentamicin and co-trimoxazole but high level of resistance was demonstrated against penicillin, cefotaxime, ofloxacin, cefoxitin, tetracycline and oxacillin. Majority of the isolates were multiantibiotic/multidrug resistant. Of the isolates which were phenotypically methicillin resistant, mecA gene was present in the 16 isolates subjected to molecular analysis. Sequence analysis showed that isolates 3 and 14 have immediate common lineage with the 8 BWI strain while isolate 7 has with the S1123 and 363LF strains. Isolate 11 has the same lineage with CLRSA1 strain. Compared to the N315 strain, not all the sequenced isolates had significant values of sequence similarities. Isolate 2, 3, 4 and 12 were not found on the phylogenetic tree and are probably of a different lineage not yet documented in the data bank.

6.2 CONCLUSION

A high perentage of healthy student volunteers from the University of Jos community are carriers of multi-drug resistant *Staphylococcus aureus*. The isolates were better characterised to species level by the use of the Microgen ID rapid test kit. They were generally resistant to the beta-lactam antibiotics, ofloxacin and tetracycline. Majority of the isolates had an MAR index of >0.3. Many of the isolates were phenotypically methicillin resistant and carried *mecA* gene. Some of the resistance were plasmid mediated. Four of the isolates have common lineage

with 8 BWI (2), S1123 and 363LF (1) and CLRSA1 (1) strains. Isolates 7, 13; 8, 14; and 1, 10, 15 are closely related to the N315 strain in lineage. Isolates 5, 9 and 16 had sequences that were not related to the sequences in the GenBank database even though they were identified as *S. aureus* by the Microgen ID test kit. This is the first time *mecB* gene is being reported in *S. aureus* and this may increase the level and magnitude of resistance by MRSA. The identification of *mecA* and *mecB* genes in community isolates implies that any outbreak of infections caused by these strains may pose a threat to public health as these strains are resistant to all antibiotics tested except ciprofloxacin, vancomycin and co-trimoxazole.

6.3 RECOMMENDATIONS

In the light of the findings in this study, it is recommended that

1. Further studies be done on isolates 5, 9, 16 and 11 to fully characterise them. When they were blasted in the NCBI GenBank data base, they did not show similarity to any organism.
2. Further investigation to confirm the presence or absence of the *mecB* gene is also recommended as presene of this gene in *S. aureus* has not been reported previously.
3. SCCmec typing of the isolates should be further done as more classes other than types IV and V which are traditionally associated with CA-MRSA and not usually considered multidrug resistant, have been reported.

6.4 CONTRIBUTION TO KNOWLEDGE

a. This study demonstrated *Staphyloous aureus* urine carriage of 33.6%
b. *mecA* gene was present in all the 16 multi-drug resistant *S. aureus* tested, and therefore this indicates there is a prevalence of MRSA in healthy individuals.

c. *mecB* gene presence has been demonstrated in some of the *S. aureus* isolates, the first ever such report in this species.

d. New strains/isolates (isolates 5, 9, 16 and 11) yet to be fully characterised have been demonstrated from this study as there was no match with the sequenes in the NCBI GenBank data base.

e. This study revealed the presence in humans of *Staphylococcus* species that are largely associated with animals.

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APPENDICES

Appendix A

Interpretive chart

Antibiotics (µg)	R(≤)*	I	S(≥)*
Tetracycline (10)	19	-	20
Erythromycin (15)	19	-	20
Cefoxitin (30)	21	-	22
Ciprofloacin (30)	17	-	18
Ofloxacin (5)	27	-	28
Co-trimoxazole (25)	16	-	17
Vancomycin (30)	11	-	12
Gentamicin (10)	19	-	20
Oxacillin (1)	14	-	15
Penicillin (1.5)	24	-	25
Cefotaxime (30)	21	-	22

*zone diameters in millimeters

Appendix B

Table 4.3a: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
P	157
P, CTX,	78
P, CTX, OX	96
P, CTX, OX, CN	39
P, CTX, OX, CN, VA	17
P, CTX, OX, CN, VA, STX	13
P, CTX, OX, CN, VA, STX, OFX	13
P, CTX, OX, CN, VA, STX, OFX, CIP	3
P, CTX, OX, CN, VA, STX, OFX, CIP, FOX	3
P, CTX, OX, CN, VA, STX, OFX, CIP, FOX, E	3
P, CTX, OX, CN, VA, STX, OFX, CIP, FOX, E, TE	3

P – Penicillin; CTX – Cefoxatime; OX – Oxacillin; CN – Gentamicin; VA – Vancomycin; STX – Co-trimoxazole; OFX – Ofloxacin; CIP – Ciprofloxacin; FOX – Cefoxitin; E – Erythromycin; TE – Tetracycline.

Table 4.3b: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
CTX,	138
CTX, OX	98
CTX, OX, CN	40
CTX, OX, CN, VA	17
CTX, OX, CN, VA, STX	13
CTX, OX, CN, VA, STX, OFX	13
CTX, OX, CN, VA, STX, OFX, CIP	3
CTX, OX, CN, VA, STX, OFX, CIP, FOX	3
CTX, OX, CN, VA, STX, OFX, CIP, FOX, E	3
CTX, OX, CN, VA, STX, OFX, CIP, FOX, E, TE	3
CTX, OX, CN, VA, STX, OFX, CIP, FOX, E, TE, P	3

Table 4.3c: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
OX	104
OX, CN	42
OX, CN, VA	19
OX, CN, VA, STX	14
OX, CN, VA, STX, OFX	14
OX, CN, VA, STX, OFX, CIP	4
OX, CN, VA, STX, OFX, CIP, FOX	4
OX, CN, VA, STX, OFX, CIP, FOX, E	4
OX, CN, VA, STX, OFX, CIP, FOX, E, TE	4
OX, CN, VA, STX, OFX, CIP, FOX, E, TE, P	4
OX, CN, VA, STX, OFX, CIP, FOX, E, TE, P, CTX	3

Table 4.3d: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
CN	59
CN, VA	20
CN, VA, STX	14
CN, VA, STX, OFX	14
CN, VA, STX, OFX, CIP	4
CN, VA, STX, OFX, CIP, FOX	4
CN, VA, STX, OFX, CIP, FOX, E	4
CN, VA, STX, OFX, CIP, FOX, E, TE	4
CN, VA, STX, OFX, CIP, FOX, E, TE, P	4
CN, VA, STX, OFX, CIP, FOX, E, TE, P, CTX	3
CN, VA, STX, OFX, CIP, FOX, E, TE, P, CTX, OX	3

Table 4.3e: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
VA	46
VA, STX	21
VA, STX, OFX	19
VA, STX, OFX, CIP	4
VA, STX, OFX, CIP, FOX	4
VA, STX, OFX, CIP, FOX, E	4
VA, STX, OFX, CIP, FOX, E, TE	4
VA, STX, OFX, CIP, FOX, E, TE, P	4
VA, STX, OFX, CIP, FOX, E, TE, P, CTX	3
VA, STX, OFX, CIP, FOX, E, TE, P, CTX, OX	3
VA, STX, OFX, CIP, FOX, E, TE, P, CTX, OX, CN	3

Table 4.3f: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
STX	63
STX, OFX	57
STX, OFX, CIP	15
STX, OFX, CIP, FOX	13
STX, OFX, CIP, FOX, E	8
STX, OFX, CIP, FOX, E, TE	7
STX, OFX, CIP, FOX, E, TE, P	7
STX, OFX, CIP, FOX, E, TE, P, CTX	6
STX, OFX, CIP, FOX, E, TE, P, CTX, OX	6
STX, OFX, CIP, FOX, E, TE, P, CTX, OX, CN	5
STX, OFX, CIP, FOX, E, TE, P, CTX, OX, CN, VA	3

Table 4.3g: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
OFX	136
OFX, CIP	20
OFX, CIP, FOX	17
OFX, CIP, FOX, E	11
OFX, CIP, FOX, E, TE	10
OFX, CIP, FOX, E, TE, P	10
OFX, CIP, FOX, E, TE, P, CTX	9
OFX, CIP, FOX, E, TE, P, CTX, OX	9
OFX, CIP, FOX, E, TE, P, CTX, OX, CN	7
OFX, CIP, FOX, E, TE, P, CTX, OX, CN, VA	5
OFX, CIP, FOX, E, TE, P, CTX, OX, CN, VA, STX	3

Table 4.3h: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
CIP	20
CIP, FOX	17
CIP, FOX, E	11
CIP, FOX, E, TE	10
CIP, FOX, E, TE, P	10
CIP, FOX, E, TE, P, CTX	9
CIP, FOX, E, TE, P, CTX, OX	9
CIP, FOX, E, TE, P, CTX, OX, CN	7
CIP, FOX, E, TE, P, CTX, OX, CN, VA	4
CIP, FOX, E, TE, P, CTX, OX, CN, VA, STX	3
CIP, FOX, E, TE, P, CTX, OX, CN, VA, STX, OFX	3

Table 4.3i: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
FOX	135
FOX, E	69
FOX, E, TE	54
FOX, E, TE, P	51
FOX, E, TE, P, CTX	47
FOX, E, TE, P, CTX, OX	43
FOX, E, TE, P, CTX, OX, CN	19
FOX, E, TE, P, CTX, OX, CN, VA	13
FOX, E, TE, P, CTX, OX, CN, VA, STX	10
FOX, E, TE, P, CTX, OX, CN, VA, STX, OFX	10
FOX, E, TE, P, CTX, OX, CN, VA, STX, OFX, CIP	3

Table 4.3j: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	No of Isolates
E	82
E, TE	64
E, TE, P	58
E, TE, P, CTX	51
E, TE, P, CTX, OX	43
E, TE, P, CTX, OX, CN	21
E, TE, P, CTX, OX, CN, VA	14
E, TE, P, CTX, OX, CN, VA, STX	10
E, TE, P, CTX, OX, CN, VA, STX, OFX	10
E, TE, P, CTX, OX, CN, VA, STX, OFX, CIP	3
E, TE, P, CTX, OX, CN, VA, STX, OFX, CIP, FOX	3

Table 4.3k: Antibiogram of isolates of *Staphylococcus* by the diffusion test

Antibiotic to which resistant	**No of Isolates**
TE	111
TE, P	100
TE, P, CTX	85
TE, P, CTX, OX	66
TE, P, CTX, OX, CN	32
TE, P, CTX, OX, CN, VA	16
TE, P, CTX, OX, CN, VA, STX	12
TE, P, CTX, OX, CN, VA, STX, OFX	12
TE, P, CTX, OX, CN, VA, STX, OFX, CIP	3
TE, P, CTX, OX, CN, VA, STX, OFX, CIP, FOX	3
TE, P, CTX, OX, CN, VA, STX, OFX, CIP, FOX, E	3

Appendix C

Table 1: Alignment view of blast of *Staph aureus* isolate 1 against other identical *Staph aureus*

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Query_38385  20       GGGCTCAAAGCGCGCGCAGGCGGTT-CTTAAGTC-G-T-TGAAAGCCCACGGCTCAACC-  74
JQ429749     516      ....GT.............T....T........T.A.G...................G  575
KX447584     567      ....GT..........T........T........T.A.G......T............G  626
KJ365315     460      ....GT..........T........T........T.A.G...................G  519
KU971601     420      ....GT..........T........T........T.A.G...................G  479
CP012972     493381   ....GT..........T........TT.......T.A.G...................G  493440
CP012972     498636   ....GT..........T........TT.......T.A.G...................G  498695
CP012972     449264   ....GT..........T........TT.......T.A.G...................G  449323
CP012972     1913787        ..........T........-.......T.A.G...................G  1913735
CP012972     2049208        ..........T........TT.......T.A.G...................G  2049155
CP012972     2164745        ..........T........TT.......T.A.G...................G  2164692
CP012978     445487   ....GT..........T........TT.......T.A.G...................G  445546
CP012978     489603   ....GT..........T........TT.......T.A.G...................G  489662
CP012978     494854   ....GT..........T........TT.......T.A.G...................G  494913
CP012978     1865203        ..........T........-.......T.A.G...................G  1865151
CP012978     2000940        ..........T........TT.......T.A.G...................G  2000887
CP012978     2116549        ..........T........TT.......T.A.G...................G  2116496
AB599719     265      ....GT..........T........T........T.A.G...................G  324
KT971132     547      ....GT..........T........T........T.A.G...................G  606
JQ429752     514      ....GT..........T........T........T.A.G...................G  573
JQ429751     510      ....GT..........T........T........T.A.G...................G  569

Query_38385  75       T-G-A-GGTCA-TGGAAACTGGGGGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGT  130
JQ429749     576      .G.-.G.....T...........A.....................A..............  634
KX447584     627      .G.A.-.....T...........A.....................................  685
KJ365315     520      .G.-.G.....T...........AA....................................  578
KU971601     480      .G.-.G.....T...........AA....................A..............  538
CP012972     493441   .G.-.G.....T..........AAA....................A..............  493499
CP012972     498696   .G.-.G.....T..........AAA....................A..............  498754
CP012972     449324   .G.-.G.....T..........AAA....................A..............  449382
CP012972     1913734  .G.-.G.....T..........AAA....................A..............  1913676
CP012972     2049154  .G.-.G.....T..........AAA....................A..............  2049096
CP012972     2164691  .G.-.G.....T..........AAA....................A..............  2164633
CP012978     445547   .G.-.G.....T..........AAA....................A..............  445605
CP012978     489663   .G.-.G.....T..........AAA....................A..............  489721
CP012978     494914   .G.-.G.....T..........AAA....................A..............  494972
CP012978     1865150  .G.-.G.....T..........AAA....................A..............  1865092
CP012978     2000886  .G.-.G.....T..........AAA....................A..............  2000828
CP012978     2116495  .G.-.G.....T..........AAA....................A..............  2116437
AB599719     325      .G.-.G.....T...........AA....................A..............  383
KT971132     607      .G.-.G.....T...........AA.....................................  665
JQ429752     574      .G.-.G.....T...........AA....................A..............  632
JQ429751     570      .G.-.G.....T...........AA....................A..............  628

Query_38385  131      GTAGCGGTGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGGCGACTTCTCTGGT  189
JQ429749     635      ................T....A.............................-......  693
KX447584     686      ....................A.........................G.-........  744
KJ365315     579      ....................A.........................G.-........  637
KU971601     539      ....................A.............................-......  597
CP012972     493500   ....................A.............................-......  493558
CP012972     498755   ....................A.............................-......  498813
CP012972     449383   ....................A.............................-......  449441
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CP012972     1913675  ....................A.............................-......  1913617
CP012972     2049095  ....................A.............................-......  2049037
CP012972     2164632  ....................A.............................-......  2164574
CP012978     445606   ....................A.............................-......  445664

CP012978     489722   ....................A.............................-......  489780
CP012978     494973   ....................A.............-...............-......  495030
CP012978     1865091  ....................A.............................-......  1865033
CP012978     2000827  ....................A.............................-......  2000769
CP012978     2116436  ....................A.............................-......  2116378
AB599719     384      ....................A.............................-......  442
KT971132     666      ....................A.........................G.-........  724
JQ429752     633      ....................A.............................-......  691
JQ429751     629      ....................A.............................-......  687

Query_38385  190      CTGTAACTGACGCTGAGGCGCGAAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA  249
JQ429749     694      ..........A.........-.....................................  752
KX447584     745      ..............T.T...-...........T.......................  803
KJ365315     638      ..............T.T...-...........T.......................  696
KU971601     598      ..............T.T...-...........T.......................  656
CP012972     493559   ..............T.T...-...........T.......................  493617
CP012972     498814   ..............T.T...-...........T.......................  498872
CP012972     449442   ..............T.T...-...........T.......................  449500
CP012972     1913616  ..............T.T......-.........T.......................  1913558
CP012972     2049036  ..............T.T......-.........T.......................  2048978
CP012972     2164573  ..............T.T......-.........T.......................  2164515
CP012978     445665   ..............T.T...-...........T.......................  445723
CP012978     489781   ..............T.T...-...........T.......................  489839
CP012978     495031   ..............T.T...-...........T.......................  495089
CP012978     1865032  ..............T.T......-.........T.......................  1864974
CP012978     2000768  ..............T.T......-.........T.......................  2000710
CP012978     2116377  ..............T.T......-.........T.......................  2116319
AB599719     443      ..............T.T...-...........T.......................  501
KT971132     725      ..............T.T...-...........T.......................  783
JQ429752     692      ..............T.T...-...........T.......................  750
JQ429751     688      ..............T.T...-...........T.......................  746

Query_38385  250      GTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGGTTTCCG-CCC-TTAGTGCTGG  307
JQ429749     753      ....................................-......-...T.........A  810
KX447584     804      .................................-.........C...-.........C  861
KJ365315     697      .................................-.........C...-.........C  754
KU971601     657      .................................-.........C...-.........C  714
CP012972     493618   .................................-.........C...-.........C  493675
CP012972     498873   .................................-.........C...-.........C  498930
CP012972     449501   .................................-.........C...-.........C  449558
CP012972     1913557  .................................-.........C...-.........C  1913500
CP012972     2048977  .................................-.........C...-.........C  2048920
CP012972     2164514  .................................-.........C...-.........C  2164457
CP012978     445724   .................................-.........C...-.........C  445781
CP012978     489840   .................................-.........C...-.........C  489897
CP012978     495090   .................................-.........-...-.--......C  495144
CP012978     1864973  .................................-.........C...-.........C  1864916
CP012978     2000709  .................................-.........C...-.........C  2000652
CP012978     2116318  .................................-.........C...-.........C  2116261
AB599719     502      .................................-.........C...-.........C  559
KT971132     784      ................................G.........C...-.........C  842
JQ429752     751      .................................-.........C...-.........C  808
JQ429751     747      ................................AG.........C...-.........C  805

Query_38385  308      AGCTAACGCATT-A-GCACTCCG-CCTGGGG-AGTACGGACGCA-GG-T-G-AA-CTCAA  358
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JQ429749      811      ...........A.-........-.......-.......C....A..C.-.-..A.....  865
KX447584      862      ...........A.-........-.......-......AC....A..-.T.-..A.....  916
KJ365315      755      ...........A.-........-.......-......AC....A..-.T.-..A.....  809
KU971601      715      ...........A.-........-.......-......AC....A..-.T.-..A.....  769
CP012972      493676   ...........A.-........-.......-......AC....A..-.T.-..A.....  493730
CP012972      498931   ...........A.-........-.......-......AC....A..-.T.-..A.....  498985
CP012972      449559   ...........A.-........-.......-......AC....A..-.T.-..A.....  449613
CP012972      1913499  ...........-.A........-.......-......AC....A..-.T.-..A.....  1913445
CP012972      2048919  ...........-.A........-.......-......AC....A..-.T.-..A.....  2048865
CP012972      2164456  ...........-.A........-.......-......AC....A..-.T.-..A.....  2164402
CP012978      445782   ...........A.-........-.......-......AC....A..-.T.-..A.....  445836
CP012978      489898   ...........A.-........-.......-......AC....A..-.T.-..A.....  489952
CP012978      495145   ...........A.-........-.......-......AC....A..-.T.-..A.....  495199
CP012978      1864915  ...........-.A........-.......-......AC....A..-.T.-..A.....  1864861
CP012978      2000651  ...........-.A........-.......-......AC....A..-.T.-..A.....  2000597
CP012978      2116260  ...........-.A........-.......-......AC....A..-.T.-..A.....  2116206
AB599719      560      ...........A.-........-.......-......AC....A..-.T.A..-.....  614
KT971132      843      ...........-.A........G.......G......AC....A..-.T.-..A.....  899
JQ429752      809      ...........A.-........-.......-......AC....A..-.T.-..A.....  863
JQ429751      806      ...........A.-........-.......-......AC....A..-.T.-..A.....  860

Query_38385   359      AGG-A-TTGACGGGGGACCCGCACAAGCGGGGGAGCATGTG-TTTTATTTCGGAGCAGCG  415
JQ429749      866      .A.-.A..........-............T..........-G....A....A....A..  922
KX447584      917      ...-.A.....-.................T..........-G....A....A....A..  973
KJ365315      810      ...-.A.....-.................T..........-G....A....A....A..  866
KU971601      770      ...-.A.....-.................T..........-G....A....A....A..  826
CP012972      493731   ...-.A.....-.................T..........-G....A....A....A..  493787
CP012972      498986   ...-.A.....-.................T..........-G....A....A....A..  499042
CP012972      449614   ...-.A.....-.................T..........-G....A....A....A..  449670
CP012972      1913444  ...-.A.....-.................T..........-G....A....A....A..  1913388
CP012972      2048864  ...-.A.....-.................T..........-G....A....A....A..  2048808
CP012972      2164401  ...-.A.....-.................T..........-G....A....A....A..  2164345
CP012978      445837   ...-.A.....-.................T..........-G....A....A....A..  445893
CP012978      489953   ...-.A.....-.................T..........-G....A....A....A..  490009
CP012978      495200   ...-.A.....-.................T.....-....-G....A....A....A..  495255
CP012978      1864860  ...-.A.....-.................T..........-G....A....A....A..  1864804
CP012978      2000596  ...-.A.....-.................T..........-G....A....A....A..  2000540
CP012978      2116205  ...-.A.....-.................T..........-G....A....A....A..  2116149
AB599719      615      ...-.-A....-.................T..........-G....A....A....A..  670
KT971132      900      ...A.-......................T..........G.....-....A....A..  957
JQ429752      864      ...-.A.....-.................T..........-G....A....A....A..  920
JQ429751      861      ...-.A.....-.................T..........-G....A....A....A..  917

Query_38385   416      CG-A-GAACCTTAACAGGTCTTGAAATCCTTTGA-C-G-CC-CTAGAG-TAG-GCTTTCC  467
JQ429749      923      ..A.-........C..........C...T.C...-.-AA..-......A...-.         971
KX447584      974      ..A.-........C..AA......C.........T.-.-.T-......A...A.T.....  1029
KJ365315      867      ..A.-........C..AA......C.........-.C.-.T-......A...          914
KU971601      827      ..A.-........C..AA......C.........-.-A-A.T......A...A..C....  882
CP012972      493788   ..A.-........C..AA......C.........-.-A-A.T......A...A.......  493843
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CP012972	499043	..A.-........C..AA......C.........-.-A-A.T......A...A.......	499098
CP012972	449671	..A.-........C..AA......C.........-.-A-A.T......A...A..C....	449726
CP012972	1913387	..-.A........C..AA......C.........-.-A-A.T......A...A.......	1913332
CP012972	2048807	..-.A........C..AA......C.........-.-A-A.T......A...A.......	2048752
CP012972	2164344	..-.A........C..AA......C.........-.-A-A.T......A...A.......	2164289
CP012978	445894	..A.-........C..AA......C.........-.-A-A.T......A...A.......	445949
CP012978	490010	..A.-........C..AA......C.........-.-A-A.T......A...A.......	490065
CP012978	495256	..A.-........C..AA......C.........-.-A-A.T......A...A.......	495311
CP012978	1864803	..-.A........C..AA......C.........-.-A-A.T......A...A.......	1864748
CP012978	2000539	..-.A........C..AA......C.........-.-A-A.T......A...A.......	2000484
CP012978	2116148	..-.A........C..AA......C.........-.-A-A.T......A...A.......	2116093
AB599719	671	..A.-........C..AA......C.........-.-A-A.T......A...A..C....	726
KT971132	958	..A.-........C..A-......C.........-.C.-.T-......A...	1004
JQ429752	921	..A.-........C..AA......C.........-.-A-A.T......A...	968
JQ429751	918	..A.-........C..AA......C.........-.-A-A.T......A...A..C....	973

Query_38385	468	ACTT	471
KX447584	1030	C...	1033
KU971601	883	C...	886
CP012972	493844	C...	493847
CP012972	499099	C...	499102
CP012972	449727	C...	449730
CP012972	1913331	C...	1913328
CP012972	2048751	C...	2048748
CP012972	2164288	C...	2164285
CP012978	445950	C...	445953
CP012978	490066	C...	490069
CP012978	495312	T...	495315
CP012978	1864747	C...	1864744
CP012978	2000483	C...	2000480
CP012978	2116092	C...	2116089
AB599719	727	C...	730
JQ429751	974	C...	977

Table 2: Alignment view of blast of *Staph aureus* isolate 2 against other identical *Staph aureus*

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Query_180747  7        CCGG-TT-TTCGGGCG-AAAGCGCGCG-AGGCGG--TTC--TAAGTC-GA--TGAAAGCC  55
CP002110      48622              .....T.........T......T-..T--......T..TG........  48577
CP002110      1203136            .....T.........T......T-..T--......T..TG........  1203181
CP002110      4560               .....T.........T......TT..T--......T..TG........  4514
CP002110      1088386            .....T.........T......TT..T--......T..TG........  1088432
CP002110      1357804            .....T.........T......--...TT......T..TG........  1357850
CP002110      2801902            .....T.........T......TT..T--......T..TG........  2801856
CP012015      552439             .....T.........T......T-...--......T..TG........  552484
CP012015      596902             .....T.........T......TT..T--......T..TG........  596948
CP012015      2268921            .....T.........T......TT..T--......T..TG........  2268875
CP012015      2389834            .....T.........T......TT..T--......T..TG........  2389788
CP012015      2090279            .....T.........T......TT..T--......T..TG........  2090233
LC107798      19           ..-..-.....-T.........T......TT..T--......T..TG........  68
LC107791      12       ....A..-A.T.....T.........T......TT..T--......T..TG........  68
LC107786      12       ....A..A..G.....-T.........T......TT..T--......T..TG........  68
CP009681      2004791            .....T.........T......TT..T--......T..TG........  2004745
CP009681      2049075            .....T.........T......TT..T--......T..TG........  2049029
CP009681      2325569            .....T.........T......TT..T--......T..TG........  2325523
CP009681      2446698            .....T.........T......TT..T--......T..TG........  2446652
CP009681      504978             .....T.........T......TT..T--......T..TG........  505024
FN393795      562                .....T.........T......TT..T--......T..TG........  608
CP012692      571781             .....T.........T......TT..T--......T..TG........  571827
CP012692      2215645            .....T.........T......TT..T--......T..TG........  2215599
CP012692      2330431            .....T.........T......TT..T--......T..TG........  2330385
CP012692      527420             .....T.........T......TT..T--......T..TG........  527466
CP012692      2069606            .....T.........T......TT..T--......T..TG........  2069560
CP017094      579168             .....T.........T......TT..T--......T..TG........  579214
CP017094      695453             .....T.........T......TT..T--......T..TG........  695499
CP017094      2318356            .....T.........T......TT..T--......T..TG........  2318310
CP017094      2323566            .....T.........T......TT..T--......T..TG........  2323520
CP017094      2367833            .....T.........T......TT..T--......T..TG........  2367787
CP017094      873403             .....T.........T......TT..T--......T..TG........  873449
CP016855      509507             .....T.........T......TT..T--......T..TG........  509553
CP016855      558990             .....T.........T......TT..T--......T..TG........  559036
CP016855      2179146            .....T.........T......TT..T--......T..TG........  2179100
CP016855      2295434            .....T.........T......TT..T--......T..TG........  2295388
CP016855      553778             .....T.........T......TT..T--......T..TG........  553824
CP016855      1957888            .....T.........T......TT..T--......T..TG........  1957842

Query_180747  56       CACGG-TCAACC-TGGA-GGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAAA-T  111
CP002110      48576    .....C......G....G.............A.....................G.  48517
CP002110      1203182  .....C......G....G.............A.....................G.  1203241
CP002110      4513     .....C......G....G.............A.....................G.  4454
CP002110      1088433  .....C......G....G.............A.....................G.  1088492
CP002110      1357851  .....C......G....G.............A.....................G.  1357910
CP002110      2801855  .....C......G....G.............A.....................G.  2801796
CP012015      552485   ..G.C-......G....-.............A.....................G.  552542
CP012015      596949   .....C......G....G.............A.....................G.  597008
CP012015      2268874  .....C......G....G.............A.....................G.  2268815
CP012015      2389787  .....C......G....G.............A.....................G.  2389728
CP012015      2090232  .....C......G....G.............A.....................G.  2090173
LC107798      69       .....C......G....G.............A.....................G.  128
LC107791      69       .....C......G....G.............A.....................G.  128
LC107786      69       .....C......G....G.............A.....................G.  128
CP009681      2004744  .....C......G....G.............A.....................G.  2004685
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CP009681      2049028  .....C......G....G..............A.....................G.  2048969
CP009681      2325522  .....C......G....G..............A.....................G.  2325463
CP009681      2446651  .....C......G....G..............A.....................G.  2446592
CP009681      505025   .....C......G....G..............A.....................G.  505084
FN393795      609      .....C......G....G..............A.....................G.  668
CP012692      571828   .....C......G....G..............A.....................G.  571887
CP012692      2215598  .....C......G....G..............A.....................G.  2215539
CP012692      2330384  .....C......G....G..............A.....................G.  2330325
CP012692      527467   .....C......G....G..............A.....................G.  527526
CP012692      2069559  .....C......G....G..............A.....................G.  2069500
CP017094      579215   .....C......G....G..............A.....................G.  579274
CP017094      695500   .....C......G....G..............A.....................G.  695559
CP017094      2318309  .....C......G....G..............A.....................G.  2318250
CP017094      2323519  .....C......G....G..............A.....................G.  2323460
CP017094      2367786  .....C......G....G..............A.....................G.  2367727
CP017094      873450   .....C......G....G..............A.....................G.  873509
CP016855      509554   .....C......G....G..............A.....................G.  509613
CP016855      559037   .....C......G....G..............A.....................G.  559096
CP016855      2179099  .....C......G....G..............A.....................G.  2179040
CP016855      2295387  .....C......G....G..............A.....................G.  2295328
CP016855      553825   .....C......G....G..............A.....................G.  553884
CP016855      1957841  .....C......G....G..............A.....................G.  1957782

Query_180747  112      GGAATTCCATGTGTAGC-G-TGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGG  168
CP002110      48516    ................-.G.............A.......................  48458
CP002110      1203242  ................G.-.............A.......................  1203300
CP002110      4453     ................-.G.............A.......................  4395
CP002110      1088493  ................G.-.............A.......................  1088551
CP002110      1357911  ................G.-.............A.......................  1357969
CP002110      2801795  ................-.G.............A.......................  2801737
CP012015      552543   ................G.-.............A.......................  552601
CP012015      597009   ................G.-.............A.......................  597067
CP012015      2268814  ................-.G.............A.......................  2268756
CP012015      2389727  ................-.G.............A.......................  2389669
CP012015      2090172  ................-.G.............A.......................  2090114
LC107798      129      ................G.-.............A.......................  187
LC107791      129      ................G.-.............A.......................  187
LC107786      129      ................G.-.............A.......................  187
CP009681      2004684  ................-.G.............A.......................  2004626
CP009681      2048968  ................-.G.............A.......................  2048910
CP009681      2325462  ................-.G.............A.......................  2325404
CP009681      2446591  ................-.G.............A.......................  2446533
CP009681      505085   ................G.-.............A.......................  505143
FN393795      669      ................G.-.............A.......................  727
CP012692      571888   ................G.-.............A.......................  571946
CP012692      2215538  ................-.G.............A.......................  2215480
CP012692      2330324  ................-.G.............A.......................  2330266
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CP012692      527527   ................G.-.............A.......................  527585
CP012692      2069499  ................-.G.............A.......................  2069441
CP017094      579275   ................G.-.............A.......................  579333
CP017094      695560   ................G.-.............A.......................  695618
CP017094      2318249  ................-.G.............A.......................  2318191
CP017094      2323459  ................-.G.............A.......................  2323401
CP017094      2367726  ................-.G.............A.......................  2367668
CP017094      873510   ................G.-.............A.......................  873568
CP016855      509614   ................G.-.............A.......................  509672
CP016855      559097   ................G.-.............A.......................  559155
CP016855      2179039  ................-.G.............A.......................  2178981
CP016855      2295327  ................-.G.............A.......................  2295269
CP016855      553885   ................G.-.............A.......................  553943
CP016855      1957781  ................-.G.............A.......................  1957723

Query_180747  169      CGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCTTGGGGGATCAAAC-GGATTA  227
CP002110      48457    ....................................G.....-.......A......  48399

CP002110      1203301  ....................................G.....-.......A......  1203359
CP002110      4394     ....................................G.....-.......A......  4336
CP002110      1088552  ....................................G.....-.......A......  1088610
CP002110      1357970  ....................................G.....-.......A......  1358028
CP002110      2801736  ....................................G.....-.......A......  2801678
CP012015      552602   ....................................G.....-.......A......  552660
CP012015      597068   ....................................G.....-.......A......  597126
CP012015      2268755  ....................................G.....-.......A......  2268697
CP012015      2389668  ....................................G.....-.......A......  2389610
CP012015      2090113  ....................................G.....-.......A......  2090055
LC107798      188      ....................................G.....-.......A......  246
LC107791      188      ....................................G.....-.......A......  246
LC107786      188      ....................................G.....-.......A......  246
CP009681      2004625  ....................................G.....-.......A......  2004567
CP009681      2048909  ....................................G.....-.......A......  2048851
CP009681      2325403  ....................................G.....-.......A......  2325345
CP009681      2446532  ....................................G.....-.......A......  2446474
CP009681      505144   ....................................G.....-.......A......  505202
FN393795      728      ....................................G.....-.......A......  786
CP012692      571947   ....................................G.....-.......A......  572005
CP012692      2215479  ....................................G.....-.......A......  2215421
CP012692      2330265  ....................................G.....-.......A......  2330207
CP012692      527586   ....................................G.....-.......A......  527644
CP012692      2069440  ....................................G.....-.......A......  2069382
CP017094      579334   ....................................G.....-.......A......  579392
CP017094      695619   ....................................G.....-.......A......  695677
CP017094      2318190  ....................................G.....-.......A......  2318132
CP017094      2323400  ....................................G.....-.......A......  2323342
CP017094      2367667  ....................................G.....-.......A......  2367609
CP017094      873569   G...................................G.....-.......A......  873627
CP016855      509673   ....................................G.....-.......A......  509731
CP016855      559156   ....................................G.....-.......A......  559214
CP016855      2178980  ....................................G.....-.......A......  2178922
CP016855      2295268  ....................................G.....-.......A......  2295210
CP016855      553944   ....................................G.....-.......A......  554002
CP016855      1957722  ....................................G.....-.......A......  1957664

Query_180747  228      GATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCCGCCC  287
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CP002110      48398    ..................................................-....  48340
CP002110      1203360  ..................................................-....  1203418
CP002110      4335     ..................................................-....  4277
CP002110      1088611  ..................................................-....  1088669
CP002110      1358029  ..................................................-....  1358087
CP002110      2801677  ..................................................-....  2801619
CP012015      552661   ..................................................-....  552719
CP012015      597127   ..................................................-....  597185
CP012015      2268696  ..................................................-....  2268638
CP012015      2389609  ..................................................-....  2389551
CP012015      2090054  ..................................................-....  2089996
LC107798      247      ..................................................-....  305
LC107791      247      ..................................................-....  305
LC107786      247      ..................................................-....  305
CP009681      2004566  ..................................................-....  2004508
CP009681      2048850  ..................................................-....  2048792
CP009681      2325344  ..................................................-....  2325286
CP009681      2446473  ..................................................-....  2446415
CP009681      505203   ..................................................-....  505261
FN393795      787      ..................................................-....  845
CP012692      572006   ..................................................-....  572064
CP012692      2215420  ..................................................-....  2215362
CP012692      2330206  ..................................................-....  2330148
CP012692      527645   ..................................................-....  527703
CP012692      2069381  ..................................................-....  2069323

CP017094      579393   ..................................................-....  579451
CP017094      695678   ..................................................-....  695736
CP017094      2318131  ..................................................-....  2318073
CP017094      2323341  ..................................................-....  2323283
CP017094      2367608  ..................................................-....  2367550
CP017094      873628   ..................................................-....  873686
CP016855      509732   ..................................................-....  509790
CP016855      559215   ..................................................-....  559273
CP016855      2178921  ..................................................-....  2178863
CP016855      2295209  ..................................................-....  2295151
CP016855      554003   ..................................................-....  554061
CP016855      1957663  ..................................................-....  1957605

Query_180747  288      CTTAGTGGCTGCAGCAAACGCATTAAGCACTCCCGCCTGGGGAGTACGACCGCAAGTTTG  347
CP002110      48339    ......-........T.................-.....................G...  48282
CP002110      1203419  ......-........T.................-.....................G...  1203476
CP002110      4276     ......-........T.................-.....................G...  4219
CP002110      1088670  ......-........T.................-.....................G...  1088727
CP002110      1358088  ......-........T.................-.....................G...  1358145
CP002110      2801618  ......-........T.................-.....................G...  2801561
CP012015      552720   ......-........T.......-.......-.....................G...  552776
CP012015      597186   ......-........T.................-.....................G...  597243
CP012015      2268637  ......-........C.................-.....................G...  2268580
CP012015      2389550  ......-........T.................-.....................G...  2389493
CP012015      2089995  ......-........T.................-.....................G...  2089938
LC107798      306      ......-........T.................-.....................G...  363
LC107791      306      ......-........T.................-.....................G...  363
LC107786      306      ......-........T.................-.....................G...  363
CP009681      2004507  ......-........T.................-.....................G...  2004450
CP009681      2048791  ......-........T.................-.....................G...  2048734
CP009681      2325285  ......-........T.................-.....................G...  2325228
CP009681      2446414  ......-........T.................-.....................G...  2446357
CP009681      505262   ......-........T.................-.....................G...  505319
FN393795      846      ......-........T.................-.....................G...  903
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CP012692      572065   ......-........T..............-.....................G...  572122
CP012692      2215361  ......-........T..............-.....................G...  2215304
CP012692      2330147  ......-........T..............-.....................G...  2330090
CP012692      527704   ......-........T..............-.....................G...  527761
CP012692      2069322  ......-........T..............-.....................G...  2069265
CP017094      579452   ......-........T..............-.....................G...  579509
CP017094      695737   ......-........T..............-.....................G...  695794
CP017094      2318072  ......-........T..............-.....................G...  2318015
CP017094      2323282  ......-........T..............-.....................G...  2323225
CP017094      2367549  ......-........T..............-.....................G...  2367492
CP017094      873687   ......-........T..............-.....................G...  873744
CP016855      509791   ......-........T..............-.....................G...  509848
CP016855      559274   ......-........T..............-.....................G...  559331
CP016855      2178862  ......-........T..............-.....................G...  2178805
CP016855      2295150  ......-........T..............-.....................G...  2295093
CP016855      554062   ......-........T..............-.....................G..A  554119
CP016855      1957604  ......-........T..............-.....................G...  1957547

Query_180747  348      AAACTCAAAAGGAATGGACGGGGACCCCCACAAGCGGTGGGAGCATGTGGGTTAATTCCG  407
CP002110      48281    .........-.....T...........G............-.........T.......-.  48225
CP002110      1203477  ......-........T...........G..........-...........T.......-.  1203533
CP002110      4218     .........-.....T...........G............-.........T.......-.  4162
CP002110      1088728  ......-........T...........G..........-...........T.......-.  1088784
CP002110      1358146  ......-........T...........G..........-...........T.......-.  1358202
CP002110      2801560  .........-.....T...........G............-.........T.......-.  2801504
CP012015      552777   ......-........T...........G..........-...........T.......-.  552833
CP012015      597244   ......-........T...........G..........-...........T.......-.  597300
CP012015      2268579  .........-.....T...........G............-.........T.......-.  2268523
CP012015      2389492  .........-.....T...........G............-.........T.......-.  2389436

CP012015      2089937  .........-.....T...........G............-.........T.......-.  2089881
LC107798      364      ......-........T...........G..........-...........T.......-.  420
LC107791      364      ......-........T...........G..........-...........T.......-.  420
LC107786      364      ......-........T...........G..........-...........T.......-.  420
CP009681      2004449  .........-.....T...........G............-.........T.......-.  2004393
CP009681      2048733  .........-.....T...........G............-.........T.......-.  2048677
CP009681      2325227  .........-.....T...........G............-.........T.......-.  2325171
CP009681      2446356  .........-.....T...........G............-.........T.......-.  2446300
CP009681      505320   ......-........T...........G..........-...........T.......-.  505376
FN393795      904      ......-........T...........G..........-...........T.......-.  960
CP012692      572123   ......-........T...........G..........-...........T.......-.  572179
CP012692      2215303  .........-.....T...........G............-.........T.......-.  2215247
CP012692      2330089  .........-.....T...........G............-.........T.......-.  2330033
CP012692      527762   ......-........T...........G..........-...........T.......-.  527818
CP012692      2069264  .........-.....T...........G............-.........T.......-.  2069208
CP017094      579510   ......-........T...........G..........-...........T.......-.  579566
CP017094      695795   ......-........T...........G..........-...........T.......-.  695851
CP017094      2318014  .........-.....T...........G............-.........T.......-.  2317958
CP017094      2323224  .........-.....T...........G............-.........T.......-.  2323168
CP017094      2367491  .........-.....T...........G............-.........T.......-.  2367435
CP017094      873745   ......-........T...........G..........-...........T.......-.  873801
CP016855      509849   ......-........T...........G..........-...........T.......-.  509905
CP016855      559332   ......-........T...........G..........-...........T.......-.  559388
CP016855      2178804  .........-.....T...........G............-.........T.......-.  2178748
CP016855      2295092  .........-.....T...........G............-.........T.......-.  2295036
CP016855      554120   ......-........T...........G..........-...........T.......-.  554176
CP016855      1957546  .........-.....T...........G............-.........T.......-.  1957490

Query_180747  408      AAGCAACCGCGAAGAACCTTACCAAATCTTGACTTCTTTTGAAAACTCCTAGAGATAGAA  467
CP002110      48224    ......-............................A..C.....C.....-..........-  48168
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CP002110      1203534  ......-.......................A..C.....C.....-..........-  1203590
CP002110      4161     ......-.......................A..C.....C.....-..........-  4105
CP002110      1088785  ......-.......................A..C.....C.....-..........-  1088841
CP002110      1358203  ......-.......................A..C.....C.....-..........-  1358259
CP002110      2801503  ......-.......................A..C.....C.....-..........-  2801447
CP012015      552834   ......-.......................A..C.....C.....-..........-  552890
CP012015      597301   ......-.......................A..C.....C.....-..........-  597357
CP012015      2268522  ......-.......................A..C.....C.....-..........-  2268466
CP012015      2389435  ......-.......................A..C.....C.....-..........-  2389379
CP012015      2089880  ......-.......................A..C.....C.....-..........-  2089824
LC107798      421      ......-.......................A..C.....C.....-..........-  477
LC107791      421      ......-.......................A..C.....C.....-..........-  477
LC107786      421      ......-.......................A..C.....C.....-..........-  477
CP009681      2004392  ......-.......................A..C.....C.....-.........-.  2004336
CP009681      2048676  ......-.......................A..C.....C.....-..........-  2048620
CP009681      2325170  ......-.......................A..C.....C.....-..........-  2325114
CP009681      2446299  ......-.......................A..C.....C.....-..........-  2446243
CP009681      505377   ......-.......................A..C.....C.....-..........-  505433
FN393795      961      ......-.......................A..C.....C.....-..........-  1017
CP012692      572180   ......-.......................A..C.....C.....-..........-  572236
CP012692      2215246  ......-.......................A..C.....C.....-..........-  2215190
CP012692      2330032  ......-.......................A..C.....C.....-..........-  2329976
CP012692      527819   ......-.......................A..C.....C.....-..........-  527875
CP012692      2069207  ......-.......................A..C.....C.....-..........-  2069151
CP017094      579567   ......-.......................A..C.....C.....-..........-  579623
CP017094      695852   ......-.......................A..C.....C.....-..........-  695908
CP017094      2317957  ......-.......................A..C.....C.....-..........-  2317901
CP017094      2323167  ......-.......................A..C.....C.....-..........-  2323111
CP017094      2367434  ......-.......................A..C.....C.....-..........-  2367378
CP017094      873802   ......-.......................A..C.....C.....-..........-  873858
CP016855      509906   ......-.......................A..C.....C.....-..........-  509962
CP016855      559389   ......-.......................A..C.....C.....-..........-  559445
CP016855      2178747  ......-.......................A..C.....C.....-..........-  2178691

CP016855      2295035  ......-.......................A..C.....C.....-..........-  2294979
CP016855      554177   ......-.......................A..C.....C.....-..........-  554233
CP016855      1957489  ......-.......................A..C.....C.....-..........-  1957433

Query_180747  468      GCCTTCCCCCTTCCGGGAACAAAAGTGACAGGTGGTGCATGGTTGTCCGTCAGCTCGTGT  527
CP002110      48167    .........-...G...G..-........................-.............  48111
CP002110      1203591  .....-.......G...G..-........................-.............  1203647
CP002110      4104     .........-...G...G..-........................-.............  4048
CP002110      1088842  .....-.......G...G..-........................-.............  1088898
CP002110      1358260  .....-.......G...GG.-........................-.............  1358316
CP002110      2801446  .........-...G...G..-........................-.............  2801390
CP012015      552891   .....-.......G...G..-........................-.............  552947
CP012015      597358   .....-.......G...G..-........................-.............  597414
CP012015      2268465  .........-...G...G..-........................-.............  2268409
CP012015      2389378  .........-...G...G..-........................-.............  2389322
CP012015      2089823  ..T.....-...G...G..-........................-.............  2089767
LC107798      478      .....-.......G...G..-........................-.............  534
LC107791      478      .....-.......G...G..-........................-.............  534
LC107786      478      .....-.......G...G..-........................-.............  534
CP009681      2004335  .........G...G...G.....-......................-.............  2004278
CP009681      2048619  .........-...G...G..-........................-.............  2048563
CP009681      2325113  .........-...G...G..-........................-.............  2325057
CP009681      2446242  .........-...G...G..-........................-.............  2446186
CP009681      505434   ..T..T-......G...G..-........................-.............  505490
FN393795      1018     .....-.......G...G..-........................-.............  1074
CP012692      572237   .....-.......G...G..-........................-.............  572293
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CP012692      2215189  .........-...G...G..-.........................-.....  2215133
CP012692      2329975  .........-...G...G..-.........................-.....  2329919
CP012692      527876   .......T-....G...G..-.........................-.....  527932
CP012692      2069150  ..T.....-...G...G..-.........................-.....  2069094
CP017094      579624   .....-.......G...G..-.........................-.....  579680
CP017094      695909   .....-.......G...G..-.........................-.....  695965
CP017094      2317900  .........-...G...G..-.........................-.....  2317844
CP017094      2323110  .........-...G....-.-.........................-.....  2323055
CP017094      2367377  .........-...G...G..-.........................-.....  2367321
CP017094      873859   ..T..-.......G...G..-.........................-.....  873915
CP016855      509963   .....-.......G...G..-.........................-.....  510019
CP016855      559446   .....-.......G...G..-.........................-.....  559502
CP016855      2178690  .........-...G...G..-.........................-.....  2178634
CP016855      2294978  .........-...G...G..-.........................-.....  2294922
CP016855      554234   .....-.......G...G..-.........................-.....  554290
CP016855      1957432  ..T.....-...G...G..-.........................-.....  1957376

Query_180747  528      CGTGAAATGTTGGTTTAAGTCCCCGCACCGAGCCCCACCTCTTAAGCTTAGTTCCCATCA  587
CP002110      48110    .....G.......G.........-...A.....G.A...-.............G......  48053
CP002110      1203648  .....G.......G......-......A.....G.A...-.............G......  1203705
CP002110      4047     .....G.......G.........-...A.....G.A...-.............G......  3990
CP002110      1088899  .....G.......G......-......A.....G.A...-.............G......  1088956
CP002110      1358317  .....G.......G......-......A.....G.A...-.............G......  1358374
CP002110      2801389  .....G.......G.........-...A.....G.A...-.............G......  2801332
CP012015      552948   .....G.......G......-......A.....G.A...-.............G......  553005
CP012015      597415   .....G.......G......-......A.....G.A...-.............G......  597472
CP012015      2268408  .....G.......G.........-...A.....G.A...-.............G......  2268351
CP012015      2389321  .....G.......G.........-...A.....G.A...-.............G......  2389264
CP012015      2089766  .....G.......G.........-...A.....G.A...-.............G......  2089709
LC107798      535      .....G.......G......-......A.....G.A...-.............G......  592
LC107791      535      .....G.......G......-......A.....G.A...-.............G......  592
LC107786      535      .....G.......G......-......A.....G.A...-.............G......  592
CP009681      2004277  .....G.......G.........-...A.....G.A...-.............G......  2004220
CP009681      2048562  .....G.......G.........-...A.....G.A...-.............G......  2048505
CP009681      2325056  .....G.......G.........-...A.....G.A...-.............G......  2324999
CP009681      2446185  .....G.......G.........-...A.....G.A...-.............G......  2446128
CP009681      505491   .....G.......G......-......A.....G.A...-.............G......  505548

FN393795      1075     .....G.......G......-......A.....G.A...-.............G......  1132
CP012692      572294   .....G.......G......-......A.....G.A...-.............G......  572351
CP012692      2215132  .....G.......G.........-...A.....G.A...-.............G......  2215075
CP012692      2329918  .....G.......G.........-...A.....G.A...-.............G......  2329861
CP012692      527933   .....G.......G......-......A.....G.A...-.............G......  527990
CP012692      2069093  .....G.......G.........-...A.....G.A...-.............G......  2069036
CP017094      579681   .....G.......G......-......A.....G.A...-.............G......  579738
CP017094      695966   .....G.......G......-......A.....G.A...-.............G......  696023
CP017094      2317843  .....G.......G.........-...A.....G.A...-.............G......  2317786
CP017094      2323054  .....G.......G.........-...A.....G.A...-.............G......  2322997
CP017094      2367320  .....G.......G.........-...A.....G.A...-.............G......  2367263
CP017094      873916   .....G.......G......-......A.....G.A...-.............G......  873973
CP016855      510020   .....G.......G......-......A.....G.A...-.............G......  510077
CP016855      559503   .....G.......G......-......A.....G.A...-.............G......  559560
CP016855      2178633  .....G.......G.........-...A.....G.A...-.............G......  2178576
CP016855      2294921  .....G.......G.........-...A.....G.A...-.............G......  2294864
CP016855      554291   .....G.......G......-......A.....G.A...-.............G......  554348
CP016855      1957375  .....G.......G.........-...A.....G.A...-.............G......  1957318

Query_180747  588      TTAATGTGGGCCTCTCTAGGTTGGACTGGCGGGTGACAAACCGGAGGAAGGGGGGGATAA  647
CP002110      48052    ....-..T..G.A.....A....-....C.-...................T......G.  47996
CP002110      1203706  ....GT.....A-.....A....-....C.-...................T......G.  1203762
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CP002110      3989     ....-..T..G.A.....A....-....C.-...................T......G.  3933
CP002110      1088957  ....GT.....A-.....A....-....C.-...................T......G.  1089013
CP002110      1358375  ....GT.....A-.....A....-....C.-...................T......G.  1358431
CP002110      2801331  ....-..T..G.A.....A....-....C.-...................T......G.  2801275
CP012015      553006   ....GT.....A-.....A....-....C.-...................T......G.  553062
CP012015      597473   ....GT.....A-.....A....-....C.-...................T......G.  597529
CP012015      2268350  ....-..T..G.A.....A....-....C.-...................T......G.  2268294
CP012015      2389263  ....-..T..G.A.....A....-....C.-...................T......G.  2389207
CP012015      2089708  ....-..T..G.A.....A....-....C.-...................T......G.  2089652
LC107798      593      ....GT.....A-.....A....-....C.-...................T......G.  649
LC107791      593      ....GT.....A-.....A....-....C.-...................T......G.  649
LC107786      593      ....GT.....A-.....A....-....C.-...................T......G.  649
CP009681      2004219  ....-..T..G.A.....A....-....C.-...................T......G.  2004163
CP009681      2048504  ....-..T..G.A.....A....-....C.-...................T......G.  2048448
CP009681      2324998  ....-..T..G.A.....A....-....C.-...................T......G.  2324942
CP009681      2446127  ....-..T..G.A.....A....-....C.-...................T......G.  2446071
CP009681      505549   ....GT.....A-.....A....-....C.-...................T......G.  505605
FN393795      1133     ....GT.....A-.....A...T.....C.-...................T......G.  1190
CP012692      572352   ....GT.....A-.....A....-....C.-...................T......G.  572408
CP012692      2215074  ....-..T..G.A.....A....-....C.-...................T......G.  2215018
CP012692      2329860  ....-..T..G.A.....A....-....C.-...................T......G.  2329804
CP012692      527991   ....GT.....A-.....A....-....C.-...................T......G.  528047
CP012692      2069035  ....-..T..G.A.....A....-....C.-...................T......G.  2068979
CP017094      579739   ....GT.....A-.....A....-....C.-...................T......G.  579795
CP017094      696024   ....GT.....A-.....A....-....C.-...................T......G.  696080
CP017094      2317785  ....-..T..G.A.....A....-....C....-.................T......G.  2317729
CP017094      2322996  ....-..T..G.A.....A....-....C.-...................T......G.  2322940
CP017094      2367262  ....-..T..G.A.....A....-....C.-...................T......G.  2367206
CP017094      873974   ....GT.....A-.....A....-....C.-...................T......G.  874030
CP016855      510078   ....GT.....A-.....A....-....C.-...................T......G.  510134
CP016855      559561   ....GT.....A-.....A....-....C.-...................T......G.  559617
CP016855      2178575  ....-..T..G.A.....A....-....C.-...................T......G.  2178519
CP016855      2294863  ....-..T..G.A.....A....-....C.-...................T......G.  2294807
CP016855      554349   ....GT.....A-.....A....-....C.-...................T......G.  554405
CP016855      1957317  ....-..T..G.A.....A....-....C.-...................T......G.  1957261

Query_180747  648      CGTCAAATCATCATCGCCCCTTAATGATTTGGGCTACACACGTGCTACAATGGACAATAC  707
CP002110      47995    ..............-.......-.....................................  47938
CP002110      1203763  ..............-.......-.....................................  1203820
CP002110      3932     ..............-.......-.....................................  3875
CP002110      1089014  ..............-.......-.....................................  1089071

CP002110      1358432  ..............-.......-.....................................  1358489
CP002110      2801274  ..............-.......-.....................................  2801217
CP012015      553063   ..............-.......-.....................................  553120
CP012015      597530   ..............-.......-.....................................  597587
CP012015      2268293  ..............-.......-.....................................  2268236
CP012015      2389206  ..............-.......-.....................................  2389149
CP012015      2089651  ..............-.......-.....................................  2089594
LC107798      650      ..............-.......-.....................................  707
LC107791      650      ..............-.......-.....................................  707
LC107786      650      ..............-.......-.....................................  707
CP009681      2004162  ..............-.......-.....................................  2004105
CP009681      2048447  ..............-.......-.....................................  2048390
CP009681      2324941  ..............-.......-.....................................  2324884
CP009681      2446070  ..............-.......-.....................................  2446013
CP009681      505606   ..............-.......-.....................................  505663
FN393795      1191     ..............-.......-.....................................  1248
CP012692      572409   ..............-.......-.....................................  572466
CP012692      2215017  ..............-.......-.....................................  2214960
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CP012692      2329803  .............-.......-.....................................  2329746
CP012692      528048   .............-.......-.....................................  528105
CP012692      2068978  .............-.......-.....................................  2068921
CP017094      579796   .............-.......-.....................................  579853
CP017094      696081   .............-.......-.....................................  696138
CP017094      2317728  .............-.......-.....................................  2317671
CP017094      2322939  .............-.......-.....................................  2322882
CP017094      2367205  .............-.......-.....................................  2367148
CP017094      874031   .............-.......-.....................................  874088
CP016855      510135   .............-.......-.....................................  510192
CP016855      559618   .............-.......-.....................................  559675
CP016855      2178518  .............-.......-.....................................  2178461
CP016855      2294806  .............-.......-.....................................  2294749
CP016855      554406   .............-.......-.....................................  554463
CP016855      1957260  .............-.......-.....................................  1957203

Query_180747  708      AAAGGGCAGCTAAACCCGCGA-GTCATGCAAATCCCATAAAGTTGTTCTCAGTTCGGATT  766
CP002110      47937    ..........G.....-....G....A.................................  47879
CP002110      1203821  ..........G.....-....G....A.................................  1203879
CP002110      3874     ..........G.....-....G....A.................................  3816
CP002110      1089072  ..........G.....-....G....A.................................  1089130
CP002110      1358490  ..........G.....-....G....A.................................  1358548
CP002110      2801216  ..........G.....-....G....A.................................  2801158
CP012015      553121   ..........G.....-....G....A.................................  553179
CP012015      597588   ..........G.....-....G....A.................................  597646
CP012015      2268235  ..........G.....-....G....A.................................  2268177
CP012015      2389148  ..........G.....-....G....A.................................  2389090
CP012015      2089593  ..........G.....-.T..G....A.................................  2089535
LC107798      708      ..........G.....-....G....A.................................  766
LC107791      708      ..........G.....-....G....A.................................  766
LC107786      708      ..........G.....-....G....A.................................  766
CP009681      2004104  ..........G.....-....G....A.................................  2004046
CP009681      2048389  ..........G.....-....G....A.................................  2048331
CP009681      2324883  ..........G.....-....G....A.................................  2324825
CP009681      2446012  ..........G.....-....G....A.................................  2445954
CP009681      505664   ..........G.....-.T..G....A.................................  505722
FN393795      1249     ..........G.....-....G....A.................................  1307
CP012692      572467   ..........G.....-....G....A.................................  572525
CP012692      2214959  ..........G.....-....G....A.................................  2214901
CP012692      2329745  ..........G.....-....G....A.................................  2329687
CP012692      528106   ..........G.....-....G....A.................................  528164
CP012692      2068920  ..........G.....-.T..G....A.................................  2068862
CP017094      579854   ..........G.....-....G....A.................................  579912
CP017094      696139   ..........G.....-....G....A.................................  696197
CP017094      2317670  ..........G.....-....G....A.................................  2317612
CP017094      2322881  ..........G.....-....G....A.................................  2322823
CP017094      2367147  ..........G.....-....G....A.................................  2367089
CP017094      874089   ..........G.....-.T..G....A.................................  874147
CP016855      510193   ..........G.....-....G....A.................................  510251
CP016855      559676   ..........G.....-....G....A.................................  559734
CP016855      2178460  ..........G.....-....G....A.................................  2178402
CP016855      2294748  ..........G.....-....G....A.................................  2294690
CP016855      554464   ..........G.....-....G....A.................................  554522
CP016855      1957202  ..........G.....-.T..G....A.................................  1957144
Query_180747  767      G-AGTCTGCA-CTCGACTAC-TGAAGCTGGAATCGCTAGTATTCGTAGAT            813
CP002110      47878    .T........A.........A...................A........            47829
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CP002110	1203880	.T........A.........A...................A........	1203929
CP002110	3815	.T........A.........A...................A........	3766
CP002110	1089131	.T........A.........A...................A........	1089180
CP002110	1358549	.T........A.........A...................A........	1358598
CP002110	2801157	.T........A.........A...................A........	2801108
CP012015	553180	.T........A.........A...................A........	553229
CP012015	597647	.T........A.........A...................A........	597696
CP012015	2268176	.T........A.........A...................A........	2268127
CP012015	2389089	.T........A.........A...................A........	2389040
CP012015	2089534	.T........A.........A...................A........	2089485
LC107798	767	.T........A.........A...................A........	816
LC107791	767	.T........A.........A...................A........	816
LC107786	767	.T........A.........A...................A........	816
CP009681	2004045	.T........A.........A...................A........	2003996
CP009681	2048330	.T........A.........A...................A........	2048281
CP009681	2324824	.T........A.........A...................A........	2324775
CP009681	2445953	.T........A.........A...................A........	2445904
CP009681	505723	.T........A.........A...................A........	505772
FN393795	1308	.T........A.........A...................A........	1357
CP012692	572526	.T........A.........A...................A........	572575
CP012692	2214900	.T........A.........A...................A........	2214851
CP012692	2329686	.T........A.........A...................A........	2329637
CP012692	528165	.T........A.........A...................A........	528214
CP012692	2068861	.T........A.........A...................A........	2068812
CP017094	579913	.T........A.........A...................A........	579962
CP017094	696198	.T........A.........A...................A........	696247
CP017094	2317611	.T........A.........A...................A........	2317562
CP017094	2322822	.T........A.........A...................A........	2322773
CP017094	2367088	.T........A.........A...................A........	2367039
CP017094	874148	.T........A.........A...................A........	874197
CP016855	510252	.T........A.........A...................A........	510301
CP016855	559735	.T........A.........A...................A........	559784
CP016855	2178401	.T........A.........A...................A........	2178352
CP016855	2294689	.T........A.........A...................A........	2294640
CP016855	554523	.T........A.........A...................A........	554572
CP016855	1957143	.T........A.........A...................A........	1957094

Table 3: Alignment view of blast of *Staph aureus* isolate 3 against other identical *Staph aureus*

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Query_56641  7        TTACCGGGCG-AAAGCGCACGCAGGCGGTT--G-TAAGTC-GA--TG-AA-TCCC-CGGG  56
KX456108     531      ....T.....T..................T-.T......A..TG..A..-....-....  587
KX447584     567           .....T.......G..T........TCT-......T..TG..A..G...A-...C  619
KJ365315     460           .....T.......G..T........TCT-......T..TG..A..-G...A...C  512
CP016863     2321874       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2321822
CP016863     577453        .....T.......G..T........TTT-......T..TG..A..-G...A...C  577505
CP016863     693746        .....T.......G..T........TTT-......T..TG..A..-G...A...C  693798
CP016863     2316667       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2316615
CP016863     871640        .....T.......G..T........TTT-......T..TG..A..-G...A...C  871692
CP016863     2366149       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2366097
CP017094     2323566       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2323514
CP017094     579168        .....T.......G..T........TTT-......T..TG..A..-G...A...C  579220
CP017094     695453        .....T.......G..T........TTT-......T..TG..A..-G...A...C  695505
CP017094     2367833       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2367781
CP017094     2318356       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2318304
CP017094     873403        .....T.......G..T........TTT-......T..TG..A..-G...A...C  873455
CP016855     553778        .....T.......G..T........TTT-......T..TG..A..-G...A...C  553830
CP016855     509507        .....T.......G..T........TTT-......T..TG..A..-G...A...C  509559
CP016855     558990        .....T.......G..T........TTT-......T..TG..A..-G...A...C  559042
CP016855     2179146       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2179094
CP016855     2295434       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2295382
CP016855     1957888       .....T.......G..T........TTT-......T..TG..A..-G...A...C  1957836
CP016856     860641        .....T.......G..T........TTT-......T..TG..A..-G...A...C  860693
CP016856     2280860       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2280808
CP016856     563193        .....T.......G..T........TTT-......T..TG..A..-G...A...C  563245
CP016856     677969        .....T.......G..T........TTT-......T..TG..A..-G...A...C  678021
CP016856     2231284       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2231232
CP016856     2236616       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2236564
LN854556     427760        .....T.......G..T........TTT-......T..TG..A..-G...A...C  427812
LN854556     472480        .....T.......G..T........TTT-......T..TG..A..-G...A...C  472532
LN854556     2096102       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2096050
LN854556     2210953       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2210901
LN854556     1920910       .....T.......G..T........TTT-......T..TG..A..-G...A...C  1920858
CP005288     2384155       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2384103
CP005288     596407        .....T.......G..T........TTT-......T..TG..A..-G...A...C  596459
CP005288     2086093       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2086041
CP005288     2263211       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2263159
CP005288     552122        .....T.......G..T........TTT-......T..TG..A..-G...A...C  552174
CP003045     1840073       .....T.......G..T........TTT-......T..TG..A..-G...A...C  1840021
CP003045     449914        .....T.......G..T........TTT-......T..TG..A..-G...A...C  449966
CP003045     494267        .....T.......G..T........TTT-......T..TG..A..-G...A...C  494319
CP003045     499507        .....T.......G..T........TTT-......T..TG..A..-G...A...C  499559
CP003045     2024667       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2024615
CP003045     2151833       .....T.......G..T........TTT-......T..TG..A..-G...A...C  2151781

Query_56641  57       -TCAA-CTG-GGAA-TG-C-TTTGAAACTGGCAAGCTAGAGT-C-C-TAGA-GG-GGGTA  105
KX456108     588      C....C...-....C..-.A.....................-.T.G....G..-.....  643
KX447584     620      -....-.C.T....-G.T.A..G........GG.A..T....G.AG-A...-..A.A..G  674
KJ365315     513      -....-.C.T...G-G.T.A..G........G..A..T....G.AG-A...-..A.A..G  567
CP016863     2321821  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2321767
CP016863     577506   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  577560
CP016863     693799   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  693853
CP016863     2316614  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2316560
CP016863     871693   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  871747
CP016863     2366096  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2366042
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CP017094     2323513  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2323459

CP017094     579221   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  579275
CP017094     695506   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  695560
CP017094     2367780  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2367726
CP017094     2318303  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2318249
CP017094     873456   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  873510
CP016855     553831   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  553885
CP016855     509560   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  509614
CP016855     559043   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  559097
CP016855     2179093  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2179039
CP016855     2295381  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2295327
CP016855     1957835  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  1957781
CP016856     860694   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  860748
CP016856     2280807  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2280753
CP016856     563246   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  563300
CP016856     678022   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  678076
CP016856     2231231  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2231177
CP016856     2236563  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2236509
LN854556     427813   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  427867
LN854556     472533   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  472587
LN854556     2096049  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2095995
LN854556     2210900  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2210846
LN854556     1920857  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  1920803
CP005288     2384102  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2384048
CP005288     596460   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  596514
CP005288     2086040  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2085986
CP005288     2263158  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2263104
CP005288     552175   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  552229
CP003045     1840020  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  1839966
CP003045     449967   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  450021
CP003045     494320   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  494374
CP003045     499560   -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  499614
CP003045     2024614  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2024560
CP003045     2151780  -....-.C.T...G-G.T.A..G........A..A..T....G.AG-A...-..AAA..G  2151726

Query_56641  106      G-ATTCCAGGTG-AGCGGTGAAATGC-TAGAG-TCTGGAGGAATACCGGTGGCGAAGGCG  161
KX456108     644      .A..........T.............G.....A............................  703
KX447584     675      .A......T...T.............GC....A.A........C...A............  734
KJ365315     568      .A......T...T.............GC....A.A........C...A............  627
CP016863     2321766  .A......T...T.............GC....A.A........C...A............  2321707
CP016863     577561   .A......T...T.............GC....A.A........C...A............  577620
CP016863     693854   .A......T...T.............GC....A.A........C...A............  693913
CP016863     2316559  .A......T...T.............GC....A.A........C...A............  2316500
CP016863     871748   .A......T...T.............GC....A.A........C...A............  871807
CP016863     2366041  .A......T...T.............GC....A.A........C...A............  2365982
CP017094     2323458  .A......T...T.............GC....A.A........C...A............  2323399
CP017094     579276   .A......T...T.............GC....A.A........C...A............  579335
CP017094     695561   .A......T...T.............GC....A.A........C...A............  695620
CP017094     2367725  .A......T...T.............GC....A.A........C...A............  2367666
CP017094     2318248  .A......T...T.............GC....A.A........C...A............  2318189
CP017094     873511   .A......T...T.............GC....A.A........C...A..........G.  873570
CP016855     553886   .A......T...T.............GC....A.A........C...A............  553945
CP016855     509615   .A......T...T.............GC....A.A........C...A............  509674
CP016855     559098   .A......T...T.............GC....A.A........C...A............  559157
CP016855     2179038  .A......T...T.............GC....A.A........C...A............  2178979
CP016855     2295326  .A......T...T.............GC....A.A........C...A............  2295267
CP016855     1957780  .A......T...T.............GC....A.A........C...A............  1957721
CP016856     860749   .A......T...T.............GC....A.A........C...A............  860808
CP016856     2280752  .A......T...T.............GC....A.A........C...A............  2280693
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CP016856      563301   .A......T...T.............GC....A.A........C...A.............  563360
CP016856      678077   .A......T...T.............GC....A.A........C...A.............  678136
CP016856      2231176  .A......T...T.............GC....A.A........C...A.............  2231117
CP016856      2236508  .A......T...T.............GC....A.A........C...A.............  2236449
LN854556      427868   .A......T...T.............GC....A.A........C...A.............  427927

LN854556      472588   .A......T...T.............GC....A.A........C...A.............  472647
LN854556      2095994  .A......T...T.............GC....A.A........C...A.............  2095935
LN854556      2210845  .A......T...T.............GC....A.A........C...A.............  2210786
LN854556      1920802  .A......T...T.............GC....A.A........C...A.............  1920743
CP005288      2384047  .A......T...T.............GC....A.A........C...A.............  2383988
CP005288      596515   .A......T...T.............GC....A.A........C...A.............  596574
CP005288      2085985  .A......T...T.............GC....A.A........C...A.............  2085926
CP005288      2263103  .A......T...T.............GC....A.A........C...A.............  2263044
CP005288      552230   .A......T...T.............GC....A.A........C...A.............  552289
CP003045      1839965  .A......T...T.............GC....A.A........C...A.............  1839906
CP003045      450022   .A......T...T.............GC....A.A........C...A.............  450081
CP003045      494375   .A......T...T.............GC....A.A........C...A.............  494434
CP003045      499615   .A......T...T.............GC....A.A........C...A.............  499674
CP003045      2024559  .A......T...T.............GC....A.A........C...A.............  2024500
CP003045      2151725  .A......T...T.............GC....A.A........C...A.............  2151666

Query_56641   162      GCCCCCTGGAC-GAAGACTGACGCTCAGGTGCGAAAGC-TGGGGAGCAAACAGGAT-AG-  217
KX456108      704      ...........-.........................G................T..A  762
KX447584      735      ..T.T....T.T.T.-.........G.T..........G......T..........T..A  793
KJ365315      628      ..T.T....T.T.T.-.........G.T..........G......T..........T..A  686
CP016863      2321706  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2321648
CP016863      577621   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  577679
CP016863      693914   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  693972
CP016863      2316499  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2316441
CP016863      871808   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  871866
CP016863      2365981  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2365923
CP017094      2323398  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2323340
CP017094      579336   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  579394
CP017094      695621   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  695679
CP017094      2367665  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2367607
CP017094      2318188  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2318130
CP017094      873571   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  873629
CP016855      553946   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  554004
CP016855      509675   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  509733
CP016855      559158   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  559216
CP016855      2178978  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2178920
CP016855      2295266  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2295208
CP016855      1957720  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  1957662
CP016856      860809   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  860867
CP016856      2280692  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2280634
CP016856      563361   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  563419
CP016856      678137   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  678195
CP016856      2231116  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2231058
CP016856      2236448  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2236390
LN854556      427928   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  427986
LN854556      472648   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  472706
LN854556      2095934  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2095876
LN854556      2210785  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2210727
LN854556      1920742  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  1920684
CP005288      2383987  A.TT.....T.T.T.-.........G.T..........G......T..........T..A  2383929
CP005288      596575   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  596633
CP005288      2085925  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2085867
CP005288      2263043  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2262985
CP005288      552290   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  552348
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CP003045     1839905  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  1839847
CP003045     450082   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  450140
CP003045     494435   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  494493
CP003045     499675   A.TTT....T.T.T.-.........G.T..........G......T..........T..A  499733
CP003045     2024499  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2024441
CP003045     2151665  A.TTT....T.T.T.-.........G.T..........G......T..........T..A  2151607

Query_56641  218      TACCCTGGTAGTCCACGCTGTAAACGATG--T-C-GA-T-TT-GGAGG-TTGTGCCCTTG  269
KX456108     763      ............................--.-.-..-.-..-.....-...........  814

KX447584     794      ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  853
KJ365315     687      ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  746
CP016863     2321647  ..................C..........AG.G.TA.G.G..A..G..T..CC......-  2321589
CP016863     577680   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  577739
CP016863     693973   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  694032
CP016863     2316440  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2316381
CP016863     871867   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  871926
CP016863     2365922  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2365863
CP017094     2323339  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2323280
CP017094     579395   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  579454
CP017094     695680   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  695739
CP017094     2367606  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2367547
CP017094     2318129  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2318070
CP017094     873630   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  873689
CP016855     554005   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  554064
CP016855     509734   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  509793
CP016855     559217   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  559276
CP016855     2178919  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2178860
CP016855     2295207  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2295148
CP016855     1957661  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  1957602
CP016856     860868   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  860927
CP016856     2280633  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2280574
CP016856     563420   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  563479
CP016856     678196   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  678255
CP016856     2231057  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2230998
CP016856     2236389  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2236330
LN854556     427987   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  428046
LN854556     472707   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  472766
LN854556     2095875  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2095816
LN854556     2210726  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2210667
LN854556     1920683  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  1920624
CP005288     2383928  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2383869
CP005288     596634   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  596693
CP005288     2085866  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2085807
CP005288     2262984  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2262925
CP005288     552349   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  552408
CP003045     1839846  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  1839787
CP003045     450141   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  450200
CP003045     494494   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  494553
CP003045     499734   ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  499793
CP003045     2024440  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2024381
CP003045     2151606  ..................C..........AG.G.TA.G.G..A..G..T..CC....C.T  2151547

Query_56641  270      AGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGA-CCGCCTGGGGAGTACG-G-CGCAAG  326
KX456108     815      ................................-................-.C......  872
KX447584     854      .---..-..-.--.C.........A....-G.ACT................AC-......  904
KJ365315     747      .---..-..-.--.C.........A....-G.ACT................AC-......  797
CP016863     2321588  .---..-..-.--.C.........A....-G.ACT................AC-......  2321538
CP016863     577740   .---..-..-.--.C.........A....-G.ACT................AC-......  577790
CP016863     694033   .---..-..-.--.C.........A....-G.ACT................AC-......  694083
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CP016863	2316380	.---..-..-.--.C.........A....-G.ACT..............AC-......	2316330
CP016863	871927	.---..-..-.--.C.........A....-G.ACT..............AC-......	871977
CP016863	2365862	.---..-..-.--.C.........A....-G.ACT..............AC-......	2365812
CP017094	2323279	.---..-..-.--.C.........A....-G.ACT..............AC-......	2323229
CP017094	579455	.---..-..-.--.C.........A....-G.ACT..............AC-......	579505
CP017094	695740	.---..-..-.--.C.........A....-G.ACT..............AC-......	695790
CP017094	2367546	.---..-..-.--.C.........A....-G.ACT..............AC-......	2367496
CP017094	2318069	.---..-..-.--.C.........A....-G.ACT..............AC-......	2318019
CP017094	873690	.---..-..-.--.C.........A....-G.ACT..............AC-......	873740
CP016855	554065	.---..-..-.--.C.........A....-G.ACT..............AC-......	554115
CP016855	509794	.---..-..-.--.C.........A....-G.ACT..............AC-......	509844
CP016855	559277	.---..-..-.--.C.........A....-G.ACT..............AC-......	559327
CP016855	2178859	.---..-..-.--.C.........A....-G.ACT..............AC-......	2178809
CP016855	2295147	.---..-..-.--.C.........A....-G.ACT..............AC-......	2295097
CP016855	1957601	.---..-..-.--.C.........A....-G.ACT..............AC-......	1957551
CP016856	860928	.---..-..-.--.C.........A....-G.ACT..............AC-......	860978
CP016856	2280573	.---..-..-.--.C.........A....-G.ACT..............AC-......	2280523
CP016856	563480	.---..-..-.--.C.........A....-G.ACT..............AC-......	563530
CP016856	678256	.---..-..-.--.C.........A....-G.ACT..............AC-......	678306
CP016856	2230997	.---..-..-.--.C.........A....-G.ACT..............AC-......	2230947
CP016856	2236329	.---..-..-.--.C.........A....-G.ACT..............AC-......	2236279
LN854556	428047	.---..-..-.--.C.........A....-G.ACT..............AC-......	428097
LN854556	472767	.---..-..-.--.C.........A....-G.ACT..............AC-......	472817
LN854556	2095815	.---..-..-.--.C.........A....-G.ACT..............AC-......	2095765
LN854556	2210666	.---..-..-.--.C.........A....-G.ACT..............AC-......	2210616
LN854556	1920623	.---..-..-.--.C.........A....-G.ACT..............AC-......	1920573
CP005288	2383868	.---..-..-.--.C.........A....-G.ACT..............AC-......	2383818
CP005288	596694	.---..-..-.--.C.........A....-G.ACT..............AC-......	596744
CP005288	2085806	.---..-..-.--.C.........A....-G.ACT..............AC-......	2085756
CP005288	2262924	.---..-..-.--.C.........A....-G.ACT..............AC-......	2262874
CP005288	552409	.---..-..-.--.C.........A....-G.ACT..............AC-......	552459
CP003045	1839786	.---..-..-.--.C.........A....-G.ACT..............AC-......	1839736
CP003045	450201	.---..-..-.--.C.........A....-G.ACT..............AC-......	450251
CP003045	494554	.---..-..-.--.C.........A....-G.ACT..............AC-......	494604
CP003045	499794	.---..-..-.--.C.........A....-G.ACT..............AC-......	499844
CP003045	2024380	.---..-..-.--.C.........A....-G.ACT..............AC-......	2024330
CP003045	2151546	.---..-..-.--.C.........A....-G.ACT..............AC-......	2151496
Query_56641	327	GTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTC	386
KX456108	873	.....	932
KX447584	905	...G.........G............A.....	964
KJ365315	798	...G.........G............A.....	857
CP016863	2321537	...G.........G............A.....	2321478
CP016863	577791	...G.........G............A.....	577850
CP016863	694084	...G.........G............A.....	694143
CP016863	2316329	...G.........G............A.....	2316270
CP016863	871978	...G.........G............A.....	872037
CP016863	2365811	...G.........G...........-A.....	2365753
CP017094	2323228	...G.........G............A.....	2323169
CP017094	579506	...G.........G............A.....	579565
CP017094	695791	...G.........G............A.....	695850
CP017094	2367495	...G.........G............A.....	2367436
CP017094	2318018	...G.........G............A.....	2317959
CP017094	873741	...G.........G............A.....	873800
CP016855	554116	............G............A.....	554175
CP016855	509845	...G.........G............A.....	509904
CP016855	559328	...G.........G............A.....	559387
CP016855	2178808	...G.........G............A.....	2178749
CP016855	2295096	...G.........G............A.....	2295037

CP016855	1957550	...G.....G.....A.....	1957491
CP016856	860979	...G.....G.....A.....	861038
CP016856	2280522	...G.....G.....A.....	2280463
CP016856	563531	...G.....G.....A.....	563590
CP016856	678307	...G.....G.....A.....	678366
CP016856	2230946	...G.....G.....A.....	2230887
CP016856	2236278	...G.....G.....A.....	2236219
LN854556	428098	...G.....G.....A.....	428157
LN854556	472818	...G.....G.....A.....	472877
LN854556	2095764	...G.....G.....A.....	2095705
LN854556	2210615	...G.....G.....A.....	2210556
LN854556	1920572	...G.....G.....A.....	1920513
CP005288	2383817	...G.....G.....A.....	2383758
CP005288	596745	...G.....G.....A.....	596804
CP005288	2085755	...G.....G.....A.....	2085696
CP005288	2262873	...G.....G.....A.....	2262814
CP005288	552460	...G.....G.....A.....	552519

Table 4: Alignment view of blast of *Staph aureus* isolate 4 against other identical *Staph aureus*

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Query_119755  11       TATTCGGGTCGTCAAGGCGTCGCGTCAGGCTGTCTTCTTTCAGGTCCAGAGGTGCAAAGG  70
JF431908      509      ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  557
JX987337      16       ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  64
CP012012      2084260  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2084212
CP012012      2377756  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2377708
CP012012      552663   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  552711
CP012012      596951   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  596999
CP012119      1365825  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  1365777
CP012119      1371037  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  1370989
CP012119      1415316  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  1415268
CP012119      2617757  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2617805
CP012119      2501450  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2501498
CP012119      2839211  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2839259
JN315154      430      ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  478
CP017094      2323570  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2323522
CP017094      2318360  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2318312
CP017094      2367837  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2367789
CP017094      579164   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  579212
CP017094      695449   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  695497
CP016863      2321878  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2321830
CP016863      2316671  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2316623
CP016863      2366153  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2366105
CP016863      577449   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  577497
CP016863      693742   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  693790
CP016863      871636   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  871684
CP007539      2129020  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2128972
CP007539      2245506  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  2245458
CP007539      1993886  ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  1993838
CP007539      577998   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  578046
CP007539      529158   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  529206
CP007539      572788   ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  572836
EU604544      515      ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  563
KT695872      552      ....-...-...-..A...-.....-....G..-..-...-.A..-.T..T...-...-.  600

Query_119755  71       CCTCACGGCTCAACCGTGGTAGGTCCTTTGAAACTCGTGAGGGCTCTGAGTGCCGGAAAG  130
JF431908      558      ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  611
JX987337      65       ..C.............AG....A..G.....-.-..AAA..-.....-.A.-...  119
CP012012      2084211  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2084158
CP012012      2377707  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2377654
CP012012      552712   ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  552765
CP012012      597000   ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  597053
CP012119      1365776  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  1365723
CP012119      1370988  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  1370935
CP012119      1415267  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  1415214
CP012119      2617806  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2617859
CP012119      2501499  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2501552
CP012119      2839260  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2839313
JN315154      479      ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  532
CP017094      2323521  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2323468
CP017094      2318311  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2318258
CP017094      2367788  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2367735
CP017094      579213   ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  579266
CP017094      695498   ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  695551
CP016863      2321829  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2321776
CP016863      2316622  ..-..............AG....A..G.....-.-..AAA..-.....-.A.-...  2316569
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CP016863      2366104  ..-................AG....A..G......-.-..AAA..-......-.A.-...  2366051

CP016863      577498   ..-................AG....A..G......-.-..AAA..-......-.A.-...  577551
CP016863      693791   ..-................AG....A..G......-.-..AAA..-......-.A.-...  693844
CP016863      871685   ..-................AG....A..G......-.-..AAA..-......-.A.-...  871738
CP007539      2128971  ..-................AG....A..G......-.-..AAA..-......-.A.-...  2128918
CP007539      2245457  ..-................AG....A..G......-.-..AAA..-......-.A.-...  2245404
CP007539      1993837  ..-................AG....A..G......-.-..AAA..-......-.A.-...  1993784
CP007539      578047   ..-................AG....A..G......-.-..AAA..-......-.A.-...  578100
CP007539      529207   ..-................AG....A..G......-.-..AAA..-......-.A.-...  529260
CP007539      572837   ..-................AG....A..G......-.-..AAA..-......-.A.-...  572890
EU604544      564      ..-................AG....A..G......-G..AAA..-......-.A.-...  618
KT695872      601      ..-................AG....A..G......-.-..AAA..-......-.A.-...  654

Query_119755  131      A-GAAAGTGGAATTTCCCA-GGGTAGCGGTGAAATGC-CTAAAATTA-GGAGGAACACCA  186
JF431908      612      .G.........-....AT-.T.............-GC.G.GA..T...........  668
JX987337      120      .G.........-......T.T.............-GC.G.GA..T...........  177
CP012012      2084157  .G.........-....AT-.T.............G.AG.G..AT-...........  2084101
CP012012      2377653  .G.........-....AT-.T.............G.AG.G..AT-...........  2377597
CP012012      552766   .G.........-....AT-.T.............-GC.G.GA..T...........  552822
CP012012      597054   .G.........-....AT-.T.............-GC.G.GA..T...........  597110
CP012119      1365722  .G.........-....AT-.T.............G.AG.G..AT-...........  1365666
CP012119      1370934  .G.........-....AT-.T.............G.AG.G..AT-...........  1370878
CP012119      1415213  .G.........-....AT-.T.............G.AG.G..AT-...........  1415157
CP012119      2617860  .G.........-....AT-.T.............-GC.G.GA..T...........  2617916
CP012119      2501553  .G.........-....AT-.T.............-GC.G.GA..T...........  2501609
CP012119      2839314  .G.........-....AT-.T.............-GC.G.GA..T...........  2839370
JN315154      533      .G.............AT-.T.............-GC.G.GA..T...........  590
CP017094      2323467  .G.........-....AT-.T.............G.AG.G..AT-...........  2323411
CP017094      2318257  .G.........-....AT-.T.............G.AG.G..AT-...........  2318201
CP017094      2367734  .G.........-....AT-.T.............G.AG.G..AT-...........  2367678
CP017094      579267   .G.........-....AT-.T.............-GC.G.GA..T...........  579323
CP017094      695552   .G.........-....AT-.T.............-GC.G.GA..T...........  695608
CP016863      2321775  .G.........-....AT-.T.............G.AG.G..AT-...........  2321719
CP016863      2316568  .G.........-....AT-.T.............G.AG.G..AT-...........  2316512
CP016863      2366050  .G.........-....AT-.T.............G.AG.G..AT-...........  2365994
CP016863      577552   .G.........-....AT-.T.............-GC.G.GA..T...........  577608
CP016863      693845   .G.........-....AT-.T.............-GC.G.GA..T...........  693901
CP016863      871739   .G.........-....AT-.T.............-GC.G.GA..T...........  871795
CP007539      2128917  .G.........-....AT-.T.............G.AG.G..AT-...........  2128861
CP007539      2245403  .G.........-....AT-.T.............G.AG.G..AT-...........  2245347
CP007539      1993783  .G.........-....AT-.T.............G.AG.G..AT-...........  1993727
CP007539      578101   .G.........-....AT-.T.............-GC.G.GA..T...........  578157
CP007539      529261   .G.........-....AT-.T.............-GC.G.GA..T...........  529317
CP007539      572891   .G.........-....AT-.T.............-GC.G.GA..T...........  572947
EU604544      619      .G.........-....AT-.T.............-GC.G.GA..T...........  675
KT695872      655      .G.........-....AT-.T.............-GC.G.GA..T...........  711

Query_119755  187      GTGGCGAAGGCGACTTTTTGGACCGGAACTGAAGCCGAAGCACGAAAGCGGGGGGAGCGA  246
JF431908      669      ................C...T.T.T......C..T..T.TG........T.....T.A.  728
JX987337      178      ................C...T.T.T......C..T..T.TG........T.....T.A.  237
CP012012      2084100  ................C...T.T.T......C..T..T.TG........T.....T.A.  2084041
CP012012      2377596  ................C...T.T.T......C..T..T.TG........T.....T.A.  2377537
CP012012      552823   ................C...T.T.T......C..T..T.TG........T.....T.A.  552882
CP012012      597111   ................C...T.T.T......C..T..T.TG........T.....T.A.  597170
CP012119      1365665  ................C...T.T.T......C..T..T.TG........T.....T.A.  1365606
CP012119      1370877  ................C...T.T.T......C..T..T.TG........T.....T.A.  1370818
CP012119      1415156  ................C...T.T.T......C..T..T.TG........T.....T.A.  1415097
CP012119      2617917  ................C...T.T.T......C..T..T.TG........T.....T.A.  2617976
CP012119      2501610  ................C...T.T.T......C..T..T.TG........T.....T.A.  2501669
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CP012119      2839371  .................C...T.T.T......C..T..T.TG........T.....T.A.  2839430
JN315154      591      .................C...T.T.T......C..T..T.TG........T.....T.A.  650
CP017094      2323410  .................C...T.T.T......C..T..T.TG........T.....T.A.  2323351
CP017094      2318200  .................C...T.T.T......C..T..T.TG........T.....T.A.  2318141
CP017094      2367677  .................C...T.T.T......C..T..T.TG........T.....T.A.  2367618

CP017094      579324   .................C...T.T.T......C..T..T.TG........T.....T.A.  579383
CP017094      695609   .................C...T.T.T......C..T..T.TG........T.....T.A.  695668
CP016863      2321718  .................C...T.T.T......C..T..T.TG........T.....T.A.  2321659
CP016863      2316511  .................C...T.T.T......C..T..T.TG........T.....T.A.  2316452
CP016863      2365993  .................C...T.T.T......C..T..T.TG........T.....T.A.  2365934
CP016863      577609   .................C...T.T.T......C..T..T.TG........T.....T.A.  577668
CP016863      693902   .................C...T.T.T......C..T..T.TG........T.....T.A.  693961
CP016863      871796   .................C...T.T.T......C..T..T.TG........T.....T.A.  871855
CP007539      2128860  .................C...T.T.T......C..T..T.TG........T.....T.A.  2128801
CP007539      2245346  .................C...T.T.T......C..T..T.TG........T.....T.A.  2245287
CP007539      1993726  .................C...T.T.T......C..T..T.TG........T.....T.A.  1993667
CP007539      578158   .................C...T.T.T......C..T..T.TG........T.....T.A.  578217
CP007539      529318   .................C...T.T.T......C..T..T.TG........T.....T.A.  529377
CP007539      572948   .................C...T.T.T......C..T..T.TG........T.....T.A.  573007
EU604544      676      .................C...T.Y.T......C..T..T.TG........T.....T.A.  735
KT695872      712      .................C...T.T.T......C..T..T.TG........T.....T.A.  771

Query_119755  247      ACAGGGTTAAAAACCCCGGGAGGCCC-CGCCGGAAACGAAGAAGGCCAAGGGGTGGGGGG  305
JF431908      729      .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  787
JX987337      238      .....A...G.T....T..T..T...A.....T......T..GT..T...T.T.A.....  297
CP012012      2084040  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2083982
CP012012      2377536  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2377478
CP012012      552883   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  552941
CP012012      597171   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  597229
CP012119      1365605  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  1365547
CP012119      1370817  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  1370759
CP012119      1415096  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  1415038
CP012119      2617977  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2618035
CP012119      2501670  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2501728
CP012119      2839431  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2839489
JN315154      651      .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  709
CP017094      2323350  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2323292
CP017094      2318140  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2318082
CP017094      2367617  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2367559
CP017094      579384   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  579442
CP017094      695669   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  695727
CP016863      2321658  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2321600
CP016863      2316451  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2316393
CP016863      2365933  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2365875
CP016863      577669   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  577727
CP016863      693962   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  694020
CP016863      871856   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  871914
CP007539      2128800  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2128742
CP007539      2245286  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  2245228
CP007539      1993666  .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  1993608
CP007539      578218   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  578276
CP007539      529378   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  529436
CP007539      573008   .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  573066
EU604544      736      .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  794
KT695872      772      .....A...G.T....T..T..T..A-.....T......T..GT..T...T.T.A.....  830

Query_119755  306      ----CGAACCTCGGCGCCGAAT-TAACGCAATAAGCACTCCGCCCGGCGAGGACGGACC-  359
JF431908      788      TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  846
JX987337      298      TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  356
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CP012012      2083981  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2083923
CP012012      2377477  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2377419
CP012012      552942   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  553000
CP012012      597230   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  597288
CP012119      1365546  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  1365488
CP012119      1370758  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  1370700
CP012119      1415037  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  1414979
CP012119      2618036  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2618094
CP012119      2501729  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2501787

CP012119      2839490  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2839548
JN315154      710      TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  768
CP017094      2323291  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2323233
CP017094      2318081  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2318023
CP017094      2367558  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2367500
CP017094      579443   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  579501
CP017094      695728   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  695786
CP016863      2321599  TTT-.CGC...TA.T..T.C.GC.......T.............T..G...T...-...G  2321542
CP016863      2316392  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2316334
CP016863      2365874  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2365816
CP016863      577728   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  577786
CP016863      694021   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  694079
CP016863      871915   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  871973
CP007539      2128741  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2128683
CP007539      2245227  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  2245169
CP007539      1993607  TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  1993549
CP007539      578277   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  578335
CP007539      529437   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  529495
CP007539      573067   TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  573125
EU604544      795      TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  853
KT695872      831      TTTC..CC...TA.T..T.C.GC.......T.............T..G...T...-...G  889

Query_119755  360      CAAGG-TGAAACTAAAAGGAA-T-GACGGGGACCCGCCCAAGCCGCGGAGCGGG-GGTTT  415
JF431908      847      .....T.......C.......T.-.............A.....G.T.....AT.T.....  905
JX987337      357      .....T.......C.......T.-.............A.....G.T.....AT.T.....  415
CP012012      2083922  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2083864
CP012012      2377418  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2377360
CP012012      553001   .....T.......C.......T.-.............A.....G.T.....AT.T.....  553059
CP012012      597289   .....T.......C.......T.-.............A.....G.T.....AT.T.....  597347
CP012119      1365487  .....T.......C.......-.T.............A.....G.T.....AT.T.....  1365429
CP012119      1370699  .....T.......C.......-.T.............A.....G.T.....AT.T.....  1370641
CP012119      1414978  .....T.......C.......-.T.............A.....G.T.....AT.T.....  1414920
CP012119      2618095  .....T.......C.......T.-.............A.....G.T.....AT.T.....  2618153
CP012119      2501788  .....T.......C.......T.-.............A.....G.T.....AT.T.....  2501846
CP012119      2839549  .....T.......C.......T.-.............A.....G.T.....AT.T.....  2839607
JN315154      769      .....T.......C.......T.-.............A.....G.T.....AT.T.....  827
CP017094      2323232  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2323174
CP017094      2318022  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2317964
CP017094      2367499  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2367441
CP017094      579502   .....T.......C.......T.-.............A.....G.T.....AT.T.....  579560
CP017094      695787   .....T.......C.......T.-.............A.....G.T.....AT.T.....  695845
CP016863      2321541  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2321483
CP016863      2316333  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2316275
CP016863      2365815  .....T.......C.......-.T...-.........A.....G.T.....AT.T.....  2365758
CP016863      577787   .....T.......C.......T.-.............A.....G.T.....AT.T.....  577845
CP016863      694080   .....T.......C.......T.-.............A.....G.T.....AT.T.....  694138
CP016863      871974   .....T.......C.......T.-.............A.....G.T.....AT.T.....  872032
CP007539      2128682  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2128624
CP007539      2245168  .....T.......C.......-.T.............A.....G.T.....AT.T.....  2245110
CP007539      1993548  .....T.......C.......-.T.............A.....G.T.....AT.T.....  1993490
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CP007539      578336   .....T.......C.......T.-.............A.....G.T.....AT.T.....  578394
CP007539      529496   .....T.......C.......T.-.............A.....G.T.....AT.T.....  529554
CP007539      573126   .....T.......C........-.T...-.........A.....G.T.....AT.T.....  573183
EU604544      854      .....T.......C.......T.-.............A.....G.T.....AT.T.....  912
KT695872      890      .....T.......C.......T.-.............A.....G.T.....AT.K.....  948

Query_119755  416      AATTCAAAGCAACGCGAAGAACCT-ACCTGGACTTGACATCCCTTGACCGATCTA-ACC-  472
JF431908      906      .....G..................T...AAAT..........T.......C....G.GAT  965
JX987337      416      .....G..................T...AAAT..........T.....AAC....G.GAT  475
CP012012      2083863  .....G..................T...AAAT..........T.....AAC....G.GAT  2083804
CP012012      2377359  .....G..................T...AAAT..........T.....AAC....G.GAT  2377300
CP012012      553060   .....G..................T...AAAT..........T.....AAC....G.GAT  553119
CP012012      597348   .....G..................T...AAAT..........T.....AAC....G.GAT  597407

CP012119      1365428  .....G..................T...AAAT..........T.....AAC....G.GAT  1365369
CP012119      1370640  .....G..................T...AAAT..........T.....AAC....G.GAT  1370581
CP012119      1414919  .....G..................T...AAAT..........T.....AAC....G.GAT  1414860
CP012119      2618154  .....G..................T...AAAT..........T.....AA.....G.GAT  2618213
CP012119      2501847  .....G..................T...AAAT..........T.....AAC....G.GAT  2501906
CP012119      2839608  .....G..................T...AAAT..........T.....AAC....G.GAT  2839667
JN315154      828      .....G..................T...AAAT..........T.....AAC....G.GAT  887
CP017094      2323173  .....G..................T...AAAT..........T.....AAC....G.GAT  2323114
CP017094      2317963  .....G..................T...AAAT..........T.....AAC....G.GAT  2317904
CP017094      2367440  .....G..................T...AAAT..........T.....AAC....G.GAT  2367381
CP017094      579561   .....G..................T...AAAT..........T.....AAC....G.GAT  579620
CP017094      695846   .....G..................T...AAAT..........T.....AAC....G.GAT  695905
CP016863      2321482  .....G..................T...AAAT..........T.....AAC....G.GAT  2321423
CP016863      2316274  .....G..................T...AAAT..........T.....AAC....G.GAT  2316215
CP016863      2365757  .....G..................T...AAAT..........T.....AAC....G.GAT  2365698
CP016863      577846   .....G..................T...AAAT..........T.....AAC....G.GAT  577905
CP016863      694139   .....G..................T...AAAT..........T.....AAC....G.GAT  694198
CP016863      872033   .....G..................T...AAAT..........T.....AAC....G.GAT  872092
CP007539      2128623  .....G..................T...AAAT..........T.....AAC....G.GAT  2128564
CP007539      2245109  .....G..................T...AAAT..........T.....AAC....G.GAT  2245050
CP007539      1993489  .....G..................T...AAAT..........T.....AAC....G.GAT  1993430
CP007539      578395   .....G..................T...AAAT..........T.....AAC....G.GAT  578454
CP007539      529555   .....G..................T...AAAT..........T.....AAC....G.GAT  529614
CP007539      573184   .....G..................T...AAAT..........T.....AAC....G.GAT  573243
EU604544      913      .....G..................T...AAAT..........T.....AAC....G.GAT  972
KT695872      949      .....G..................T...AAAT..........T.....AAC....G.GAT  1008

Query_119755  473      -GA-TCCT-CCC-TTCCT--G--ACAGAGAAAACAGGAG-TGCATGGTGG-C-TCAGCTC  521
JF431908      966      A..G..T.-...C...GG--.GG...A..TG.-....T.G........T.T.G.......  1021
JX987337      476      A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  531
CP012012      2083803  A..-G...T...C...GGGG.--...A..T.G.....T.G........T.T.G.......  2083747
CP012012      2377299  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  2377244
CP012012      553120   A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  553175
CP012012      597408   A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  597463
CP012119      1365368  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  1365313
CP012119      1370580  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  1370525
CP012119      1414859  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  1414804
CP012119      2618214  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  2618269
CP012119      2501907  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  2501962
CP012119      2839668  A..-G.T.T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  2839723
JN315154      888      A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  943
CP017094      2323113  A..-G...T...C...GG--.G-...A..TG.-....T.G........T.T.G.......  2323059
CP017094      2317903  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  2317848
CP017094      2367380  A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  2367325
CP017094      579621   A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  579676
CP017094      695906   A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......  695961
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CP016863	2321422	A..-G...T...C...GG--.G-...A..TG.-....T.G........T.T.G.......	2321368
CP016863	2316214	A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	2316159
CP016863	2365697	A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	2365642
CP016863	577906	A..-G...T...C...GG--.G-...A..TG.-....T.G........T.T.G.......	577960
CP016863	694199	A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	694254
CP016863	872093	A..-G.T.T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	872148
CP007539	2128563	A..-G...T...C...GG--.G-...A..TG.-....T.G........T.T.G.......	2128509
CP007539	2245049	A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	2244994
CP007539	1993429	A..-G.T.T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	1993374
CP007539	578455	A..-G...T...C...GG--.G-...A..TG.-....T.G........T.T.G.......	578509
CP007539	529615	A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	529670
CP007539	573244	A..-G...T...C...GG--.G-...A..TG.-....T.G........T.T.G.......	573298
EU604544	973	A..-G...T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	1028
KT695872	1009	A..-G.Y.T...C...GG--.GG...A..TG.-....T.G........T.T.G.......	1064

Table 5: Alignment view of blast of *Staph aureus* isolate 6 against other identical *Staph aureus*

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Query_79091  16       GGGCGCAAAGGGCGCGCAGGCGGTT-T-T-T-AAGTCTCGA--TGAAAGCCCACGG-TCA  68
LC121094     442      .....T....C.....T.........-.-.-T.......-..TG.............C...  497
CP017094     2318356  .....T....C.....T.........-.-.-T.......-..TG.............C...  2318301
CP017094     2323566  .....T....C.....T.........-.-.-T.......-..TG.............C...  2323511
CP017094     2367833  .....T....C.....T.........-.-.-T.......-..TG.............C...  2367778
CP017094     579168   .....T....C.....T.........-.-.-T.......-..TG.............C...  579223
CP017094     695453   .....T....C.....T.........-.-.-T.......-..TG.............C...  695508
CP017094     873403   .....T....C.....T.........-.-.-T.......-..TG.............C...  873458
CP014064     1291567  .....T....C.....T.........-.-.-T.......-..TG.............C...  1291512
CP014064     1406131  .....T....C.....T.........-.-.-T.......-..TG.............C...  1406076
CP014064     1118343  .....T....C.....T.........-.-.-T.......-..TG.............C...  1118288
CP014064     2451097  .....T....C.....T.........-.-.-T.......-..TG.............C...  2451152
CP014064     2495375  .....T....C.....T.........-.-.-T.......-..TG.............C...  2495430
CP014064     2500587  .....T....C.....T.........-.-.-T.......-..TG.............C...  2500642
KR265361     491      .....T....C.....T.........-.-.-T.......-..TG.............C...  546
CP007672     2149565  .....T....C.....T.........-.-.-T.......-..TG.............C...  2149510
CP007672     2265875  .....T....C.....T.........-.-.-T.......-..TG.............C...  2265820
CP007672     1971566  .....T....C.....T.........-.-.-T.......-..TG.............C...  1971511
CP007672     552607   .....T....C.....T.........-.-.-T.......-..TG.............C...  552662
CP007672     503115   .....T....C.....T.........-.-.-T.......-..TG.............C...  503170
CP007672     547395   .....T....C.....T.........-.-.-T.......-..TG.............C...  547450
KJ365315     460      .....T....C.....T.........-.C.-.-.......-..TG.............C...  515
KF983325     548      .....T....C.....T.........-.-.-T.......-..TG.............C...  603
CP002110     48622    .....T....C.....T.........-.-.-.-.......-..TG.............C...  48568
CP002110     4560     .....T....C.....T.........-.-.-T.......-..TG.............C...  4505
CP002110     2801902  .....T....C.....T.........-.-.-T.......-..TG.............C...  2801847
CP002110     1203136  .....T....C.....T.........-.-.-.-.......-..TG.............C...  1203190
CP002110     1088386  .....T....C.....T.........-.-.-T.......-..TG.............C...  1088441
CP002110     1357804  .....T....C.....T........C.-.-.-.......-..TG.............C...  1357859
AB599719     265      .....T....C.....T.........-.C.-.-.......-..TG.............C...  320
KX373891     540      .....T....C.....T.........-.-.C.-.......-..T-.............C...  594

Query_79091  69       ACCGGGGA-GGTC-TTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCA  126
LC121094     498      ....T...G....A...........AA...................A............  557
CP017094     2318300  ....T...G....A...........AA...................A............  2318241
CP017094     2323510  ....T...G....A...........AA...................A............  2323451
CP017094     2367777  ....T...G....A...........AA...................A............  2367718
CP017094     579224   ....T...G....A...........AA...................A............  579283
CP017094     695509   ....T...G....A...........AA...................A............  695568
CP017094     873459   ....T...G....A...........AA...................A............  873518
CP014064     1291511  ....T...G....A...........AA...................A............  1291452
CP014064     1406075  ....T...G....A...........AA...................A............  1406016
CP014064     1118287  ....T...G....A...........AA...................A............  1118228
CP014064     2451153  ....T...G....A...........AA...................A............  2451212
CP014064     2495431  ....T...G....A...........AA...................A............  2495490
CP014064     2500643  ....T...G....A...........AA...................A............  2500702
KR265361     547      ....T...G....A...........AA...................A............  606
CP007672     2149509  ....T...G....A...........AA...................A............  2149450
CP007672     2265819  ....T...G....A...........AA...................A............  2265760
CP007672     1971510  ....T...G....A...........AA...................A............  1971451
CP007672     552663   ....T...G....A...........AA...................A............  552722
CP007672     503171   ....T...G....A...........AA...................A............  503230
CP007672     547451   ....T...G....A...........AA...................A............  547510
KJ365315     516      ....T...G....A...........A.....................................  575
KF983325     604      ....T...G....A...........AA...................A............  663
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CP002110     48567    ....T...G....A..........AA..................A.....  48508

CP002110     4504     ....T...G....A..........AA..................A.....  4445
CP002110     2801846  ....T...G....A..........AA..................A.....  2801787
CP002110     1203191  ....T...G....A..........AA..................A.....  1203250
CP002110     1088442  ....T...G....A..........AA..................A.....  1088501
CP002110     1357860  ....T...G....A..........AA..................A.....  1357919
AB599719     321      ....T...G....A...........A..................A.....  380
KX373891     595      ....T...G....A..........AA..................A.....  654

Query_79091  127      TCGTGTAGCGGTGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGGCGACTCTCT  185
LC121094     558      .-....................A.............................T...  616
CP017094     2318240  .-....................A.............................T...  2318182
CP017094     2323450  .-....................A.............................T...  2323392
CP017094     2367717  .-....................A.............................T...  2367659
CP017094     579284   .-....................A.............................T...  579342
CP017094     695569   .-....................A.............................T...  695627
CP017094     873519   .-....................A.........................G....T...  873577
CP014064     1291451  .-....................A.............................T...  1291393
CP014064     1406015  .-....................A.............................T...  1405957
CP014064     1118227  .-....................A.............................T...  1118169
CP014064     2451213  .-....................A.............................T...  2451271
CP014064     2495491  .-....................A.............................T...  2495549
CP014064     2500703  .-....................A.............................T...  2500761
KR265361     607      .-....................A.............................T...  665
CP007672     2149449  .-....................A.............................T...  2149391
CP007672     2265759  .-....................A.............................T...  2265701
CP007672     1971450  .-....................A.............................T...  1971392
CP007672     552723   .-....................A.............................T...  552781
CP007672     503231   .-....................A.............................T...  503289
CP007672     547511   .-....................A.............................T...  547569
KJ365315     576      .-....................A...........................G.....  634
KF983325     664      .-....................A.............................T...  722
CP002110     48507    .-....................A.............................T...  48449
CP002110     4444     .-....................A.............................T...  4386
CP002110     2801786  .-....................A.............................T...  2801728
CP002110     1203251  .-....................A.............................T...  1203309
CP002110     1088502  .-....................A.............................T...  1088560
CP002110     1357920  .-....................A.............................T...  1357978
AB599719     381      .-....................A.............................T...  439
KX373891     655      .-....................A.............................T...  713

Query_79091  186      GGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG  245
LC121094     617      ...................T.T..............T.....................  676
CP017094     2318181  ...................T.T..............T.....................  2318122
CP017094     2323391  ...................T.T..............T.....................  2323332
CP017094     2367658  ...................T.T..............T.....................  2367599
CP017094     579343   ...................T.T..............T.....................  579402
CP017094     695628   ...................T.T..............T.....................  695687
CP017094     873578   ...................T.T..............T.....................  873637
CP014064     1291392  ...................T.T..............T.....................  1291333
CP014064     1405956  ...................T.T..............T.....................  1405897
CP014064     1118168  ...................T.T..............T.....................  1118109
CP014064     2451272  ...................T.T..............T.....................  2451331
CP014064     2495550  ...................T.T..............T.....................  2495609
CP014064     2500762  ...................T.T..............T.....................  2500821
KR265361     666      ...................T.T..............T.....................  725
CP007672     2149390  ...................T.T..............T.....................  2149331
CP007672     2265700  ...................T.T..............T.....................  2265641
CP007672     1971391  ...................T.T..............T.....................  1971332
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CP007672     552782   ..................T.T..............T.....................  552841
CP007672     503290   ..................T.T..............T.....................  503349
CP007672     547570   ..................T.T..............T.....................  547629
KJ365315     635      ..................T.T..............T.....................  694
KF983325     723      ..................T.T..............T.....................  782

CP002110     48448    ..................T.T..............T.....................  48389
CP002110     4385     ..................T.T..............T.....................  4326
CP002110     2801727  ..................T.T..............T.....................  2801668
CP002110     1203310  ..................T.T..............T.....................  1203369
CP002110     1088561  ..................T.T..............T.....................  1088620
CP002110     1357979  ..................T.T..............T.....................  1358038
AB599719     440      ..................T.T..............T.....................  499
KX373891     714      ..................T.T..............T.....................  773

Query_79091  246      TAGTCCACGCC-TAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGC  304
LC121094     677      ...........G.............................................  736
CP017094     2318121  ...........G.............................................  2318062
CP017094     2323331  ...........G.............................................  2323272
CP017094     2367598  ...........G.............................................  2367539
CP017094     579403   ...........G.............................................  579462
CP017094     695688   ...........G.............................................  695747
CP017094     873638   ...........G.............................................  873697
CP014064     1291332  ...........G.............................................  1291273
CP014064     1405896  ...........G.............................................  1405837
CP014064     1118108  ...........G.............................................  1118049
CP014064     2451332  ...........G.............................................  2451391
CP014064     2495610  ...........G.............................................  2495669
CP014064     2500822  ...........G.............................................  2500881
KR265361     726      ...........G.............................................  785
CP007672     2149330  ...........G.............................................  2149271
CP007672     2265640  ...........G.............................................  2265581
CP007672     1971331  ...........G.............................................  1971272
CP007672     552842   ...........G.............................................  552901
CP007672     503350   ...........G.............................................  503409
CP007672     547630   ...........G.............................................  547689
KJ365315     695      ...........G.............................................  754
KF983325     783      ...........G.............................................  842
CP002110     48388    ...........G.............................................  48329
CP002110     4325     ...........G.............................................  4266
CP002110     2801667  ...........G.............................................  2801608
CP002110     1203370  ...........G.............................................  1203429
CP002110     1088621  ...........G.............................................  1088680
CP002110     1358039  ...........G.............................................  1358098
AB599719     500      ...........G.............................................  559
KX373891     774      ..A........G.............................................  833

Query_79091  305      AGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGACTGAAACTCAAAGGAT  364
LC121094     737      ........................................GT.............A  796
CP017094     2318061  ........................................GT.............A  2318002
CP017094     2323271  ........................................GT.............A  2323212
CP017094     2367538  ........................................GT.............A  2367479
CP017094     579463   ........................................GT.............A  579522
CP017094     695748   ........................................GT.............A  695807
CP017094     873698   ........................................GT.............A  873757
CP014064     1291272  ........................................GT.............A  1291213
CP014064     1405836  ........................................GT.............A  1405777
CP014064     1118048  ........................................GT.............A  1117989
CP014064     2451392  ........................................GT.............A  2451451
CP014064     2495670  ........................................GT.............A  2495729
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CP014064     2500882  ........................................GT.............A  2500941
KR265361     786      ........................................GT.............A  845
CP007672     2149270  ........................................GT.............A  2149211
CP007672     2265580  ........................................GT.............A  2265521
CP007672     1971271  ........................................GT.............A  1971212
CP007672     552902   ........................................GT.............A  552961
CP007672     503410   ........................................GT.............A  503469
CP007672     547690   ........................................GT.............A  547749
KJ365315     755      ........................................GT.............A  814

KF983325     843      ........................................GT.............A  902
CP002110     48328    ........................................GT.............A  48269
CP002110     4265     ........................................GT.............A  4206
CP002110     2801607  ........................................GT.............A  2801548
CP002110     1203430  ........................................GT.............A  1203489
CP002110     1088681  ........................................GT.............A  1088740
CP002110     1358099  ........................................GT.............A  1358158
AB599719     560      ........................................GT.............A  619
KX373891     834      ........................................GT.............A  893

Query_79091  365      TTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTTAATTCGAAGCAACGCGAAGAA  424
LC121094     797      ................................-.......................  855
CP017094     2318001  ................................-.......................  2317943
CP017094     2323211  ................................-.......................  2323153
CP017094     2367478  ................................-.......................  2367420
CP017094     579523   ................................-.......................  579581
CP017094     695808   ................................-.......................  695866
CP017094     873758   ................................-.......................  873816
CP014064     1291212  ................................-.......................  1291154
CP014064     1405776  ................................-.......................  1405718
CP014064     1117988  ................................-.......................  1117930
CP014064     2451452  ................................-.......................  2451510
CP014064     2495730  ................................-.......................  2495788
CP014064     2500942  ................................-.......................  2501000
KR265361     846      ................................-.......................  904
CP007672     2149210  ................................-.......................  2149152
CP007672     2265520  ................................-.......................  2265462
CP007672     1971211  ................................-.......................  1971153
CP007672     552962   ................................-.......................  553020
CP007672     503470   ................................-.......................  503528
CP007672     547750   ................................-.......................  547808
KJ365315     815      ................................-.......................  873
KF983325     903      ................................-.......................  961
CP002110     48268    ................................-.......................  48210
CP002110     4205     ................................-.......................  4147
CP002110     2801547  ................................-.......................  2801489
CP002110     1203490  ................................-.......................  1203548
CP002110     1088741  ................................-.......................  1088799
CP002110     1358159  ................................-.......................  1358217
AB599719     620      -...............................-.......................  677
KX373891     894      ................................-.......................  952

Query_79091  425      CCTTACCAGGTCTTGACATCCTCTGACAGTCCTAGAGATAG-GACTT-CCCCTCTCGGGG  482
LC121094     856      ........AA............T.....ACT.........A.C...-.....-......  913
CP017094     2317942  ........AA............T.....ACT.........A.C...-.....-......  2317885
CP017094     2323152  ........AA............T.....ACT.........A.C...-.....-......  2323095
CP017094     2367419  ........AA............T.....ACT.........A.C...-.....-......  2367362
CP017094     579582   ........AA............T.....ACT.........A.C...-.....-......  579639
CP017094     695867   ........AA............T.....ACT.........A.C...-.....-......  695924
CP017094     873817   ........AA............T.....ACT.........-AG...T.....-......  873874
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CP014064     1291153  ........AA............T.....ACT..........A.C...-.....-......  1291096
CP014064     1405717  ........AA............T.....ACT..........A.C...-.....-......  1405660
CP014064     1117929  ........AA............T.....ACT..........A.CT..-.....-......  1117872
CP014064     2451511  ........AA............T.....ACT..........A.C...-.....-......  2451568
CP014064     2495789  ........AA............T.....ACT..........A.C...-.....-......  2495846
CP014064     2501001  ........AA............T.....ACT..........A.C...-.....-......  2501058
KR265361     905      ........AA............T.....ACT..........A.C...-.....-......  962
CP007672     2149151  ........AA............T.....ACT..........A.C...-.....-......  2149094
CP007672     2265461  ........AA............T.....ACT..........A.C...-.....-......  2265404
CP007672     1971152  ........AA............T.....ACT..........A.CT..-.....-......  1971095
CP007672     553021   ........AA............T.....ACT..........A.C...-.....-......  553078
CP007672     503529   ........AA............T.....ACT..........A.C...-.....-......  503586
CP007672     547809   ........AA............T.....ACT..........A.C...-.....-......  547866

KJ365315     874      ........AA............T....C.CT..........A.T...-.....-......  931
KF983325     962      ........AA............T.....ACT..........A.C...-.....-......  1019
CP002110     48209    ........AA............T.....ACT..........A.C...-.....-......  48152
CP002110     4146     ........AA............T.....ACT..........A.C...-.....-......  4089
CP002110     2801488  ........AA............T.....ACT..........A.C...-.....-......  2801431
CP002110     1203549  ........AA............T.....ACT..........A.C...-.....-......  1203606
CP002110     1088800  ........AA............T.....ACT..........A.C...-.....-......  1088857
CP002110     1358218  ........AA............T.....ACT..........A.C...-.....-......  1358275
AB599719     678      ........AA............T.....ACT..........A.C...-.....-......  735
KX373891     953      ........AA............T.....C..-.........A.C...-.....-......  1009

Query_79091  483      CACAAAGA-ACGGG-GG-GCACGGTTTCCGCCCCCTCGCG-CTTGAGGAGATTGGGGTAA  538
LC121094     914      G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  972
CP017094     2317884  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  2317826
CP017094     2323094  -......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  2323037
CP017094     2367361  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  2367303
CP017094     579640   G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  579698
CP017094     695925   G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  695983
CP017094     873875   G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  873933
CP014064     1291095  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  1291037
CP014064     1405659  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  1405601
CP014064     1117871  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  1117813
CP014064     2451569  G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  2451627
CP014064     2495847  G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  2495905
CP014064     2501059  G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  2501117
KR265361     963      G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  1021
CP007672     2149093  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  2149035
CP007672     2265403  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  2265345
CP007672     1971094  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  1971036
CP007672     553079   G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  553137
CP007672     503587   G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  503645
CP007672     547867   G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  547925
KJ365315     932      G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  990
KF983325     1020     G......TG..A..-..T...T....GT..T.AG....T.T.G....AT.-.....T...  1077
CP002110     48151    G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  48093
CP002110     4088     G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  4030
CP002110     2801430  G......TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...  2801372
CP002110     1203607  G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  1203665
CP002110     1088858  G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  1088916
CP002110     1358276  GG.....TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  1358334
AB599719     736      G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  794
KX373891     1010     G......TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...  1068

Query_79091  539      ATCCCGCAACGAGCGC-A-CCCTTTAA-CTTATTTGGCCCCCTTTAGTTGGGCACTCTAG  595
LC121094     973      G..............A.-.....A..-....G...C.AG.A.................  1030
CP017094     2317825  G..............-.A.....A.G-....G...C.AT.A..A.............A  2317768
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CP017094	2323036	G.............-.A.....A.G-....G...C.AT.A..A.............A	2322979
CP017094	2367302	G.............-.A.....A.G-....G...C.AT.A..A.............A	2367245
CP017094	579699	G.............-.-A..C....G....G...C.AT.A..A.............A	579756
CP017094	695984	G.............-.-A..C....G....G...C.AT.A..A.............A	696041
CP017094	873934	G.............-.-A..C....G....G...C.AT.A..A.............A	873991
CP014064	1291036	G.............-.A.....A.G-....G...C.AT.A..A.............A	1290979
CP014064	1405600	G.............-.A.....A.G-....G...C.AT.A..A.............A	1405543
CP014064	1117812	G.............-.A.....A.G-....G...C.AT.A..A.............A	1117755
CP014064	2451628	G.............-.-A..C....G....G...C.AT.A..A.............A	2451685
CP014064	2495906	G.............-.-A..C....G....G...C.AT.A..A.............A	2495963
CP014064	2501118	G.....A.......-.-A..C....G....G...C.AT.A..A.............A	2501175
KR265361	1022	G.............-.-A..C....G....G...C.AT.A..A.............A	1079
CP007672	2149034	G.............-.A.....A.G-....G...C.AT.A..A.............A	2148977
CP007672	2265344	G.............-.A.....A.G-....G...C.AT.A..A.............A	2265287
CP007672	1971035	G.............-.A.....A.G-....G...C.AT.A..A.............A	1970978
CP007672	553138	G.............-.-A..C....G....G...C.AT.A..A.............A	553195
CP007672	503646	G.............-.-A..C....G....G...C.AT.A..A.............A	503703

Appendix D

Table 1: Alignment view of blast of isolate 2 against *Staph aureus* N315

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Query_169227  25       GGGCG-AAAGCGCGCG-AGGCGG--TTCTAAGTC-GA--TGAAAGCCCACGG-TCAACC-  75
NC_002745     551162   .....T.........T......TT..T......T..TG.............C......G  551221
NC_002745     506734   .....T.........T......TT..T......T..TG.............C......G  506793
NC_002745     1923310  .....T.........T......TT..T......T..TG.............C......G  1923251
NC_002745     2113603  .....T.........T......TT..T......T..TG.............C......G  2113544
NC_002745     2235302  .....T.........T......TT..T......T..TG.............C......G  2235243

Query_169227  76       TGGA-GGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAAA-TGGAATTCCATGTG  133
NC_002745     551222   ....G.............A.....................G.............  551281
NC_002745     506794   ....G.............A.....................G.............  506853
NC_002745     1923250  ....G.............A.....................G.............  1923191
NC_002745     2113543  ....G.............A.....................G.............  2113484
NC_002745     2235242  ....G.............A.....................G.............  2235183

Query_169227  134      TAGC-G-TGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTC  190
NC_002745     551282   ....G.-............A.....................................  551340
NC_002745     506854   ....G.-............A.....................................  506912
NC_002745     1923190  ....-.G............A.....................................  1923132
NC_002745     2113483  ....-.G............A.....................................  2113425
NC_002745     2235182  ....-.G............A.....................................  2235124

Query_169227  191      TGTAACTGACGCTGATGTGCGAAAGCTTGGGGGATCAAACAGGATTAGATACCCTGGTAG  250
NC_002745     551341   ........................G.-.............................  551399
NC_002745     506913   ........................G.-.............................  506971
NC_002745     1923131  ........................G.....-.........................  1923073
NC_002745     2113424  ........................G.....-.........................  2113366
NC_002745     2235123  ........................G.....-.........................  2235065

Query_169227  251      TCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCCGCCCCTTAGTGGCTGCA  310
NC_002745     551400   ..........................................-.........-.....  551457
NC_002745     506972   ..........................................-.........-.....  507029
NC_002745     1923072  ..........................................-.........-.....  1923015
NC_002745     2113365  ..........................................-.........-.....  2113308
NC_002745     2235064  ..........................................-.........-.....  2235007

Query_169227  311      GCAAACGCATTAAGCACTCCCGCCTGGGGAGTACGACCGCAAGTTTGAAACTCAAAAGGA  370
NC_002745     551458   ..T.............-.....................G.........-.....  551515
NC_002745     507030   ..T.............-.....................G.........-.....  507087
NC_002745     1923014  ..T.............-.....................G...........-...  1922957
NC_002745     2113307  ..T.............-.....................G...........-...  2113250
NC_002745     2235006  ..T.............-.....................G...........-...  2234949

Query_169227  371      ATGGACGGGGACCCCGCACAAGCGGTGGGAGCATGTGGGTTAATTCCGAAGCAACCGCGA  430
NC_002745     551516   ..T........-............-...........T.......-.......-.....  551571
NC_002745     507088   ..T........-............-...........T.......-.......-.....  507143
NC_002745     1922956  ..T.........-...........-...........T.......-.......-.....  1922901
NC_002745     2113249  ..T.........-...........-...........T.......-.......-.....  2113194
NC_002745     2234948  ..T.........-...........-...........T.......-.......-.....  2234893

Query_169227  431      AGAACCTTACCAAATCTTGACTTCTTTTGAAAACTCCTAGAGATAGAAGCCTTCCCCCTT  490
NC_002745     551572   ..................A..C.....C.....-.........-.....-......  551628
NC_002745     507144   ..................A..C.....C.....-.........-..T..-......  507200
NC_002745     1922900  ..................A..C.....C.....-.........-.........-..  1922844
NC_002745     2113193  ..................A..C.....C.....-.........-.........-..  2113137
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NC_002745     2234892  ....................A..C.....C.....-.........-.........-..  2234836

Query_169227  491      CCGGGAACAAAAGTGACAGGTGGTGCATGGTTGTCCGTCAGCTCGTGTCGTGAAATGTTG  550
NC_002745     551629   .G...G..-.......................-.................G......  551686
NC_002745     507201   .G...G..-.......................-.................G......  507258
NC_002745     1922843  .G...G..-.......................-.................G......  1922786
NC_002745     2113136  .G...G..-.......................-.................G......  2113079
NC_002745     2234835  .G...G..-.......................-.................G......  2234778

Query_169227  551      GTTTAAGTCCCCGCACCGAGCCCCACCTCTTAAGCTTAGTTCCCATCATTAATGTGGGCC  610
NC_002745     551687   .G......-......A.....G.A...-.............G..........GT.....A  551744
NC_002745     507259   .G......-......A.....G.A...-.............G..........GT.....A  507316
NC_002745     1922785  .G.........-...A.....G.A...-.............G..........-..T..G.  1922729
NC_002745     2113078  .G.........-...A.....G.A...-.............G..........-..T..G.  2113022
NC_002745     2234777  .G.........-...A.....G.A...-.............G..........-..T..G.  2234721

Query_169227  611      TCTCTAGGTTGGACTGGCGGGTGACAAACCGGAGGAAGGGGGGGATAACGTCAAATCATC  670
NC_002745     551745   -.....A....-....C.-.....................T......G.............  551801
NC_002745     507317   -.....A....-....C.-.....................T......G.............  507373
NC_002745     1922728  A.....A....-....C.-.....................T......G.............  1922671
NC_002745     2113021  A.....A....-....C.-.....................T......G.............  2112964
NC_002745     2234720  A.....A....-....C.-.....................T......G.............  2234663

Query_169227  671      ATCGCCCCTTAATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCTA  730
NC_002745     551802   ..-........-................................................G.  551859
NC_002745     507374   ..-........-................................................G.  507431
NC_002745     1922670  ..-........-................................................G.  1922613
NC_002745     2112963  ..-........-................................................G.  2112906
NC_002745     2234662  ..-........-................................................G.  2234605

Query_169227  731      AACCCGCGA-GTCATGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTG-AGTCTGCA-C  787
NC_002745     551860   ....-....G....A.................................T........A.  551918
NC_002745     507432   .....T..G....A.................................T........A.  507490
NC_002745     1922612  ....-....G....A.................................T........A.  1922554
NC_002745     2112905  ....-....G....A.................................T........A.  2112847
NC_002745     2234604  ....-....G....A.................................T........A.  2234546

Query_169227  788      TCGACTAC-TGAAGCTGGAATCGCTAGTATTCGTAGATCAGC-TGCTACG-TGA-TAC  841
NC_002745     551919   ........A...................A............A.......G...A...  551976
NC_002745     507491   ........A...................A............A.......G...A...  507548
NC_002745     1922553  ........A...................A............A.......G...A...  1922496
NC_002745     2112846  ........A...................A............A.......G...A...  2112789
NC_002745     2234545  ........A...................A............A.......G...A...  2234488
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Table 2: Alignment view of blast of isolate 6 against *Staph aureus* N315

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Query_67941  25       GGGCGCAAAGGGCGCGCAGGCGGTTTTT-AAGTCTCGA--TGAAAGCCCACGG-TCAACC  80
NC_002745    1923310  .....T....C.....T...........T......-..TG.............C......  1923252
NC_002745    2113603  .....T....C.....T...........T......-..TG.............C......  2113545
NC_002745    2235302  .....T....C.....T...........T......-..TG.............C......  2235244
NC_002745    551162   .....T....C.....T...........T......-..TG.............C......  551220
NC_002745    506734   .....T....C.....T...........T......-..TG.............C......  506792

Query_67941  81       GGGGA-GGTC-TTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATCG  138
NC_002745    1923251  .T...G....A...........AA.....................A.............-.  1923193
NC_002745    2113544  .T...G....A...........AA.....................A.............-.  2113486
NC_002745    2235243  .T...G....A...........AA.....................A.............-.  2235185
NC_002745    551221   .T...G....A...........AA.....................A.............-.  551279
NC_002745    506793   .T...G....A...........AA.....................A.............-.  506851

Query_67941  139      TGTAGCGGTGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGT  197
NC_002745    1923192  ......................A.............................T......  1923133
NC_002745    2113485  ......................A.............................T......  2113426
NC_002745    2235184  ......................A.............................T......  2235125
NC_002745    551280   ......................A.............................T......  551339
NC_002745    506852   ......................A.............................T......  506911

Query_67941  198      CTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG  257
NC_002745    1923132  ................T.T..............T.......................  1923073
NC_002745    2113425  ................T.T..............T.......................  2113366
NC_002745    2235124  ................T.T..............T.......................  2235065
NC_002745    551340   ................T.T..............T.......................  551399
NC_002745    506912   ................T.T..............T.......................  506971

Query_67941  258      TCCACGCC-TAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGC  316
NC_002745    1923072  ........G.....................................................  1923013
NC_002745    2113365  ........G.....................................................  2113306
NC_002745    2235064  ........G.....................................................  2235005
NC_002745    551400   ........G.....................................................  551459
NC_002745    506972   ........G.....................................................  507031

Query_67941  317      TAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGACTGAAACTCAAAGGATTTG  376
NC_002745    1923012  ....................................GT.............A...  1922953
NC_002745    2113305  ....................................GT.............A...  2113246
NC_002745    2235004  ....................................GT.............A...  2234945
NC_002745    551460   ....................................GT.............A...  551519
NC_002745    507032   ....................................GT.............A...  507091

Query_67941  377      ACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTTAATTCGAAGCAACGCGAAGAACCT  436
NC_002745    1922952  ................................-.....................  1922894
NC_002745    2113245  ................................-.....................  2113187
NC_002745    2234944  ................................-.....................  2234886
NC_002745    551520   ................................-.....................  551578
NC_002745    507092   ................................-.....................  507150

Query_67941  437      TACCAGGTCTTGACATCCTCTGACAGTCCTAGAGATAG-GACTT-CCCCTCTCGGGGCAC  494
NC_002745    1922893  .....AA...........T.....ACT.........A.C...-.....-......G..  1922836
NC_002745    2113186  .....AA...........T.....ACT.........A.C...-.....-......G..  2113129
NC_002745    2234885  .....AA...........T.....ACT.........A.C...-.....-......G..  2234828
NC_002745    551579   .....AA...........T.....ACT.........A.C...-.....-......G..  551636
NC_002745    507151   .....AA...........T.....ACT.........-AG...T.....-......G..  507208

Query_67941  495      AAAGA-ACGGG-GG-GCACGGTTTCCGCCCCCTCGCG-CTTGAGGAGATTGGGGTAAATC  550
NC_002745    1922835  ....TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...G..  1922777
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NC_002745    2113128  ....TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...G..  2113070
NC_002745    2234827  ....TG..A..T..T...T....GT..T.AG....T.T.G....-.TG.....T...G..  2234769
NC_002745    551637   ....TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...G..  551695
NC_002745    507209   ....TG..A..T..T...T....GT..T.AG....T.T.G....AT.-.....T...G..  507267

Query_67941  551      CCGCAACGAGCGCA-CCCTTTAA-CTTATTTGGCCCCCTTTAGTTGGGCACTCTAGGG-G  607
NC_002745    1922776  ..............A.....A.G-....G...C.AT.A..A..............A.TT.  1922718
NC_002745    2113069  ..............A.....A.G-....G...C.AT.A..A..............A.TT.  2113011
NC_002745    2234768  ..............A.....A.G-....G...C.AT.A..A..............A.TT.  2234710
NC_002745    551696   ..............-A..C....G....G...C.AT.A..A..............A.TT.  551754
NC_002745    507268   ..............-A..C....G....G...C.AT.A..A..............A.TT.  507326

Query_67941  608      ACTGCGGGGGACAAAACGGAGGAGGGGGGGG-TAA-GTCAAA-CATCATGCCCCTTATAA  664
NC_002745    1922717  .....C..T......C.......A..T....A.G.C......T..............G.  1922658
NC_002745    2113010  .....C..T......C.......A..T....A.G.C......T..............G.  2112951
NC_002745    2234709  .....C..T......C.......A..T....A.G.C......T..............G.  2234650
NC_002745    551755   .....C..T......C.......A..T....A.G.C......T..............G.  551814
NC_002745    507327   .....C..T......C.......A..T....A.G.C......T..............G.  507386

Query_67941  665      C-T-GGGCTACACACGTGCTACA-TGGACAGAACACAGGGCAGCGAAACCCCGAGGGTAA  721
NC_002745    1922657  T-.T...................A......AT...A..............G.....TC..  1922599
NC_002745    2112950  T-.T...................A......AT...A..............G.....TC..  2112892
NC_002745    2234649  T-.T...................A......AT...A..............G.....TC..  2234591
NC_002745    551815   TT.-...................A......AT...A..............G.....      551869
NC_002745    507387   TT.-...................A......AT...A.............             507435

Query_67941  722      GC  723
NC_002745    1922598  ..  1922597
NC_002745    2112891  ..  2112890
NC_002745    2234590  ..  2234589
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Table 3: Alignment view of blast of isolate 7 against *Staph aureus* N315

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Query_241767  23       GGGCG-AAAGCGCGCG-AGGCGG--TTCTAAGTC-GA--TGAAAGCCCACGG-TCAACC-  73
NC_002745     1923310  .....T.........T......TT..T......T..TG.............C......G  1923251
NC_002745     2113603  .....T.........T......TT..T......T..TG.............C......G  2113544
NC_002745     2235302  .....T.........T......TT..T......T..TG.............C......G  2235243
NC_002745     506734   .....T.........T......TT..T......T..TG.............C......G  506793
NC_002745     551162   .....T.........T......TT..T......T..TG.............C......G  551221

Query_241767  74       TGGA-GGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGA-TGGAATTCCATGTG  131
NC_002745     1923250  ....G..............AA.....................A.G.............  1923191
NC_002745     2113543  ....G..............AA.....................A.G.............  2113484
NC_002745     2235242  ....G..............AA.....................A.G.............  2235183
NC_002745     506794   ....G..............AA.....................A.G.............  506853
NC_002745     551222   ....G..............AA.....................A.G.............  551281

Query_241767  132      TAGCGGTGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCT  190
NC_002745     1923190  ....................A...........................A..T........  1923131
NC_002745     2113483  ....................A...........................A..T........  2113424
NC_002745     2235182  ....................A...........................A..T........  2235123
NC_002745     506854   ....................A...........................A..T........  506913
NC_002745     551282   ....................A...........................A..T........  551341

Query_241767  191      GTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTC  250
NC_002745     1923130  ............................................................  1923071
NC_002745     2113423  ............................................................  2113364
NC_002745     2235122  ............................................................  2235063
NC_002745     506914   ............................................................  506973
NC_002745     551342   ............................................................  551401

Query_241767  251      CACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTA  310
NC_002745     1923070  ............................................................  1923011
NC_002745     2113363  ............................................................  2113304
NC_002745     2235062  ............................................................  2235003
NC_002745     506974   ............................................................  507033
NC_002745     551402   ............................................................  551461

Query_241767  311      ACGCATTAAGCACTCCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGA  370
NC_002745     1923010  ..............-.............................................  1922952
NC_002745     2113303  ..............-.............................................  2113245
NC_002745     2235002  ..............-.............................................  2234944
NC_002745     507034   .............-..............................................  507092
NC_002745     551462   .............-..............................................  551520

Query_241767  371      CGGGGACCCCGCACAAGCGGTGGGAGCATGTGGTTTAATTCCGAAGCAACGCGAAGAAAC  430
NC_002745     1922951  .........-............-...............-..............-.....  1922896
NC_002745     2113244  .........-............-...............-..............-.....  2113189
NC_002745     2234943  .........-............-...............-..............-.....  2234888
NC_002745     507093   ......-.............-.................-.............-...  507148
NC_002745     551521   ......-.............-.................-.............-...  551576

Query_241767  431      CTTACCAAATCTTGACATCCTTTGACCCGCTCCTAGAGATAGAGTTTTCCCCCTTC-GGG  489
NC_002745     1922895  ........................-AA..-............CC......-...G...  1922839
NC_002745     2113188  ........................-AA..-............CC......-...G...  2113132
NC_002745     2234887  ........................-AA..-............CC......-...G...  2234831
NC_002745     507149   .......................AA-..-............C.......-...G...  507205
NC_002745     551577   .......................AA-..-............CC......-...G...  551633

Query_241767  490      GACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAAATGTTGGGTTAAA  549
NC_002745     1922838  ..........................................G.............-  1922780
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NC_002745     2113131  ..........................................G.............-  2113073
NC_002745     2234830  ..........................................G.............-  2234772
NC_002745     507206   ..........................................G..........-..  507264
NC_002745     551634   ..........................................G..........-..  551692

Query_241767  550      GTCCCGCAACGAGGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGGCACTCTAG  609
NC_002745     1922779  ............-.......................................-.......A  1922722
NC_002745     2113072  ............-.......................................-.......A  2113015
NC_002745     2234771  ............-.......................................-.......A  2234714
NC_002745     507265   ............-.......................................-.......A  507322
NC_002745     551693   ............-.......................................-.......A  551750

Query_241767  610      GTTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCT  669
NC_002745     1922721  ...-.....................................................  1922663
NC_002745     2113014  ...-.....................................................  2112956
NC_002745     2234713  ...-.....................................................  2234655
NC_002745     507323   .-......................................................  507381
NC_002745     551751   .-......................................................  551809

Query_241767  670      TATGATTTGGGCTACACACGTGCTACAATGGATAATAC-AA-GGGCAGCGAACCCGCGAG  727
NC_002745     1922662  ..............................C.....-..A.........A.......  1922604
NC_002745     2112955  ..............................C.....-..A.........A.......  2112897
NC_002745     2234654  ..............................C.....-..A.........A.......  2234596
NC_002745     507382   ..............................C.....A..-.........A...T...  507440
NC_002745     551810   ..............................C.....A..-.........A.......  551868

Query_241767  728      GTCAAGCAA-TCCCATAAA-TTATTCTCAGTTC-G-ATTGTAGTCTGCAACTCGACTAC-  782
NC_002745     1922603  .........A.........G..G..........-.G.....................A  1922545
NC_002745     2112896  .........A.........G..G..........-.G.....................A  2112838
NC_002745     2234595  .........A.........G..G..........-.G.....................A  2234537
NC_002745     507441   .........A.........G..G.........G.-.....................A  507499
NC_002745     551869   .........A.........G..G.........G.-.....................A  551927

Query_241767  783      TGAAGCTGGGATCGCTAGTAATCGTAGATCAGCATGCTACG-TGA-TACG  830
NC_002745     1922544  .........A.............................G...A....  1922495
NC_002745     2112837  .........A.............................G...A....  2112788
NC_002745     2234536  .........A.............................G...A....  2234487
NC_002745     507500   .........A.............................G...A....  507549
NC_002745     551928   .........A.............................G...A....  551977
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Table 4: Alignment view of blast of isolate 10 against *Staph aureus* N315

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Query_225391  26       GGGCG-AAAGCGCGCGCAGGCGGTT-CTTAAGTC-GA--TGAAAGCCCACGGCTCAACC-  79
NC_002745     1923310  .....T.........T........TT.......T..TG...................G  1923251
NC_002745     2113603  .....T.........T........TT.......T..TG...................G  2113544
NC_002745     2235302  .....T.........T........TT.......T..TG...................G  2235243
NC_002745     506734   .....T.........T........TT.......T..TG...................G  506793
NC_002745     551162   .....T.........T........TT.......T..TG...................G  551221

Query_225391  80       TGGA-GGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTG  138
NC_002745     1923250  ....G.............A.A..................A.................  1923191
NC_002745     2113543  ....G.............A.A..................A.................  2113484
NC_002745     2235242  ....G.............A.A..................A.................  2235183
NC_002745     506794   ....G.............A.A..................A.................  506853
NC_002745     551222   ....G.............A.A..................A.................  551281

Query_225391  139      TAGCGGTGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGGCGGCTTCTCTGGTC  197
NC_002745     1923190  ..................A.........................A...-.......  1923132
NC_002745     2113483  ..................A.........................A...-.......  2113425
NC_002745     2235182  ..................A.........................A...-.......  2235124
NC_002745     506854   ..................A.........................A...-.......  506912
NC_002745     551282   ..................A.........................A...-.......  551340

Query_225391  198      TGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGT  257
NC_002745     1923131  ..............T.T..............T.........................  1923072
NC_002745     2113424  ..............T.T..............T.........................  2113365
NC_002745     2235123  ..............T.T..............T.........................  2235064
NC_002745     506913   ..............T.T..............T.........................  506972
NC_002745     551341   ..............T.T..............T.........................  551400

Query_225391  258      CCACGCCTAAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGGCTGCAGC  317
NC_002745     1923071  .......GT.............................................-.......  1923013
NC_002745     2113364  .......GT.............................................-.......  2113306
NC_002745     2235063  .......GT.............................................-.......  2235005
NC_002745     506973   .......GT..............................................-........  507031
NC_002745     551401   .......GT..............................................-........  551459

Query_225391  318      TAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCCAAAGGATTT  377
NC_002745     1923012  ...................................................-......A..  1922954
NC_002745     2113305  ...................................................-......A..  2113247
NC_002745     2235004  ...................................................-......A..  2234946
NC_002745     507032   ....................................................-.......A..  507090
NC_002745     551460   ....................................................-.......A..  551518

Query_225391  378      GACGGGGACCCCGCACAAGCGGTGGGAGCATGTGGTTTATTTCCGAAGCAACCGCGAAGA  437
NC_002745     1922953  ..........-..........-.............A...-.......-........  1922898
NC_002745     2113246  ..........-..........-.............A...-.......-........  2113191
NC_002745     2234945  ..........-..........-.............A...-.......-........  2234890
NC_002745     507091   ........-............-.............A...-.......-........  507146
NC_002745     551519   ........-............-.............A...-.......-........  551574

Query_225391  438      ACCTTACCAGACTCTTGACATTCCTTTGACCGCCCTAGAAGTAAGAGTCTCTCCCCCTTC  497
NC_002745     1922897  .........A.-........-.........AA.T.....GA..-...C..-.....-...  1922843
NC_002745     2113190  .........A.-........-.........AA.T.....GA..-...C..-.....-...  2113136
NC_002745     2234889  .........A.-........-.........AA.T.....GA..-...C..-.....-...  2234835
NC_002745     507147   .........A.-........-.........AA.T.....GA..-...-..T.....-...  507201
NC_002745     551575   .........A.-........-.........AA.T.....GA..-...C..-.....-...  551629

Query_225391  498      CGGGGCAAAAAAGAGACAGGGGGGCGCAGGGTTGTCCGCCCCCTCCTGCTCGGGAGATTG  557
NC_002745     1922842  G....-.C-....T......T..T-...T........-.T.AG...G..-...T.....GT  1922788
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NC_002745     2113135  G....-.C-....T......T..T-...T........-.T.AG...G..-...T.....GT  2113081
NC_002745     2234834  G....-.C-....T......T..T-...T........-.T.AG...G..-...T.....GT  2234780
NC_002745     507202   G....-.C-....T......T..T-...T........-.T.AG...G..-...T.....GT  507256
NC_002745     551630   G....-.C-....T......T..T-...T........-.T.AG...G..-...T.....GT  551684

Query_225391  558      TGGTTTAAGTCGCGAAACGGGCGCAACTTTTACTTTTATTTCCCATCTTTAATTGGGGCA  617
NC_002745     1922787  ...G.......C..C....A.......CC...AGC...G..G.....A....G.T.....  1922728
NC_002745     2113080  ...G.......C..C....A.......CC...AGC...G..G.....A....G.T.....  2113021
NC_002745     2234779  ...G.......C..C....A.......CC...AGC...G..G.....A....G.T.....  2234720
NC_002745     507257   ...G.......C..C....A.......CC...AGC...G..G.....A....G.T.....  507316
NC_002745     551685   ...G.......C..C....A.......CC...AGC...G..G.....A....G.T.....  551744

Query_225391  618      CTCTGGGG-GAGAGCGCGG-GAGAAAACCCGGAGGAGGGGGGGG-TGAC-TCAAAACATC  673
NC_002745     1922727  ....AA.TT..CT..-...T..C....-........A..T....A....G.....T....  1922671
NC_002745     2113020  ....AA.TT..CT..-...T..C....-........A..T....A....G.....T....  2112964
NC_002745     2234719  ....AA.TT..CT..-...T..C....-........A..T....A....G.....T....  2234663
NC_002745     507317   ....AA.TT..CT..-...T..C....-........A..T....A....G.....T....  507373
NC_002745     551745   ....AA.TT..CT..-...T..C....-........A..T....A....G.....T....  551801

Query_225391  674      GCGCCCTTTAAGATGGGGGCAACACACGCGCTACTGTGTGTTAACACAGAGGGCAGCAAA  733
NC_002745     1922670  AT....C...T...TT....T.......T.....AA..-.AC..T...A........G..  1922612
NC_002745     2112963  AT....C...T...TT....T.......T.....AA..-.AC..T...A........G..  2112905
NC_002745     2234662  AT....C...T...TT....T.......T.....AA..-.AC..T...A........G..  2234604
NC_002745     507374   AT....C...T...TT....T.......T.....AA..-.AC..T...A........G..  507432
NC_002745     551802   AT....C...T...TT....T.......T.....AA..-.AC..T...A........G..  551860

Query_225391  734      TCCCGAGAGGCCGATCAAATCCTATAAAAATTGTTCTCAGCGCAGAGTGGAGTGTGCCTC  793
NC_002745     1922611  A-...C....T.A.G.......C.....G-..........TT.G..T..T...C...AA.  1922554
NC_002745     2112904  A-...C....T.A.G.......C.....G-..........TT.G..T..T...C...AA.  2112847
NC_002745     2234603  A-...C....T.A.G.......C.....G-..........TT.G..T..T...C...AA.  2234546
NC_002745     507433   A-...T....T.A.G.......C.....G-..........TT.G..T..T...C...AA.  507490
NC_002745     551861   A-...C....T.A.G.......C.....G-..........TT.G..T..T...C...AA.  551918

Query_225391  794      TGCGACCACCTAAAGCGGGAATCTCTAGT-A-TCGAAGATCAACATGC  839
NC_002745     1922553  .-....T..A.G....T......G.....-.A...T......G.....  1922508
NC_002745     2112846  .-....T..A.G....T......G.....-.A...T......G.....  2112801
NC_002745     2234545  .-....T..A.G....T......G.....-.A...T......G.....  2234500
NC_002745     507491   .-....T..A.G....T......G.....A.-...T......G.....  507536
NC_002745     551919   .-....T..A.G....T......G.....A.-...T......G.....  551964
```

Table 5: Alignment view of blast of isolate 13 against *Staph aureus* N315

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Query_159737  21       GGGCGCAAAGCGCGCG-AGGC-GTTTCTTAATCCCGA-GCGAAAGCCCACGGCCCAACC-  76
NC_002745     1923310  .....T.........T....G....T....GT.T..T.T.............T.....G  1923251
NC_002745     2113603  .....T.........T....G....T....GT.T..T.T.............T.....G  2113544
NC_002745     2235302  .....T.........T....G....T....GT.T..T.T.............T.....G  2235243
NC_002745     506734   .....T.........T....G....T....GT.T..T.T.............T.....G  506793
NC_002745     551162   .....T.........T....G....T....GT.T..T.T.............T.....G  551221

Query_159737  77       TGGA-GGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAA-TGG-ATTCCATGTG  133
NC_002745     1923250  ....G..............A.A.....................G...A..........  1923191
NC_002745     2113543  ....G..............A.A.....................G...A..........  2113484
NC_002745     2235242  ....G..............A.A.....................G...A..........  2235183
NC_002745     506794   ....G..............A.A.....................G...A..........  506853
NC_002745     551222   ....G..............A.A.....................G...A..........  551281

Query_159737  134      -AGCGGTGAAATGCGCAGAG-TATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCT  191
NC_002745     1923190  T...................A.....................................  1923131
NC_002745     2113483  T...................A.....................................  2113424
NC_002745     2235182  T...................A.....................................  2235123
NC_002745     506854   T...................A.....................................  506913
NC_002745     551282   T...................A.....................................  551341

Query_159737  192      GTAACTGACGCTGATGTGCGAAAGCGTGGGGGATCAAACAGGATTAGATACCCTGGTAGT  251
NC_002745     1923130  ............................-............................  1923072
NC_002745     2113423  ............................-............................  2113365
NC_002745     2235122  ............................-............................  2235064
NC_002745     506914   ...........................-............................  506972
NC_002745     551342   ...........................-............................  551400

Query_159737  252      CCCACGCCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCCGCCCCCTTAGTGGCTG  311
NC_002745     1923071  ..-...-.....................................-.....-......-...  1923017
NC_002745     2113364  ..-...-.....................................-.....-......-...  2113310
NC_002745     2235063  ..-...-.....................................-.....-......-...  2235009
NC_002745     506973   ..-...-.....................................-.....-......-...  507027
NC_002745     551401   ..-...-.....................................-.....-......-...  551455

Query_159737  312      CAGCTAAACGCATTAAGCACTCCCGCCTGGGGGAGTACGGACCGCAAGGTTGAAACTCAA  371
NC_002745     1923016  .....-.................-........-.....-.....................  1922961
NC_002745     2113309  .....-.................-........-.....-.....................  2113254
NC_002745     2235008  .....-.................-........-.....-.....................  2234953
NC_002745     507028   .....-.................-........-.....-...................-.  507082
NC_002745     551456   .....-.................-........-.....-...................-.  551510

Query_159737  372      AAGGAATTGACGGGGGACCCCGCACAAGCGGTGGAGCATGTGGTTTTATTTCCGAAGCAC  431
NC_002745     1922960  .-.............-.-.............................A...--........A  1922906
NC_002745     2113253  .-.............-.-.............................A...--........A  2113199
NC_002745     2234952  .-.............-.-.............................A...--........A  2234898
NC_002745     507083   ...............-.-.............................A...--........A  507138
NC_002745     551511   ...............-.-.............................A...--........A  551566

Query_159737  432      CGCGAAGAACCTTACCCAAATCTTGACATCCTTTTGACCACTCCTAGGAGATA-AGACTT  490
NC_002745     1922905  ................-.................-...A....-...-.....G..C...  1922850
NC_002745     2113198  ................-.................-...A....-...-.....G..C...  2113143
NC_002745     2234897  ................-.................-...A....-...-.....G..C...  2234842
NC_002745     507139   ..............-...................-...A....-...-.....G..-...  507193
NC_002745     551567   ..............-...................-...A....-...-.....G..C...  551622

Query_159737  491      TTCCCCTTTCCGGGGAACAAA-TGACCAGGTGGTGGCATGTTTGTCGGCAGCTCCGTGTC  549
NC_002745     1922849  --.......-.G.....-....G....-........-.....G......T......-.....  1922797
```

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NC_002745     2113142  --.......-.G.....-....G....-.......-.....G......T......-.....  2113090
NC_002745     2234841  --.......-.G.....-....G....-.......-.....G......T......-.....  2234789
NC_002745     507194   .-.......-.G.....-....G....-.......-.....G......T......-.....  507247
NC_002745     551623   --.......-.G.....-....G....-.......-.....G......T......-.....  551675

Query_159737  550      GGGAGGATGTTGGGTTAAGTCCCGGCAACGGAGCCGCAACCTTTAAACTCTAGTTGCCAG  609
NC_002745     1922796  .T..-...................-.....-..-.......C....G..-.........T  1922742
NC_002745     2113089  .T..-...................-.....-..-.......C....G..-.........T  2113035
NC_002745     2234788  .T..-...................-.....-..-.......C....G..-.........T  2234734
NC_002745     507248   .T..-...................-.....-..-.......C....G..-.........T  507302
NC_002745     551676   .T..-...................-.....-..-.......C....G..-.........T  551730

Query_159737  610      CATTTTAGTTTGGGCACTCCTAGTGTGGACTGGCCGGTGACAAACCGGAGGAAGGTGGGG  669
NC_002745     1922741  ....A-....-........TA...-..-...-............................  1922687
NC_002745     2113034  ....A-....-........TA...-..-...-............................  2112980
NC_002745     2234733  ....A-....-........TA...-..-...-............................  2234679
NC_002745     507303   ....A-....-........TA...-..-...-............................  507357
NC_002745     551731   ....A-....-........TA...-..-...-............................  551785

Query_159737  670      ATGAACGTCAAATCATCATGCCC-TTATG-TTTGGGCTACACACGTGCTAACAATGGACA  727
NC_002745     1922686  ....-..................C.....A...................-.........  1922629
NC_002745     2112979  ....-..................C.....A...................-.........  2112922
NC_002745     2234678  ....-..................C.....A...................-.........  2234621
NC_002745     507358   ...-...................C.....A...................-.........  507415
NC_002745     551786   ...-...................C.....A...................-.........  551843

Query_159737  728      -TAC-AAGGGCAGCTAAACCGCGAAGTCATGCAAATCCCATAAAGTTGTTCTCAGTTCGG  785
NC_002745     1922628  A...A.........G.........G....A.............................  1922569
NC_002745     2112921  A...A.........G.........G....A.............................  2112862
NC_002745     2234620  A...A.........G.........G....A.............................  2234561
NC_002745     507416   A...A.........G......T..G....A.............................  507475
NC_002745     551844   A...A.........G.........G....A.............................  551903

Query_159737  786      ATT-TAGTCTGC-ACTCGACTACATGAAGCTGGGATCGCTAGTAATCGGAGATCAGC-TG  842
NC_002745     1922568  ...G........A...................A..............T........A..  1922509
NC_002745     2112861  ...G........A...................A..............T........A..  2112802
NC_002745     2234560  ...G........A...................A..............T........A..  2234501
NC_002745     507476   ...G........A...................A..............T........A..  507535
NC_002745     551904   ...G........A...................A..............T........A..  551963

Query_159737  843      CTACG-TGA-TAC   853
NC_002745     1922508  .....G...A...   1922496
NC_002745     2112801  .....G...A...   2112789
NC_002745     2234500  .....G...A...   2234488
NC_002745     507536   .....G...A...   507548
NC_002745     551964   .....G...A...   551976
```

Appendix E

Nucleotide sequences of the isolates

ISOLATE 1

TAGACGTCTCCCGGTTTTGGGGCTCAAAGCGCGCGCAGGCGGTTCTTAAGTCGTT
GAAAGCCCACGGCTCAACCTGAGGTCATGGAAACTGGGGGACTTGAGTGCAGAA
GAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGTATGGAGGAACA
CCAGTGGCGAAGGCGACTTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT
GCTAAGTGTTAGAGGGGTTTCCGCCCTTAGTGCTGGAGCTAACGCATTAGCACTC
CGCCTGGGGAGTACGGACGCAGGTGAACTCAAAGGATTGACGGGGGACCCGCAC
AAGCGGGGGAGCATGTGTTTTATTTCGGAGCAGCGCGAGAACCTTAACAGGTCT
TGAAATCCTTTGACGCCCTAGAGTAGGCTTTCCACTTTCCGGGCCAGATAGACAC
GGCGCAAGGTTGCGACACCTTCTGGTGCGGGGAATGTGGGTAATAGCCGACAGG
GAACTGACTTGTGCACAATAGTGGGACTTAGGTGTGCGGGAAAACCGGTAGGCG
TGTGAACCAATATAGCCTTAACCTGGCCACACGTCATATGTTGGCACAAGTGCAG
TCTGAGGACCAAAAACTGAAGCCGTCTAAAACCGAATGAAGACCA

ISOLATE 2

GTCACACCGGTTTTCGGGCGAAAGCGCGCGAGGCGGTTCTAAGTCGATGAAAGC
CCACGGTCAACCTGGAGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGA
AATGGAATTCCATGTGTAGCGTGAAATGCGCAGAGTATGGAGGAACACCAGTGG
CGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCTTGGGGGATC
AAACGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT
AGGGGGTTTCCCGCCCCTTAGTGGCTGCAGCAAACGCATTAAGCACTCCCGCCTG
GGGAGTACGACCGCAAGTTTGAAACTCAAAAGGAATGGACGGGGACCCCCACA
AGCGGTGGGAGCATGTGGGTTAATTCCGAAGCAACCGCGAAGAACCTTACCAAA
TCTTGACTTCTTTTGAAAACTCCTAGAGATAGAAGCCTTCCCCCTTCCGGGAACA
AAAGTGACAGGTGGTGCATGGTTGTCCGTCAGCTCGTGTCGTGAAATGTTGGTTT
AAGTCCCCGCACCGAGCCCCACCTCTTAAGCTTAGTTCCCATCATTAATGTGGGC
CTCTCTAGGTTGGACTGGCGGGTGACAAACCGGAGGAAGGGGGGGATAACGTCA
AATCATCATCGCCCCTTAATGATTTGGGCTACACACGTGCTACAATGGACAATAC
AAAGGGCAGCTAAACCCGCGAGTCATGCAAATCCCATAAAGTTGTTCTCAGTTC
GGATTGAGTCTGCACTCGACTACTGAAGCTGGAATCGCTAGTATTCGTAGAT

ISOLATE 3

AACCGGTTACCGGGCGAAAGCGCACGCAGGCGGTTGTAAGTCGATGAATCCCCG
GGTCAACTGGGAATGCTTTGAAACTGGCAAGCTAGAGTCCTAGAGGGGGTAGAT
TCCAGGTGAGCGGTGAAATGCTAGAGTCTGGAGGAATACCGGTGGCGAAGGCGG
CCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCTGGGGAGCAAACAGGATA
GTACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAG
GCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCGCAA
GGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTC
CAGAGATGCATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCG
GCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC

CTTTGTTGCCAGCGGTTCGGCCGGAACTCAAAGGAGACTGCCAGTGATAAACTG
GAGGAAGGTGGGGATGACGCCAGTCATCATGGCCCTTACGAGTAGGGCTACACA
CGTGCTACAATGGCATATACAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTC
ATACGTATGTCTACTCCGGATTGGAATCTGCACTCCGACTCCCTGAATCGCAATC
GCTTGTCAATCGAAGATCAGA

ISOLATE 4

CCCCGGTCTCTATTCGGGTCGTCAAGGCGTCGCGTCAGGCTGTCTTCTTTCAGGTC
CAGAGGTGCAAAGGCCTCACGGCTCAACCGTGGTAGGTCCTTTGAAACTCGTGA
GGGCTCTGAGTGCCGGAAAGAGAAAGTGGAATTTCCCAGGGTAGCGGTGAAATG
CCTAAAATTAGGAGGAACACCAGTGGCGAAGGCGACTTTTTGGACCGGAACTGA
AGCCGAAGCACGAAAGCGGGGGGAGCGAACAGGGTTAAAAACCCCGGGAGGCC
CCGCCGGAAACGAAGAAGGCCAAGGGGTGGGGGGCGAACCTCGGCGCCGAATT
AACGCAATAAGCACTCCGCCCGGCGAGGACGGACCCAAGGTGAAACTAAAAGG
AATGACGGGGACCCGCCCAAGCCGCGGAGCGGGGGTTTAATTCAAAGCAACGCG
AAGAACCTACCTGGACTTGACATCCCTTGACCGATCTAACCGATCCTCCCTTCCT
GACAGAGAAAACAGGAGTGCATGGTGGCTCAGCTCGCTCTGAGTGTGGTTAATC
CCGCCACGAGCGCAACCCTGCTTATTGCCTCTTAATTGGGCCTCCACAAGATGGC
GGGGAAATCGAGGAAGGGGGAGGACTCAACTCTGCCCTTCTGTAGGGTGCCACT
GCTACATGGCCGAACACGGAGCGAAAGATATCGGAGCAACCTACCAATCGTCCC
ATTCGTTGGGTGTGAACTCGCCCCTGATTGGATTGTATATCCGACAATGCCCGTG
ATACTCCCCCCCC

ISOLATE 5

AGGGGGGGCACATGTCAGAGGTCTTTTGGGGCGCAAAACGGGGGCAGGCGGTTC
CTAATCTCGAGCGAAAGCCCATTTCCAGCGGTGGGGCCTGGAACTGAGAAACTC
GTTACAGAAGAGGTAAGGAATCCATGGGAACTCAAAAGCGCAGAGTACGGGAG
GACCCCCGCCGACCCCAGTCGGACGGCAGCCGACCTGTGGCCCAAGAAGGGGCA
AAAGGATTAGGGCTGCGTCGCCGCGGATTAACACTATGTCAGGAAGGGGGTCGA
CAACCTTAACGATGTGCACTGCTCCCCCAAGGGCCGGCGCGCCCTAGATCTCAAA
AACTGCAGATTGGCGAGTCTTGCGAGGGGCAACCTAAGACAAGTCCAAAGGGGC
GGAGACCTGCATTCCTGGATTGTAGAAGCCGCGAATAGCCCTCC

ISOLATE 6

GTGTCTCCGCTTTTCGGGCGCAAAGGGCGCGCAGGCGGTTTTTAAGTCTCGATGA
AAGCCCACGGTCAACCGGGGAGGTCTTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCATCGTGTAGCGGTGAAATGCGCAGAGTATGGAGGAACA
CCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCTAAACGATGAGTGCT
AAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCG
CCTGGGGAGTACGACCGCAAGACTGAAACTCAAAGGATTTGACGGGGACCCGCA
CAAGCGGTGGAGCATGTGGTTTTAATTCGAAGCAACGCGAAGAACCTTACCAGG
TCTTGACATCCTCTGACAGTCCTAGAGATAGGACTTCCCCTCTCGGGGCACAAAG
AACGGGGGGCACGGTTTCCGCCCCCTCGCGCTTGAGGAGATTGGGGTAAATCCC
GCAACGAGCGCACCCTTTAACTTATTTGGCCCCCTTTAGTTGGGCACTCTAGGGG
ACTGCGGGGGACAAAACGGAGGAGGGGGGGGTAAGTCAAACATCATGCCCCTT

ATAACTGGGCTACACACGTGCTACATGGACAGAACACAGGGCAGCGAAACCCCG
AGGGTAAGCCCATCTCCCAATCTGTGTTCAGTCGGATAGCGTCGCGACTCGCTGC
GCGAGATGTGGTCGTCTATTCGCGAAACATGCCCGG

ISOLATE 7

TAGCAGACGTTCCCCGGTTTTCGGGCGAAAGCGCGCGAGGCGGTTCTAAGTCGA
TGAAAGCCCACGGTCAACCTGGAGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGATGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGTATGGAGGAAC
ACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCG
TGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTG
CTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC
CCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCC
CGCACAAGCGGTGGGAGCATGTGGTTTAATTCCGAAGCAACGCGAAGAAACCTT
ACCAAATCTTGACATCCTTTGACCCGCTCCTAGAGATAGAGTTTTCCCCCTTCGG
GGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAAATGTTGG
GTTAAAGTCCCGCAACGAGGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTG
GGGCACTCTAGGTTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
CAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGATAATAC
AAGGGCAGCGAACCCGCGAGGTCAAGCAATCCCATAAATTATTCTCAGTTCGAT
TGTAGTCTGCAACTCGACTACTGAAGCTGGGATCGCTAGTAATCGTAGATCAGCA
TGCTACGTGATACGCCCC

ISOLATE 8

GCAGCGTCCACCCTTATTGGGGCAAAAGCGAGCGCAGGCGGTTCTAATCTCGAG
CGAAAGCCCACGGTAAACCGGGGAGGTCATTGGAAACTGGGGGACTTGAGTGCA
GAAGAGGAGATGGATTCCATGTGTAGCGGTGAAATGCGCAGAGTATGGAGGAAC
ACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCG
GGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCTAAACGATGAGTGC
TAAGTGTTAGGAGGGTTTCCGCCCTTTCAGTGCTGCAGCAAACGCATTAAGCACT
CCGCCTGGGGAGTACGACCGCAAGGTTGGAAACTCAAAGGAATTGACGGGGACC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAGGTCTTGACATCCTTTGACCGCTCTAGAGATAGAGTTTTCCCCTTCGGGACAC
AGAGACACAGGTGGCACGGGTTCGTCACCTCTCGCTCGAGATGTTTTGGTTTAAC
CCCCGCAGAGAGCACCCCTTTTTAGTCATAGGCCCCACTTAAAGGGGCACACTCT
AGATAGTGCGGCGGAGACACAGGAGAAGGGGGGGGGTGACCAAATATCTTCGC
CCCTTAACCTTGGGAACACGGGGCACATGGATAACACAAGGCGACGAACCCGCG
AGCTCACGAAATCTATAGCTTTCTCAATGTCGTATCGAGTGCGAACTGCTCTCTA
GACGTGGTCCTATATACCGGTAGAGCTGCAGGG

ISOLATE 9

CGGTGTAGCTGTCCCCCCGCTTTTCGGGCGAAAGCGCGCGAGGCGGTTCTTAAGT
CCGAGCGAAAGCCCACGGTTCAACCGGGAGGGCTTGGAAACTGGGAAACTTGAG
GGCAGAAGAGGAGATGGATTCCTGCTAGCGGAGAATGCGCAGAGTATGGAGGA
CACCAGTGGCGAAGGCGATTTTCTGGCCGGAACTGACGCTGATGCGCAAAGCGT
GGGGGTCAAACAGGCTTAAGTACCCGGTAGTCCACGCCGTAAACCATGATGCTA
AGAGTTAGGGGGTTTCCGCCCTTTATGTGCGGAATATGATTAAGCACTCCGCCGG

GGGAGTACTACCGCTAGTTTGAAACTCAAAGGAATTGACGGGGTCCCCGCACAA
GCGTTGGAGCTAGTCGTTTTAATTCCAAAGCTCGGGAAGACCCTACCCAATTCTT
GGAATTCTTTTGAAACCCCTAGGAATAGAGCCTCCCCTTTCGGGGACAAAGTGAC
AGGGGGTGAAGGGTGGAGGCCCCCCCGAGTCGTGGGATGTTGGGGTTAAGCCCG
GAACAAGCGCAACCTTAAAGCTTAGTTGCCACCATTCAAGTTGGGCCATCCTAGG
TTGATTGCCGGCAAAAAACCGAAGTAGGCGGGGGTTGACTCCAAATCTTTATGG
CCTTTATGATTTGGGCTAGAAACGTCCTATATTGTATAATACAAAGGGTTCTAAA
CCTGCGAGGGCACGCAATCGTATAACGGTGGTCCTTAGTTCGGATTGTAGACAG
AAACTCGACTACTGTTGCGGCATCGTTAGCATCGAGGTCAGCTGCTACGTTATAC
TCCGG

ISOLATE 10

TGGGGCAGCTGTCTCCCCGGTTTTCGGGCGAAAGCGCGCGCAGGCGGTTCTTAAG
TCGATGAAAGCCCACGGCTCAACCTGGAGGTCATTGGAAACTGGGAGACTTGAG
TGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGTATGG
AGGAACACCAGTGGCGAAGGCGGCTTCTCTGGTCTGTAACTGACGCTGAGGCGC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCTAAAACG
ATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGGCTGCAGCTAACGCATT
AAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCCAAAGGATTTGAC
GGGGACCCCGCACAAGCGGTGGGAGCATGTGGTTTATTTCCGAAGCAACCGCGA
AGAACCTTACCAGACTCTTGACATTCCTTTGACCGCCCTAGAAGTAAGAGTCTCT
CCCCCTTCCGGGGCAAAAAAGAGACAGGGGGGCGCAGGGTTGTCCGCCCCCTCC
TGCTCGGGAGATTGTGGTTTAAGTCGCGAAACGGGCGCAACTTTTACTTTTATTT
CCCATCTTTAATTGGGGCACTCTGGGGGAGAGCGCGGGAGAAAACCCGGAGGAG
GGGGGGGTGACTCAAAACATCGCGCCCTTTAAGATGGGGGCAACACACGCGCTA
CTGTGTGTTAACACAGAGGGCAGCAAATCCCGAGAGGCCGATCAAATCCTATAA
AAATTGTTCTCAGCGCAGAGTGGAGTGTGCCTCTGCGACCACCTAAAGCGGGAA
TCTCTAGTATCGAAGATCAACATGCCAGGGAACCCGGCCC

ISOLATE 11

CGGGGGTAGCGTCCGCCGGATTTTGGGCGCAAAGCGCGCGCGGTGGTTCTAAGC
TCGAGCGAAAGCCCACGGTCAACCGGGAGGGCATTGGAAACTGGGAGATTGATG
CAGAAGAGGAAATGGATTCCATGCTAGCGTGAATGCGAGAGTATGGAGGAACAC
CATGGCGAAGGCGATTTTTGTCTGAACTGACACTGAGGCGCGAAAGCGTGGGGG
GCAAACAGGTTAGGTACCCTCGTAGTCCACGCCTTAAACGATGAGCGCAAAGTG
TAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTACCGCATTAGGAACTCCGCCTGGG
GAGCACGGCCGTTAGGAGGAAATTAAAAGAATTGACGGGGTCCCCCCCAACCGG
GGAAGCACCACTTTATTTCCGGGTGGCCGGGAAACCCTTAAATGGTTTTGATTTC
CTCCAAACCCCAGAAACAGGTTTCTCTTCCGGAAGCAGAGAGACAGGGGGTGAA
GGGTGGGAGCCCCCCCAAACGAGAAATTCTGGGTTACGCCCGGAACGAGCGCAC
TCTTAATCTGTTGGCCCCCAAAATTTGGGGCCTCTAGGGAGGTTCCGGCAACAAA
TCGCAGTCGGCAGTGGAGGTCCTCAAATATCTAGCCTTTTAACCCTGCGCTAGAC
ATTCCATATTCGCTTGTGAAAAGAGTTCAACTTCGAGGGGCTTCATCTTAGAACC
CGTCTCACTCGGATTGTGGTGGCCCTCGCTCGCTGAGCGGAACGTTTCATCACGA
TCAGCTGCCAGGTGATACTCGGCG

ISOLATE 12

TAGACGTCTCCCGGCTTCATCGGGCGCAAAGCGCGCGCAGGCGGTTCTTCAAGTC
TCGAGCGAAAGCCCACGGCCCAACCTGGAGGTCTTCGGAAACCGGGAAACTTGA
GTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGTATG
GAGGAACACCAGTGGCGAAGGCGACTTTCCTCGGCCTGTAACTCGACGCTCGAT
GCCCGAAAGCGTGGGGACCAAAACAGGGATTAGATACCCCGTTAGTCCAACGCC
CTAAACGATGAGTGCTAAGTGTTAGGGGGGTTTCCCGCCCCTTAGTGCTGCAGCT
AACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAGGTTGAAACTCAAAGGA
ATTGACGGGGACCCCGCACAACCGGTGGAGCATGTGTTTATTTCCGAAGCACGC
CGAAGAACCTTACCAAATCCTTGACATTCTTTTGAAACTCCTGGAGATAGAGCCT
TCCCCCTTCCGGGGGAAAAAGTGACAGGTGGTGCATGGTTTGTCGTCAGCTCGTG
TCTTGAGATGTTGGTTTAAGTCCCGCACGAGCGCAACCTTAAGCTTATTTGCCTC
ATTAATTTGGGAATTCTGGTTGACTGCGGTGACAAACCGGAAGAAGGTGGGGTT
GACTCCAATCATCATGCCCTTTATGATTTGGCTACAAACTTGCTATATTGGACAA
TACAAAGGGAGCCTAACCCGCGAGTCATGCAATTCCAACAGTGTTCTCATTCGGA
TTGTATCTGCACTCGATACTGATGCGCATCGATTGAATCTAGACAAGCTGCTACG
TGATACTCCCC

ISOLATE 13

GCAGACGTCACCCGGTTTTCGGGCGCAAAGCGCGCGAGGCGTTTCTTAATCCCGA
GCGAAAGCCCACGGCCCAACCTGGAGGTCATTGGAAACTGGGAGACTTGAGTGC
AGAAGAGGAAATGGATTCCATGTGAGCGGTGAAATGCGCAGAGTATGGAGGAA
CACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGC
GTGGGGGATCAAACAGGATTAGATACCCTGGTAGTCCCACGCCCGTAAACGATG
AGTGCTAAGTGTTAGGGGGTTTCCCGCCCCCTTAGTGGCTGCAGCTAAACGCATT
AAGCACTCCCGCCTGGGGGAGTACGGACCGCAAGGTTGAAACTCAAAAGGAATT
GACGGGGGACCCCGCACAAGCGGTGGAGCATGTGGTTTTATTTCCGAAGCACCG
CGAAGAACCTTACCCAAATCTTGACATCCTTTTGACCACTCCTAGGAGATAAGAC
TTTTCCCCTTTCCGGGGAACAAATGACCAGGTGGTGGCATGTTTGTCGGCAGCTC
CGTGTCGGGAGGATGTTGGGTTAAGTCCCGGCAACGGAGCCGCAACCTTTAAAC
TCTAGTTGCCAGCATTTTAGTTTGGGCACTCCTAGTGTGGACTGGCCGGTGACAA
ACCGGAGGAAGGTGGGGATGAACGTCAAATCATCATGCCCTTATGTTTGGGCTA
CACACGTGCTAACAATGGACATACAAGGGCAGCTAAACCGCGAAGTCATGCAAA
TCCCATAAAGTTGTTCTCAGTTCGGATTTAGTCTGCACTCGACTACATGAAGCTG
GGATCGCTAGTAATCGGAGATCAGCTGCTACGTGATACTCCCCCC

ISOLATE 14

AGTCTTACTACCGGCTTACCGGGCGCAAAGCGCACGCAGGCGGTTGTAATCGAT
GAATCCCCGGGTCAACTGGGAATGCTCTGTATGGCAAGCTTGAGTCCTAGAGGG
GGTAGATTCCAGGTGTAGCGGTGAAATGCTAGAGTCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCTGGGGAGCA
AACAGGATAGTACCCTGGTAGTCCACGCCTAAACGATGTCGACTTGGAGGTTGT
GCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGT
ACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCA
CGGAAGTTTTCCAGAGATGAAATTGGTGCCTTCGGGAACTGTGAGACAGGTGCT
GCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGC

GCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCC
AGTGATAAACTGGAGGAAGGTGGGGGATGACGTCAAGTCATCATGGCCCTTACG
ACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGA
GAGCAAGCGGACCTCATAAAGTGCGTCTATCCCGGATTGGAGTCTGCAACTCCG
ACTCCTGAAGTCGAATCGCTAGTAATCTGGATCAAATGCCCGGTGATACTCCC

ISOLATE 15

GAGGAGACTGTCTCCCGGGTTCTTTGGGCGCAAAGCGCGCGCGTGGTTTTTAAGT
CTCGAGCGAAAGCCCACGGCCCAACCTGAGGTCATTGGAAACTGGGAGACTTGA
GTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCTAGAGTATGG
AGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGAACTGACACTGAGGCGCGA
AAGCGTGGGGGAGCAAACAGGATTAGATACCCTGTAGTCCACGCCGAAAACGAT
GAGTGCTAAGTGTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGC
ACTCCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTAAAAGGAATTGACGGGGG
CCCGCACAAGCGGGGGAGCATGTGGTTAATTTCAAAGAACCGCGAAAAACTTTA
CCAGGTCTGGACATCCTTTGAAAACCCTAAGAGATAGGGTTTTCTCCTTCGGGAG
CAGAATAACAGGGGGGGCATGTTTGCCGACAGTCCGGGGCGGGGGATGTTGGTT
TAAGTCCCCGAACGAGGCGCACCCTTGGATCTAATTTGCCATCTTTAAGTTGGGC
CCTCTAAGGGGACTGCGGGGGACAAACCGAAAAAGTGGGAGAACGCCAAATCTT
CTCCCCTTTTGACCGGGCTACACACGGCTAAATGGAGGTACAAAGAGGTCCAAA
CCGCGGGGGGGAGCTAATCTCTAAAACCCGTTCTCAGTCGGATGGAGGTGGAACT
CCGCCTACTGAAGCGGAATCGCTAGAATCCCGGTCACATGCCGGGTGATACTCC
GC

ISOLATE 16

TGAGCTAGCTGTCTCCCGACTTTTCGGGCGAAAGCGCGCGAAGTCGGTTTTTAAG
TCAGAGTGAAAGCCCACGGTCAACCGAGAGGGCCATTGAAAACGGGGAGATTG
AGGGCGGAAAAGGGAAGGGGATTCCCAGGGGAGGGGGGAAAAGGCGAGAAGTT
AGGGGGGACCACCGGGGGCGAAGGGATTTTCGGGCTGGAACTTGACACTGAGGC
CCGAAGCCGGGGGGGGCAACAGAATAGAAACCTGGTAGTCCACGCCTAAACATG
AGTGTAATTTTAGGGGTGACCCCCTTAGGATGAAGTAACTCTTATCATCCGCTAG
GGATACGCACGTGATCTACTGAAAGGATTGACGCGCACGGCAGCGGGGCATGCA
TTGATTAGTTGCAAGCGCGAACATACCTGACTGTCTGACTTCTACGATACTAAAG
ATGGTCTCTCCTCGAAGAGATAACTGGGACATTTTCACTCTCGCGCGAGAATGTG
TAAACCCCACAGCACACTTTGTCTTGTCGCACATATGTGCCCTAGAGGACACGTA
CTACAGAGAGGGGAGCTAATCTCGCCCTAGACGGCAACGTGCATATGCCGTCAA
AGTGAGACAGTGACTACTAAATCTCATCGATGAGGACTCGTCATAGCCGGTCGG
TATCGTCAGTGCGGATCCGCC