ANTIBACTERIAL RESISTANCE PROFILE OF *Staphylococcus spp* IN NEONATAL BLOOD SAMPLES FROM NEONATAL INTENSIVE CARE UNIT, EKITI STATE UNIVERSITY TEACHING HOSPITAL, EKITI STATE

BY

BAMGBOSE, OLUWASEYE VICTORIA

MCB/14/2319

DEPARTMENT OF MICROBIOLOGY

IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF B.Sc (HONS) DEGREE IN MICROBIOLOGY

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CERTIFICATION

I hereby certify that **BAMGBOSE, OLUWASEYE VICTORIA** with Matric Number MCB/14/2319 carried out this project in the Department of Microbiology, Faculty of Sciences, Federal University Oye Ekiti, Ekiti State.

28-03-2019

DR S.K. OJO
SUPERVISOR

DATE

1-4-19

PROF B.O. OGENEH
HEAD OF DEPARTMENT

DATE

DEDICATION

This project work is dedicated to God Almighty for His grace and faithfulness upon my life throughout this project.

ACKNOWLEDGEMENT

First of all, I give my profound and special gratitude to my creator for his everlasting love and grace.

I also want to say a big thank you to my fatherly and wonderful supervisor, Dr S.K. Ojo for his guidance, advice, commitment, patience and help during this project work. God bless you sir.

My over-riding gratitude goes to my ever supportive parents Mr and Mrs Bamgbose and also my lovely siblings Seun, Olawunmi and Boluwatife and my caring brother Oluwole. I also want to thank my aunts and uncles for their untiring support towards the success of this journey. I want to appreciate the effort of my wonderful lecturers.

Also I'd like to say a big thank you to my project mates; Makanjuola Emmanuel and Fafiyebi Opeyemi for their cooperation and my classmates who are now brothers and sisters, those who defined the true meaning of friendship and showed me the essence of friends, it was so good having and meeting you guys, I really appreciate you all; you have been so wonderful, caring and loving.

Lastly to my wonderful friends, Quarshie Doris, Odekayo Kelvin, Mepaiyeda Sophia, Akinduntire Funmi, Ibigbami Feranmi, Odiniya Silas, thank you for being there always and pushing me to always give my best. Thank you so much! I Love you all

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ABSTRACT

Neonatal sepsis or septicaemia is a systemic disease that is present in the blood and other tissues of new born. Neonatal septicaemia is divided into two groups; primary septicaemia and secondary septicaemia associated with major anomalies, focal infection, debilitating illnesses and medical procedures. Clinical features include high fever, hypothermia, poor activity, poor feeding, vomiting, jaundice, respiratory distress, convulsion, irritability etc. Blood samples were gotten from the neonatal intensive care unit, Ekiti State University Teaching Hospital, Ekiti State into blood culture bottles. Samples were standardized and inoculated on selective media and biochemical characterization was carried out on the organisms isolated. *Staphylococcus* spp were isolated which included *S. aureus* and *S. epidermidis* which were 105 and 45 isolates respectively. *S. aureus* showed resistance to all antibiotics used against it with a high resistance pattern to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin, augmentin, and bacitracin, while few resistance pattern was recorded for ofloxacin and gentamicin. *S. epidermidis* showed high resistance to ceftazidime, cefuroxime, erythromycin, augmentin, ofloxacin, gentamicin and bacitracin, with few resistance pattern to cloxacillin and ceftriaxone. A total of 150 strains were obtained were 105(70%) strains of *Staphylococcus aureus* showed resistance percentage to the following antibiotics; ceftazidime 90.4%(95), cefuroxime 93.3%(98), gentamicin 53(50.4%), ceftriaxone 83(79.4%), erythromycin 93(88.2%), cloxacillin 88(83.8%), ofloxacin 65(61.9%), augumentin 92(87.6%) and bacitracin 103(98.0%) while a total of 45 (30%) strains of *Staphylococcus epidermidis* showed resistance percentage to the same antibiotics which includes the following; ceftazidime 38(84.8%), cefuroxime 36(80.4%), gentamicin 34(76.1%), ceftriaxone 25(54.8%), erythromycin 30(67.7%), cloxacillin 26(58.1%), ofloxacin 32(71.1%), augumentin 33(71.7%) and bacitracin 41(91.3%). In conclusion, this study indicated that *Staphylococcus* spp have over time shown increased resistance to a wide range of antibiotics making the use of these drugs ineffective. *Therefore, great caution is required in selection of antibiotic therapy.*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background study of neonatal sepsis

Neonatal sepsis is defined as a clinical syndrome in an infant younger than 90 days, manifested by systemic signs of infection and isolation of pathogenic organisms from the bloodstream. Neonatal sepsis is caused by Gram positive and Gram negative bacteria and Candida. Neonatal sepsis encompasses various systemic infections of the new born, such as septicaemia, meningitis, pneumonia, arthritis and osteomyelitis (Sankar *et al.*, 2008). The diversity of organisms causing sepsis varies from one region to another and changes over time even in the same place, this is attributed to the changing pattern of antibiotic use and changes in lifestyle of these organisms. Many factors contribute to the susceptibility of the neonate to sepsis, which can influence neonatal sepsis. (Sankar *et al.*, 2008)

Neonatal septicaemia continues to be an important cause of morbidity and mortality in spite of great advances in antimicrobial therapy, neonatal life support measures and early detection of risk factors (West and Tabansi, 2014). This is due to high susceptibility of the new born to infections, which can be attributed to compromised immune defence system especially in the preterm (Wu *et al.*, 2009). Neonatal sepsis itself is potentially treatable and preventable, yet despite considerable advances and improvements in the survival rate of new born in developed countries, there has not been a concomitant improvement in outcomes recorded in developing countries (Wu *et al.*, 2009).

Neonatal sepsis is divided into early and late onset sepsis. Both of which are associated with different distributions of pathogens. Early onset sepsis occurs less than 72 hours of life and is generally acquired from pathogens in the maternal genital tract, whereas late onset sepsis,

which occurs between 4th and 90th day of life, has its origin either in the community or in the healthcare environment (Stoll, Hanshen and Sanchez, 2011).

The aetiology of neonatal sepsis varies geographically, with different regions reporting a plethora of prevalent pathogens. Organisms such as *Escherichia coli, Klebsiella pneumoniae,* Group B *Streptococcus, Staphylococcus aureus,* coagulase-negative *Staphylococci, Enterococcus spp, Proteus spp , Acinetobacter baumanii, Acinetobacter iwoffii, Burkholderia cepacia, Candida albicans, Candida parapsilosis, Enterococcus faecalis, Klebsiella oxytoca, Klebsiella pneumonia, Proteus vulgaris, Serratia rubidaea, Listeria monocytogenes, viridians streptococci, Citrobacter spp, Aeromonas spp, Alkaligenes spp, Micrococci spp, Serratia marcescens, Haffnia alvei, Heamophilus influenza, Acinetobacter anitratus, and Pseudomonas aeruginosa* has been reported (Motara, Ballot and Perovic, 2005).

With regard to aetiology, *Klebsiella pneumoniae* was the most frequently recovered pathogen in both early onset sepsis and late onset sepsis. In addition, one organism or group of organism may over time replace another as the leading cause of neonatal septicaemia in a particular region. (West and Tabansil, 2014). Gram positive organisms have shown to cause up to 70% of nosocomial infections in neonates in many hospitals with coagulase negative staphylococci accounting for over 50% of these (Patel, Oshodi and Prasad, 2010; Van der Zwet *et al.*, 2005). In developing countries, the neonatal pathogens which were far more prevalent were Gram negative organisms (Couto *et al.*, 2007). Group B *Streptococcus* is known to be rare or not seen at all although maternal recto-vaginal carriage rates of Group B *Streptococcus* may be similar to those recorded in developed countries. In most of the African studies the incidence of Group B *Streptococcus* is low with South Africa as an exception. Group B *Streptococcus* was also reported to be extremely rare in Asia. Neonatal surveillance in developed countries generally

identifies Group B *Streptococcus* and *E. coli* as the dominant early onset sepsis pathogens and CONS as the dominant late onset.

Globally, sepsis is still one of the major causes of morbidity and mortality in neonates, in spite of recent advances in health care units (Wu *et al.*, 2009). Over 40% of deaths in children under-five occurs globally in the neonatal period, resulting in 3.1 million new born deaths each year. The majority of these deaths usually occur in low-income countries and almost one million of these deaths are attributed to infectious causes including neonatal sepsis, meningitis, and pneumonia. On the other hand, the survivors of neonatal sepsis are vulnerable to short and long-term neurodevelopmental morbidity (Dammann, Kuban and Leviton, 2002; Fanaroff *et al.*, 2002; Ferreira, Mello and Silva, 2014).

In developing countries such as Nigeria, which share 99% of the estimated 4million neonatal deaths annually, neonatal mortality resulting from neonatal sepsis is estimated to be 34/1000 live births, while in developed countries it is 5/1000. Neonatal morbidity and mortality are major public health challenges in our local environment, with a huge percentage of deaths occuring in developing countries from neonatal sepsis directly responsible for 26% of neonatal deaths. The World Health organisation (WHO) reported in 2005 that over 70% of deaths in children under age five occur within the first year of life and 40% occur within the first month (WHO, 2005). Sepsis and meningitis are responsible for most of these deaths. (Vergnano *et al.*, 2005).

The clinical signs and symptoms associated with neonatal infections are non-specific because of an over lapped disease that comes with infections occurred by other diseases, and hence prior detection and treatment becomes crucial for the better neonatal outcomes. Over- diagnosis

of neonatal infections results in an ill-suited and inappropriate usage of antibiotics, causing risks of antibiotic resistance. Recorded clinical features includes fever, hypothermia, lethargy, bulging fontanelle, irritability, vomiting, jaundice, convulsion, respiratory distress, abdominal distension seizures, apnoea and failure to thrive for the first 28 days of life (West and Tabansi, 2014)

Some factors that influence the susceptibility of neonates to sepsis include prematurity or low birth weight, preterm labour, premature or prolonged rupture of membranes, maternal chorioamnionitis, foetal hypoxia, traumatic delivery, male gender and low socio-economic status (Eman, Mohmed, Mohmed and Ramadan, 2015; Ogundare *et al.*, 2016).

Neonatal sepsis is clinically diagnosed by a combination of clinical signs, non-specific laboratory tests and microbiological confirmation by the detection of bacteria in blood culture (Marchant, Boyce, Sadarangani and Leviton, 2013). Blood culture is the gold standard for diagnosis of septicaemia. Diagnosis and treatment of new born with infection is inadequate in many developing countries, because sick new-born show non-specific signs and symptoms (Lawn, Cousens and Zupan, 2009).

Recently, over the last few decades, there has been an increase in antibiotic resistance due to the increase in mutation among common bacterial organisms due to the underuse, overuse and inappropriate use of antibiotics and poor infection control practiced in maternity and neonatal units (Aftab and Iqbal, 2006; Muhammad *et al.*, 2010; Tom-Revzon, 2004). Neonates are prone to adverse side effects of drug use due to their under-developed organ system. Precaution has to be taken to reduce number of possible intake of antibiotics. Studies reported that the unnecessary, injudicious, or excessive use of antibiotics, leads to antibiotics resistance, which is a major global public health concern. Some studies have shown that resistance is associated

directly with selection and use of inappropriate antibiotics (Truter, 2008). Improved guidelines for antibiotic treatment in neonatal sepsis from institutional aetiology and microbial sensitivity should be encouraged. (NareNdra and Jayanti, 2017). As the sensitivity pattern of common pathogen changes day by day to specific antibiotics, so is it necessary to study about their bacteriological analysis and antibiotic sensitivity pattern. Therefore, the determination of antibiotic sensitivity patterns at periodic intervals is mandatory in each region for choosing appropriate antibiotics for each infection. (Leela *et al.*, 2016).

1.2 STATEMENT OF PROBLEM

Antimicrobial resistance has shown to be a global problem which is accelerating due to the misuse and overuse of antibiotics as well as poor infection prevention and control. This study was carried out to determine the antimicrobial resistance of organisms isolated from neonate blood samples.

1.3 JUSTIFICATION OF STUDY

Studies on antimicrobial resistance profile on neonatal sepsis has not been carried out in Ekiti State, Nigeria. This is important because the spectrum of bacterial aetiology as well as the pattern of antimicrobial resistance had been demonstrated from a report in Nigeria to vary over time from one geographical area to another.

1.4 AIM OF STUDY

The aim of this study was to determine antimicrobial resistance profile of the causal organisms of neonatal sepsis from selected teaching hospitals in Ekiti State.

1.5 SPECIFIC OBJECTIVES

(1) To isolate the causal organisms of neonatal sepsis of bacterial origin from Ekiti state University Teaching Hospital Ekiti State using selective medium.

(2) To characterize isolates obtained using microbiological (colonial morphology, Gram's reaction, motility test) and various biochemical tests to species level.

(3) To determine the antimicrobial resistance pattern of the isolates obtained from positive cultures of the neonatal samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Neonatal Sepsis

Neonatal sepsis is defined as a clinical syndrome of bacteraemia with signs and symptoms of infection in the first ninety days after birth (Pius and Bello, 2017). Some authors also define neonatal septicaemia as systemic bacterial infection in a neonate documented by positive blood culture within the first 90 days of birth. It can also be referred to as a clinical syndrome in an infant 90 days younger, which brings about systemic signs of infection and isolation of pathogens from the bloodstream. Neonatal sepsis is caused by a wide range of bacteria and *Candida* spp. Neonatal sepsis encompasses various systemic infections of the new born, such as septicaemia, meningitis, pneumonia, arthritis and osteomyelitis (Pius and Bello, 2017).

2.2 Types of Neonatal Sepsis

Neonatal sepsis can be classified according to time of onset of disease: early onset and late onset. Both are associated with different pathogens. Early onset neonatal sepsis (EOS) occurs within the first 72 hours of life and late onset neonatal sepsis (LOS) occurs beyond 72 hours of life until the end of the neonatal period. Few studies differ on the definition of EOS and LOS. This is mainly on their duration of onset. EOS has been defined to range from 48 hours to 6 days and LOS from 72 hours to 28 days after delivery (Stoll, Hansen and Sanchez, 2010; Vergnano *et al.*, 2005).

2.3 Aetiology of Neonatal Sepsis

Organisms causing neonatal sepsis vary from region to region and also changes periodically in the same area or location. Consequently, the known causal pathogens in neonatal sepsis in developed countries differ from those in developing countries (Vergnano *et al.*, 2005). In developed countries, group B *Streptococcus* (GBS) is the organism mainly implicated and isolated in neonatal sepsis, followed by *E. coli* in EOS while in LOS, *Staphylococcus aureus, Enterococcus* species and group B *Streptococcus* are implicated (Bizzaro, Raskind, Baltimore and Gallaghar, 2003;Stoll *et al.*, 2011). Concurring with these findings are studies in USA and Australia where group B *Streptococcus* and *E. coli* were the most isolated organisms in early onset neonatal sepsis and Coagulase Negative *Staphylococci* (CoNS) followed by *Staphylococcus aureus* in late onset neonatal sepsis (Hyde *et al.*, 2002; Kohli-Kochhar, Omuse and Revathi., 2011). In another study done in the USA Group B *Streptococcus* was the predominant organism, followed by *E.coli* and *Staphylococcus* species. The study also reported a declining trend in occurrence of Group B *Streptococcus* and *E. coli* cases over seventy years between 1928-2003. In the United kingdom GBS was the most frequent pathogen isolated followed by Coagulase Negative *Staphylococci*, non-pyogenic *Streptococci* and *Escherichia coli* (Muller-Pebody *et al.*, 2011).

In the developing world, *Staphylococcus aureus, Klebsiella* species and *Escherichia coli* are most common pathogens causing early onset neonatal sepsis, while in case of late onset neonatal sepsis, *Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pneumoniae* are implicated (Zaidi *et al.*, 2009). The causative agents in early onset neonatal sepsis (EOS) and late onset neonatal sepsis (LONS), are similar especially in hospitals in developing countries (Sundaram *et al.*, 2009). A study done at a private hospital in Kenya found

Gram-positive organisms to be the predominant pathogens in both early and late onset sepsis. Common isolates were *Staphylococcus epidermidis* and *Staphylococcus aureus*. EOS was mainly caused by *Staphylococcus aureus, Klebsiella spp, Staphylococcus epidermidis* and LONS was caused by *Staphylococcus aureus, Streptococcus spp, Enterococcus spp* and *Staphylococcus epidermidis* (Kohli-Kochhar *et al.*, 2011). In another study done in Kenya, Gram positive causative organisms included *Streptococcus pneumoniae, Staphylococcus aureus*, Group A *Streptococcus* and Group B *Streptococcus*. The main Gram negative causative isolates were *E.coli, Klebsiella spp, Acinetobacter spp, Hemophilus influenzae* and *Pseudomonas spp* among others (Berkley *et al.*, 2005). A WHO multicentre study in developing countries that included Ethiopia, Gambia, Papua New Guinea and Philipines reported *Streptococcus pneumoniae, Staphylococcus aureus* and Group A *Streptococcus* as predominant organism and that Group B *Streptococcus* was uncommon. This differs with a study that reported Group B Streptococcus to be the most common isolate in EOS (English *et al.*, 2005). Differences in findings could be due to the different locations of the studies or difference in age of neonates recruited.

2.4 Antibiotic resistance profile of causal organisms in neonatal sepsis

The spectrum of bacteria and their susceptibility patterns may vary depending on prevailing conditions especially antimicrobial drug use. Antibiotic resistance has become a global issue. There are several reports of multi resistant bacteria causing neonatal sepsis, and the trend shows increase in resistance to antibiotics commonly used in developing countries (Vergnano *et al.*, 2005). In a review carried out in developing countries it was reported that most Gram negative bacteria are now resistant to ampicillin and cloxacillin, and many are becoming resistant to gentamicin (Vergnano *et al.*, 2005). Similarly this was also observed by another author where

it was reported that Gram negative bacteria showed a high level of resistance to commonly used antibiotics (Aurangzeb *et al.*, 2003). In other studies conducted, it was also reported that there is an emerging reduction in sensitivity to third generation cephalosporins and quinolones (Rahman, Hammed, Roghani and Ulah, 2002).

In a report on global antimicrobial resistance, with data from 114 countries, the W.H.O found that resistance observed in seven common bacteria has reached alarming levels in all regions of the world (W.H.O, 2014). It also highlights that many gaps exist in documentation of pathogens of major public health importance. This analysis concurs with the WHO's conclusion on the necessity for standards of methodologies to investigate these issues. The W.H.O report also draws attention to the fact that resistance may be overestimated in the general population because most samples reported were collected in large hospitals with less from the population which implies that data from the community are lacking. Finally, the W.H.O calls for actions to strengthen and coordinate collaboration to address these knowledge gaps (W.H.O, 2014)

Despite these levels of resistance, current recommendations state that a new born with suspicion of sepsis should be hospitalized and treated with ampicillin plus gentamicin. However, physicians must keep in mind the local resistance patterns when deciding empiric therapy (Thaver, Ali and Zaidi, 2009). Resistance of hospital-acquired infections is also very high in developing countries (Zaidi *et al.*, 2005). Around 30–90% of *Klebsiella spp* isolates in hospital setting are resistant to commonly used antibiotics against Gram-negative bacteria, and resistance rates are alarmingly high in Southeast Asia. *Escherichia coli* resistance rates are slightly lower but still very high. Overall resistance of *Staphylococcus aureus* to methicillin is

38% in developing countries but rises to 56% in South Asia (Zaidi *et al.*, 2005). High resistance levels force physicians to use broad-spectrum antibiotics, like carbapenems and vancomycin, as first-line regimens. In these low resource communities, many families cannot afford the cost of these medications. If they are obtained, health-care workers might try to prolong their use by using the leftovers on other patients, leading to contamination and outbreaks of resistant bacteria (Zaidi *et al.*, 2005).

Data from the Centre for Disease Control and Prevention (CDC) show rapid increase in the rates of infection due to methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and fluoroquinolone-resistant *Pseudomonas aeruginosa*. Furthermore, multi- and even panantibiotic-resistant infections occur (Boucher *et al.*, 2009). Several highly resistant Gram-negative pathogens, namely *Acinetobacter spp.*, multidrug-resistant (MDR) *Pseudomonas aeruginosa*, and MDR (extended spectrum β-lactamase – ESBL – and carbapenemase producing *Klebsiella spp.* and *Escherichia coli* (carbapenem resistant *Enterobacteriaceae*) have emerged as significant pathogens worldwide Altogether, antibiotic-resistant bacteria, including VRE, MRSA, ESBL- and carbapenemase-producing *Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter* species, have been referred to as "ESKAPE" pathogens [Boucher *et al.*, 2009; Rice, 2008).

These MDR 'superbugs' are often isolated in nosocomial settings, from where they can easily spread in other hospitals of the same geographical region and worldwide. *Klebsiella* resistant to carbapenems, the powerful broad-spectrum antibiotics developed in the 1980s, exemplifies how pathogenic bacteria can spread globally. *A strain of Klebsiella pneumoniae* carrying a gene called KPC, conferring resistance to carbapenems, was first discovered in 1996 from a

North Carolina hospital. In the subsequent years, KPC-positive bacteria were found spreading rapidly through hospitals across New York City, then to several other countries including Israel, Italy, Colombia, the United Kingdom and Sweden (McKenna, 2013). In 2008, a new carbapenem resistance gene, New Delhi Metallo-β-Lactamase (NDM), originating in India, was found in Sweden) (McKenna, 2013 and NDM-producing *Klebsiella spp.* has been implicated in neonatal cases of infections caused by carbapenemase resistant strains (Datta *et al.*, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Collection of samples

Blood samples were collected from the neonatal unit at the Ekiti State University Teaching Hospital Ado Ekiti, Ekiti State and 1ml each of the blood sample was dispensed into sterile universal bottles containing brain heart infusion broth and fluid thioglycolate medium for aerobic and anaerobic incubation respectively. Samples were transported in an ice pack bag containing ice packs to the laboratory and immediately incubated aerobically and anaerobic incubation was also done in an anaerobic jar.

3.2 Sample processing

Samples were incubated aerobically and anaerobically for 24hours at 37°C after which the blood culture was observed for haemolysis, air bubbles and coagulation.

The blood culture (10µl) was standardized using 0.5 McFarland turbidity standard (1-2 $x10^8$ cfu/ml) and then inoculated into selective and differential media (mannitol salt agar, MacConkey agar and blood agar) and incubated aerobically and anaerobically for 24hours at 37°C. The plates were been observed and sub cultured onto nutrient agar for further observation.

3.3 Standardization of inoculum

Isolates from culture plates were standardized using 0.5 McFarland turbidity standard (1-2 $x10^8$ cfu/ml). The test organism was picked with an inoculating loop and transferred into a sterile

test tube containing 2mls of normal saline and is continually diluted till it matches with the 0.5 McFarland turbidity standard.

3.4 Antibiotic sensitivity test

This test was carried out by Kirby-Bauer disc diffusion method using Mueller-Hinton agar following the recommendation of the Clinical and Laboratory Standards Institute (CLSI, 2017). Isolates from pure culture was transferred using an inoculating loop into a tube containing normal saline and mixed well and incubated for 2hours.The organism was distributed evenly on the agar plate and allowed to dry .

The antibiotic disc was placed on the agar and incubated overnight and the diameter of the zone of inhibition around the disc was measured. The isolates was classified according to CLSI (2017) standard as either sensitive or resistant.

CHAPTER FOUR

4.0 RESULTS

4.1 Biochemical characteristics of isolates from blood culture samples

A total of 150 isolates were gotten from this study with 105(70%) identified to be *Staphylococcus aureus* while 45(30%) isolates was identified to be *Staphylococcus epidermidis*. (Table 1)

4.2 Antibiotic resistance pattern of Staphylococcal isolates obtained from neonates

A total of 105(70%) *Staphylococcus aureus* strains obtained showed a multi- drug resistance pattern. 100% of *S. aureus* strains showed multi-drug resistance. (Table 2)

4.3 Antibiotic sensitivity test result for *Staphylococcus epidermidis*

Staphylococcus epidermidis was obtained with isolates showing multi-drug resistance pattern. A total of 45(30%) strains showed multi-drug resistance. (Table 3)

4.4 Percentage of resistance and susceptibility of *Staphylococcus aureus*

This table compares the resistance and susceptibility of the isolates obtained. (Table 4)

4.5 Percentage of resistance and susceptibility of *Staphylococcus epidermidis*

This table compares the resistance and susceptibility of the isolates obtained. (Table 5)

Table 1: Biochemical characterization of S. aureus and S. epidermidis.

Biochemical test	No of Staphylococcus isolates			
	S. aureus		*S. epidermidis*	
	Present(+)	Absent(-)	Present(+)	Absent(-)
Gram staining	105	0	45	0
Catalase	105	0	45	0
Coagulase	105	0	0	45
DNase	105	0	0	45
Oxidase	0	105	0	45
Citrate	105	0	0	45
Methyl red	105	0	0	45
Voges proskaeur	0	105	0	45
Urease	105	0	45	0

Table 2: Antibiotic sensitivity test result for *Staphylococcus aureus*

S/N	SAMPLE NAME	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	B
1	S1 AER BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
2	S1 AER MAC(1)	6R	6R	25S	6R	10R	22R	33S	6R	6R
3	S1 AER MAC(2)	6R	6R	25S	10R	15R	6R	32S	6R	12R
4	S2 AER BA	6R	6R	7R	7R	6R	6R	6R	6R	6R
5	S1 ANA BA	6R	6R	12R	6R	8R	6R	6R	7R	7R
6	S2 ANA BA	6R	8R	10R	6R	6R	6R	8R	6R	6R
7	$S1^2$ AER BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
8	$S1^2$ AER MAC G	6R	6R	25S	6R	10R	22S	35S	6R	6R
9	$S1^2$ AER MAC G	6R	10R	20S	8R	21R	6R	30S	6R	15R
10	$S1^2$ ANA BA	6R	8R	10R	12R	6R	6R	12R	11R	6R
11	$S1^2$ ANA MAC	6R	6R	25S	30S	15R	6R	30S	6R	10R
12	$S2^2$ AER BA	6R	6R	10R	6R	12R	6R	6R	6R	6R
13	$S2^2$ AER MAC(1)	6R	6R	20S	10R	6R	6R	6R	6R	6R
14	$S2^2$ AER MAC(2)	6R	10R	24S	10R	20R	6R	18R	6R	6R
15	$S2^2$ ANA BA	6R	6R	25S	10R	20R	6R	15R	6R	6R
16	$S2^2$ ANA MAC	25S	15R	25S	10R	20R	6R	18R	6R	6R
17	$S2^2$ ANA MSA	6R	6R	30S	32S	20R	6R	15R	6R	6R

S/N	SAMPLENAME	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	B
18	S3 AER BA	6R	6R	30S	35S	20R	6R	30S	6R	6R
19	S3 ANA BA	6R	6R	10R	6R	6R	6R	30S	25S	6R
20	S3 AER MSA	6R	6R	6R	19R	6R	6R	10R	6R	6R
21	S4 AER BA	10R	13R	15R	12R	6R	6R	6R	6R	9R
22	S4 AER MSA	18R	35S	35S	35S	8R	8R	35S	9R	6R
23	S4 AER MAC	22S	16R	25S	24R	7R	6R	27S	24S	6R
24	S4 ANA BA	25S	9R	25S	25S	10R	6R	15R	28S	15R
25	S5 AER BA	6R	6R	6R	9R	6R	6R	26S	6R	6R
26	S5 AER MSA	6R	6R	6R	6R	6R	6R	8R	6R	6R
27	S5 ANA BA	6R	6R	25S	6R	6R	6R	30S	6R	6R
28	S5 ANA MSA	6R	6R	6R	6R	6R	6R	8R	6R	6R
29	S6 AER BA	6R	6R	6R	20R	6R	6R	6R	6R	6R
30	S6 AER MSA (1)	20R	12R	30S	29S	6R	6R	21R	6R	6R
31	S6 AER MSA (2)	15R	9R	30S	10R	15R	26S	31S	14R	7R
32	S6 AER MAC	17R	9R	28S	26S	24R	6R	25S	7R	6R
33	S6 ANA BA	6R	6R	6R	6R	6R	6R	10R	6R	6R
34	S6 ANA MSA	6R	6R	30S	26S	6R	6R	32S	6R	6R
35	$S3^2$ AER BA	15R	6R	20S	10R	6R	6R	30S	10R	6R
36	$S3^2$ AER MAC	6R	12R	6R	10R	25S	6R	30S	6R	6R
37	$S3^2$ ANA BA	6R	6R	35S	20R	6R	6R	35S	31S	6R
38	$S4^2$ AER BA	6R	6R	24S	6R	25S	25S	30S	6R	6R
39	$S4^2$ AER MSA	9R	6R	6R	15R	6R	6R	6R	10R	6R

S/N	SAMPLE NAME	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	B
40	$S4^2$ ANA BA	8R	8R	35S	25R	35S	18R	25S	9R	6R
41	$S5^2$ AER BA	6R	6R	25S	10R	6R	25S	35S	9R	6R
42	$S5^2$ AER MSA	6R	6R	12R	10R	8R	6R	11R	13R	6R
43	$S5^2$ ANA BA	6R	6R	24S	6R	35S	30S	30S	6R	10R
44	$S6^2$ AER BA	6R	23R	35S	30S	6R	6R	32S	31S	6R
45	$S6^2$ AER MSA	10R	25R	25S	10R	6R	25S	30S	10R	6R
46	$S6^2$ ANA BA	9R	6R	30S	6R	32S	25S	30S	10R	6R
47	S7 AER MSA	30S	17R	31S	25S	31S	12R	30S	20R	6R
48	S7 AER MAC	6R	6R	6R	6R	6R	6R	6R	6R	6R
49	S7 ANA BA	18R	6R	18R	17R	6R	6R	6R	6R	6R
50	S8 AER BA	6R	34S	35S	25S	6R	19R	28S	30S	32S
51	S8 ANA BA	16R	6R	12R	6R	6R	6R	6R	6R	6R
52	S9 AER MAC	15R	6R	20S	6R	6R	6R	6R	35S	6R
53	S9 ANA BA	17R	6R	25S	6R	6R	6R	15R	6R	6R
54	$S7^2$ AER MSA	20R	20R	25S	18R	30S	6R	6R	15R	6R
55	$S7^2$ ANA BA	25S	15R	30S	25S	6R	10R	30S	18R	6R
56	$S8^2$ AER BA	21R	20R	31S	25S	31S	6R	26R	10R	6R
57	$S8^2$ AER MSA	25S	18R	24S	6R	6R	12R	30S	18R	6R
58	S10 AER BA	20R	6R	6R	6R	21R	25S	25S	20R	6R
59	S10 ANA BA	10R	8R	6R	6R	25S	25S	17R	6R	8R
60	S11 AER BA	10R	6R	6R	6R	20R	25S	25S	6R	6R
61	S11 AER MSA	20R	8R	6R	15R	23R	25S	20R	30S	6R
62	S13 AER BA	12R	6R	15R	6R	6R	25S	25S	20R	25S

S/N	SAMPLE NO	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	B
63	$S10^2$ AER MSA	25S	6R	6R	6R	25S	20R	20S	25S	6R
64	$S10^2$ ANA BA	6R	6R	6R	6R	21R	25S	27S	6R	6R
65	$S11^2$ AER BA	29S	6R	22R	30S	10R	35S	29S	6R	6R
66	$S11^2$ AER MSA	30S	10R	24R	31S	21R	34S	32S	6R	6R
67	$S11^2$ ANA BA	6R	6R	6R	6R	12R	6R	6R	6R	6R
68	$S12^2$ AER BA	25S	6R	6R	6R	25S	20R	25S	6R	6R
69	$S12^2$ AER MSA	25S	6R	6R	6R	25S	20R	20R	25S	6R
70	$S12^2$ ANA BA	6R	8R	6R	6R	20R	6R	25S	20R	20R
71	$S13^2$ ANA BA	6R	6R	6R	12R	22R	25S	15R	6R	6R
72	$S14^2$ AER BA	8R	6R	6R	6R	13R	20R	25S	6R	6R
73	$S14^2$ ANA BA	6R	6R	22R	25S	25S	18R	22S	20R	17R
74	$S15^2$ AER BA	6R	6R	20R	6R	25S	25S	20S	6R	6R
75	$S15^2$ ANA BA	6R	15R	25S	25S	25S	18R	22S	20R	17R
76	$S16^2$ AER MSA	25S	25S	25S	20R	20R	20R	25S	25S	6R
77	$S16^2$ ANA BA	6R	8R	10R	15R	20R	6R	23S	20R	18R
78	S17 AER BA	6R	25S	6R	6R	6R	6R	15R	6R	10R
79	S18 AER BA	6R	6R	6R	6R	6R	25S	6R	6R	6R
80	S18 ANA BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
81	S19 AER BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
82	S19 ANA BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
83	S17AER MSA(P)	25S	6R	6R	6R	25S	20S	20S	25S	6R
84	S18AER MSA(P)	20R	6R	6R	6R	25S	23S	15R	15R	6R

S/N	SAMPLE NO	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	B
85	S19 AER MSA(P)	6R	20R	13R	6R	20R	25S	15R	15R	6R
86	S17^2 AER MSA	25S	6R	6R	6R	25S	20S	20S	25S	6R
87	S18^2 AER MSA	30S	10R	24R	31S	21R	34S	32S	6R	6R
88	S19^2 AER MSA	6R	20R	13R	6R	23R	25S	15R	15R	6R
89	S20 AER BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
90	S20 ANA BA	18R	11R	25S	30S	6R	6R	6R	6R	6R
91	S21 AER BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
92	S21 ANA BA	6R	6R	6R	6R	6R	6R	8R	6R	6R
93	S22 AER BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
94	S22 AER MAC	6R	6R	6R	6R	6R	6R	6R	6R	6R
95	S22 ANA BA	28S	6R	6R	6R	6R	19R	15R	15R	6R
96	S20^2 AER BA	6R	10R	6R	20R	6R	6R	6R	6R	6R
97	S20^2 ANA BA	6R	17R	6R	30S	15R	6R	6R	20R	6R
98	S21^2 AER BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
99	S21^2 ANA BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
100	S22^2 AER BA	6R	6R	6R	20R	6R	6R	6R	6R	6R
101	S22^2 ANA BA	6R	6R	6R	6R	6R	6R	6R	6R	6R
102	S23 AER BA	6R	6R	14R	6R	20R	27S	6R	6R	6R
103	S23 ANA BA	6R	6R	20R	6R	23R	25R	6R	6R	6R
104	S24 AER BA	6R	6R	6R	6R	10R	15R	6R	6R	6R
105	S24 AER BA	6R	6R	15R	6R	20R	20R	6R	6R	6R

KEYS:

AUG-Augmentin; **B**-Bacitracin; **CAZ**-ceftazidime; **CRX**-Cefuroxime; **CTR**-Ceftriaxone; **CXC**-Cloxacillin; **ERY**-Erythromycin; **GEN**-Gentamicin; **OFL**- Ofloxacin; **AER**-Aerobic; **ANA**- Anaerobic; **BA**-Blood agar; **MSA**- Mannitol salt agar; **MAC**; MacConkey agar; ^{2} ; second culture, **R**- Resistant, **S**- Susceptible, S- sample, **(G)**-Green **(P)**- Pink, **(Y)**- Yellow, **S1**- sample one, **S2**- sample two, **S10 ^{2}AER BA** – sample ten second culture aerobic blood agar, **S1 AER BA**- sample one aerobic blood agar.

Table 3: Antibiotic sensitivity test result for *Staphylococcus epidermidis*

	SAMPLE NO	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	B
1	$S2^2$AER MAC(2)	6R	10R	24S	10R	20R	6R	18R	6R	6R
2	S3 ANA MSA	35S	35S	35S	30S	35S	35S	35S	25S	6R
3	S8 AER MAC	6R	6R	6R	6R	6R	6R	6R	6R	6R
4	S9 AER BA	6R	6R	6R	10R	6R	17R	6R	6R	6R
5	S9 AER MSA	20R	10R	25S	25S	30S	20R	15R	30S	15R
6	$S7^2$ AER BA	12R	30S	25S	30S	18R	25S	20R	30S	6R
7	$S8^2$ ANA BA	20R	30S	6R	25S	20R	20R	30S	25S	30S
8	$S9^2$ AER BA	15R	30S	25S	25S	15R	25S	20R	30S	6R
9	$S9^2$ AER MSA	12R	25S	20R	25S	20R	35S	20R	30S	30S
10	$S9^2$ ANA BA	15R	28S	19R	25S	18R	20R	15R	26S	6R
11	S11 ANA BA	20R	10R	6R	6R	20R	25S	25S	6R	6R
12	S12 AER BA	12R	12R	6R	23R	25S	19R	25S	25S	6R
13	S12 AER MSA	10R	8R	20R	20R	6R	10R	25S	30S	6R
14	S12 ANA BA	25S	25S	25S	25S	25S	25S	25S	25S	20R
15	S13 AER MSA	6R	6R	20R	25S	25S	25S	20R	15R	6R
16	S13 ANA BA	6R	6R	6R	12R	22R	25S	15R	6R	6R
17	S14 AER MSA	6R	6R	22R	25S	25S	18R	22R	20R	17R
18	S14 ANA BA	6R	6R	20R	6R	25S	25S	20R	6R	6R
19	S15 AER MSA	6R	6R	6R	6R	15R	18R	22R	6R	6R
20	S15 ANA BA	6R	22R	6R	6R	10R	6R	20R	8R	6R

S/N	SAMPLE NO	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	B
21	S16 AER BA	25S	25S	25S	20R	20R	20R	25S	25S	25S
22	S16 AER MSA	25S	25S	16R	25S	16R	20R	25S	25S	6R
23	S16 ANA BA	8R	6R	6R	6R	20R	6R	25S	20R	20R
24	$S10^2$ AER BA	9R	16R	10R	30S	27S	25S	30S	10R	6R
25	S17 AER MSA	25S	6R	20R	25S	6R	20R	20R	25S	6R
26	S17 ANA BA	16R	6R	6R	6R	6R	15R	9R	6R	6R
27	S18 AER MSA	20R	6R	6R	6R	25S	23R	15R	15R	6R
28	S19 AER MSA	6R	20R	13R	6R	20R	25S	15R	15R	6R
29	$S17^2$ AER BA	9R	16R	10R	30S	27S	25S	30S	10R	6R
30	$S17^2$ ANA BA	6R	6R	6R	6R	21R	25S	27S	6R	6R
31	$S18^2$ AER BA	29S	6R	22R	30S	10R	35S	29S	6R	6R
32	$S18^2$ ANA BA	6R	6R	6R	6R	21R	6R	6R	6R	6R
33	$S19^2$ AER BA	6R	6R	6R	6R	23R	6R	6R	6R	6R
34	$S19^2$ ANA BA	6R	6R	6R	6R	16R	6R	6R	6R	6R
35	S20 AER MAC	6R	10R	16R	6R	8R	6R	6R	6R	6R
36	S21 AER MAC	6R	6R	6R	6R	15R	6R	8R	10R	6R
37	S22 AER MAC	6R	20R	10R	8R	6R	15R	10R	15R	6R
38	$S20^2$ AER MAC	10R	6R	6R	8R	6R	6R	6R	6R	6R
39	$S21^2$ AER MAC	6R	6R	8R	6R	10R	6R	6R	6R	6R
40	$S22^2$ AER MAC	6R	6R	6R	6R	6R	6R	6R	6R	6R
41	S23 AER MSA	6R	6R	10R	15R	18R	6R	6R	6R	6R
42	S24AER MSA(P)	6R	6R	20R	15R	20R	6R	6R	6R	6R
43	S24AER MSA(Y)	6R	15R	10R	15R	26S	6R	15R	6R	6R

44	S23[2] AER MAC	6R	6R	6R	6R	6R	6R	6R	6R	6R
45	S24[2] AER MAC	6R	6R	6R	6R	6R	6R	6R	6R	6R

KEYS:

AUG-Augmentin; **B**-Bacitracin; **CAZ**-ceftazidime; **CRX**-Cefuroxime; **CTR**-Ceftriaxone; **CXC**-Cloxacillin; **ERY**-Erythromycin; **GEN**-Gentamicin; **OFL**- Ofloxacin; **AER**-Aerobic; **ANA**- Anaerobic; **BA**-Blood agar; **MSA**- Mannitol salt agar; **MAC**; MacConkey agar, [2] ; second culture, **R**- Resistant, **S**- Susceptible, **S1**- sample one, **S2**- sample two, **S10 [2]AER BA**– sample ten second culture aerobic blood agar, **S1 AER BA**- sample one aerobic blood agar.

Table 4: The percentage of resistance and susceptibility of *Staphylococcus aureus* from neonatal blood samples

Antibiotic	Resistant n(%)	Susceptible n(%)
CAZ	90.4%	14.2%
CRX	93.3%	6.6%
GEN	50.4%	49.5%
CTR	79.4%	20.5%
ERY	88.2%	11.7%
CXC	83.8%	16.1%
OFL	61.9%	38.0%
AUG	87.6%	12.8%
B	98.0%	1.9%

KEYS:

n- number of isolates resistant or susceptible **AUG**-Augmentin; **B**-Bacitracin; **CAZ**-ceftazidime; **CRX**-Cefuroxime; **CTR**-Ceftriaxone; **CXC**-Cloxacillin; **ERY**-Erythromycin; **GEN**-Gentamicin; **OFL**- Ofloxacin; **AER**-Aerobic; **ANA**- Anaerobic; **BA**-Blood agar; **MSA**-Mannitol salt agar; **MAC**; MacConkey agar; **R**- Resistant, **S**- Susceptible

Table 5: The percentage of resistance and susceptibility of *Stapylococcus epidermidis* from neonatal blood samples

Antibiotics	Resistant n(%)	Susceptible n(%)
CAZ	84.8%	15.2%
CRX	80.4%	19.6%
GEN	76.1%	23.9%
CTR	54.8%	45.2%
ERY	67.7%	32.3%
CXC	58.1%	41.9%
OFL	71.7%	28.3%
AUG	71.7%	28.3%
B	91.3%	8.9%

KEYS:

n- number of isolates resistant or susceptible **AUG**-Augmentin; **B**-Bacitracin; **CAZ**-ceftazidime; **CRX**-Cefuroxime; **CTR**-Ceftriaxone; **CXC**-Cloxacillin; **ERY**-Erythromycin; **GEN**-Gentamicin; **OFL-** Ofloxacin; **AER**-Aerobic; **ANA**- Anaerobic; **BA**-Blood agar; **MSA**-Mannitol salt agar; **MAC**; MacConkey agar, **R**- Resistant, **S**- Susceptible

CHAPTER FIVE

5.0 DISCUSSION

5.1 DISCUSSION

Staphylococcus spp are the most common bacterial cause for diverse range of infections from folliculitis and furuncules to life threatening infections, including sepsis, deep abscesses, pneumonia, osteomyelitis and infective endocarditis (Lowy, 2015). *Staphylococci* the most common hospital acquired organism accounts for most of the infections. Similarly its greater prevalence in neonatal septicaemia could be explained by the fact, that there is high chance of its transmission to neonates from health workers and relatives (Kayange *et al.*, 2010). *Staphylococcus aureus* was the most frequent isolate followed by *Klebsiella* (Arora and Devi. 2007; Shrestha, Rai, Khanal and Mandal, 2007).

In this study, the only organism isolated was *Staphylococcus* spp which is similar to a study carried out by Mamishi *et al.* (2005) and Douglas *et al.* (2004) which showed *Staphylococcus* spp as the commonest bacterial isolate with 74% predominance.

Staphylococcus spp which include *S. aureus* and *S epidermidis* were isolated with *S. aureus* being the predominant with 105(70%) strains followed by *S. epidermidis* consisting of 45 (30%) strains. In contrast to this study, a study carried out by Pooja *et al.*, (2015), showed that Gram negative rods were predominant comprising of 79.9% and Gram positive of 18.1% with *S.aureus* the second most common pathogen. In comparison with other studies in India by Mustafa and Ahmed (2014) *Escherichia coli* and *Klebsiella spp* was found to be the major cause of neonatal sepsis respectively.

Afrin *et al.* (2016) stated that *Staphylococcus aureus* was the predominant (51.5%) bacterial isolate. This was supported by the study of Sharma *et al.* (2013) in Amritsar India, where *Staphylococcus aureus* was 51.9%. On the contrary, this present study differed with that of

Mohamadi *et al.* (2014) in Iran which showed that isolation rate of *Staphylococcus aureus* 11.1%.

Ghosh and Basu (2018) stated that *S. epidermidis* was the most isolated Gram positive organism which accounted for 18.51% of the total isolates.

According to Pooja *et al.* (2015) 14.5% of the isolates obtained showed to be *S. aureus* which is in contrast with 70% obtained in this study.

In contrast to the present study, a total of 94 isolates was gotten with 37 (39.36%) being Gram positive and *Staphylococcus aureus* accounting for 17(18.1%) according to Shrestha *et al.* (2013). This study also indicated that Gram positive isolates showed resistance to gentamicin (31.5%), cloxacillin (50.4) and cefotaxime (59.35) which is lower than the present study.

In another study carried out in India by Kante, Lankshmi and Reddy (2015) out of 200 samples, growth was observed on 34(17%). Gram positive isolates accounted for 11(5%) and *S. aureus* showed to be the predominant accounting for 9(4.5%).

Biochemical characterization table shows that *Staphylococcus aureus* strains were positive to catalase test, urease test, coagulase test, DNase test, citrate test, H_2S production test and methyl red test while they were negative to oxidase and voges proskaeur test. *Staphylococcus epidermidis* were positive to catalase test, DNase test, citrate test, camp test, H_2S production test, methyl red test, oxidase test and Voges Proskaeur test and negative to coagulase and urease test.

In India, both *S. aureus* and *S. epidermidis* had shown resistance to cefotaxime and ceftazidime ranging from 34 to 86% and from 40 to 71.6% respectively but this study showed higher resistance to cefotaxime and ceftazidime ranging from 90.4 to 93.3% and from 83.8 to 80.4% respectively (Aurangzeb and Hammed, 2015; Mokuolu, Jiya and Adesiyan, 2015). In addition, Sheth, Patel and Tripathi (2012) reported the resistance of *S. aureus* and *S. epidermidis* to

cloxacillin to be lower than 50% as reported in India, Nepal and Saudi Arabia (Abd El Hafez. 2008; Raghunath., 2008; Shaw, Shaw and Thapalil, 2007) but was recorded in this study to be 83.4% and 58.1% respectively.

Coagulase-negative *Staphylococcus* were found to be the most frequent causative agents consisting of 17 isolates (63.0%). The following causative bacteria was *S. aureus* consisting of 3 isolates (11.1%). The highest resistance rates among *Staphylococcus* were found against Penicillin, Ampicillin and Cotrimoxazol while others with less resistance like gentamicin and erythromycin with resistance of 3(15%) and 6(30%) respectively are in contrast with the present study which recorded higher resistance to these antibiotics (Mohamadi *et al.*, 2014).

In another study, a total of 166 samples was gotten and only 4(2.4%) was recorded for *Staphylococcus aureus*. The strains obtained were resistant showing a resistance pattern of 100% (4) to cefotaxime and ceftazidime (Mohsen *et al.*, 2017).

Desai, Malek and Parikh. (2011) stated that out of a total of 140 strains isolated in the study, 25% out of 28% Gram positive strains was identified to be *Staphylococcus aureus* showing it to be to dominating organism. Erythromycin and gentamicin showed 49.12% and 45% resistance respectively.

The predominant growth in this study was Gram-positive organisms (51.92%), of which *Staphylococcus aureus* and Enterococcus (15.38%) were the predominant isolates. In the Gram-positive group, complete resistance was observed to penicillin and ampicillin. *S. aureus* showed 100% resistance to penicillin while Enterococci showed 100% resistance to cephalosporins and ampicillin which is in contrast to the present study which did not record 100% resistance to any antibiotics except bacitracin which showed 98% resistance for *Staphylococcus aureus* (Vazhayil. Stephen and Nayana Prabha. 2017).

Gram positive organism *Staphylococcus aureus* [52.7%] remained the predominate isolate followed by gram negative isolates. The gram positive organisms had greater resistance to Ampicillin and least resistance to Vancomycin, Linezolid, Pipercillin, Clindomycin and others. Resistance recorded was very low in comparison to the present study. (Srinivasa and Arunkumar, 2014).

Kabwe *et al.* (2014) also recorded a very low resistance pattern to antibiotics used. S. aureus accounted for 6% (6) of a total of 103 bacterial isolates. The following antibiotic resistance pattern gentamicin (50%), erythromycin (33%), ceftriaxone (33%), cefotaxime (0%) and ceftazidime (50%) which is very low compared to the present study.

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Staphylococci species are bacteria known for their wide distribution in the environment. Due to this reason they are prone to high resistance pattern among wide range of antibiotics which makes them multi-drug resistant organisms. *Staphylococcus aureus* and *Staphylococcus epidermidis* showed from high to moderate resistance to all the antibiotics which it was tested against. They were tested against nine antibiotics and *S. aureus* showed very high resistance to Ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin, augmentin, and bacitracin, while lesser resistance was recorded for ofloxacin and gentamicin, *S. epidermidis* showed high resistance to Ceftazidime, cefuroxime, erythromycin, augmentin, ofloxacin, gentamicin and bacitracin, with lesser resistance of cloxacillin and ceftriaxone thus indicating that the use of these drugs might be ineffective. Therefore, great caution is required in selection of antibiotic therapy.

6.2 RECOMMENDATION

Due to the challenges faced;

1) Awareness should be created to closely monitor the changes in trends, to obtain information for empiric antibiotic therapy and to act rapidly in case of emergencies.
2) Further work should be done for identification of the aetiology and surveillance of the bacterial resistance pattern and also for identifying effective antibiotic combinations against the most resistant antibiotics.
3) Awareness for global regulations to restrict the use of antimicrobials in the community as well as in the hospital setting

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APPENDIX

1.1 GRAM STAINING:

This is used to distinguish between gram-positive and gram-negative bacteria, which have distinct and consistent differences in their cell walls. The difference between gram-positive and gram-negative bacteria lies in the ability of the cell wall of the organism to retain the crystal violet.

A loop full of sterile water was dropped on a clean grease free slide. Sterile inoculating loop was used to pick a colony onto a drop of water on the slide and emulsified on a small area (smear).The smear was allowed to air dry and then heat fixed by passing it through flame two or three times without exposing the dried film directly to the flame. The slide was flooded with crystal violet solution for 60 seconds, washed off with running water for 5 seconds and drained. The slide was flooded with Gram's Iodine solution (mordant) and allowed for 60 seconds then washed off with running water. The slide was flooded with 95% alcohol for 10 seconds and washed off with running water and then drained. Slide was finally flooded with safranin solution and allowed to counterstain for 30 seconds, washed off with running water, drained and allowed to air dry. All stained slides were examined under the oil immersion lens (mag X 100).

1.2 Catalase test:

Principle: Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. Catalase mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. Enterococci are known to be catalase negative.

Procedure: A drop of 3% H_2O_2 was placed on a surface of clean and dry glass slide using a sterile inoculating loop. A colony was transferred on it and emulsified. A positive result is the

rapid evolution of oxygen (5-10 sec.) as evidence by the presence of bubbles. A negative result shows no bubbles or only a few scattered bubbles.

1.3 **Oxidase test**

Principle: The final stage of bacterial respiration involves a series of membrane embedded components, collectively known as the electron transport chain. The final step in the chain may involve the use of the enzyme cytochrome oxidase, which catalyzes the oxidation of cytochrome c while reducing oxygen to form water. The oxidase test often uses the reagent, tetra-methyl-p-phenylenediamine dihydrochloride, as an artificial electron donor for cytochrome c. When the reagent is oxidized by cytochrome c, it changes from colourless to a dark blue or purple compound, indophenol blue.

Microorganisms are oxidase positive when the colour changes to blue within 15 to 30 seconds. Microorganisms are delayed oxidase positive when the colour changes to purple within 2 to 3 minutes. Microorganisms are oxidase negative if the colour does not change at all

Procedure: The isolated bacteria was grown on a nutrient agar plate for 24 hours at 37°C.After 24 hours 0.2 ml of 1% α-naphthol followed by 0.3 ml of 1% p-aminodimethylaniline oxalate was added and observed for colour change.

1.4 Citrate utilization test

Principle: This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Ammonium dihydrogen phosphate and sodium citrate serve as the source of nitrogen and carbon, respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Utilization of citrate involves the enzyme citrase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO_2.

Production of Na2CO3 as well as NH3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in the change of medium's colour from green to blue.

Procedure: Bacterial colonies were picked with a wire loop and inoculated into slope of Simmon's citrate agar and incubated overnight at 37°C. If the organism has the ability to utilize citrate, the medium changes its colour from green to blue.

1.5 **Methyl red (MR) test:**

Principle: This test is carried out to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation because of which they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in colour at a pH of 4.4 or less.

Procedure: The bacterium to be tested was inoculated into glucose phosphate broth containing glucose and phosphate buffer, and incubated at 37°C for 48 hours. Over the 48 hours the mixed-acid producing organisms produce sufficient acid to overcome the phosphate buffer and remain acidic. The pH of the medium was tested by addition of 5 drops of MR reagent. Development of red colour was taken as positive. MR negative organisms produced yellow colour.

1.6 **Voges Proskaeur (VP) test**:

Principle: VP test is used to identify bacteria that ferment glucose, leading to 2, 3 butanediol accumulation in the medium. The addition of 40% KOH and a 5% solution of alpha-naphthol in absolute ethanol (Barritt's reagent) detects the presence of acetoin, a precursor in the synthesis of 2, 3-butanediol. In the presence of the reagents and acetoin, a cherry-red colour

develops. Development of a red colour in the culture medium 15 minutes following the addition of Barritt's reagent represents a positive VP test; absence of a red colour is a negative VP test

Procedure: Bacterium to be tested was inoculated into glucose phosphate broth and incubated for 48 hours. 0.6 ml of alpha-naphthol was added to the test broth and shaked. 0.2 ml of 40% KOH was added to the broth with shaking. The tube was allowed to stand for 15 minutes. Appearance of red colour was taken as a positive test. The tubes showing negative result were held for one hour, since maximum colour development occurs within one hour after addition of reagents.

1.7 **Urea hydrolysis test (urease)**:

Principle: The urease test identifies those organisms that are capable of hydrolysing urea to produce ammonia and carbon dioxide. Urease activity (urease test) is detected by growing bacteria in a medium containing urea and using a pH indicator such as phenol red. When urea is hydrolysed, ammonia accumulates in the medium and makes it alkaline.

This increase in pH causes the indicator to change from orange-red to deep pink or purplish red (cerise) and is a positive test for urea hydrolysis. Failure to develop a deep pink colour is an indication of negative test. Christensen's urea agar is used to determine urease activity.

Procedure: Slant of urea agar medium was prepared and inoculated with isolated bacteria on the entire surface of the slant. The tubes were inoculated at 37°C. The slant was observed for a colour change at 24 hours. Urease production was indicated by a bright pink (fuchsia) colour on the slant. Any degree of pink colour development was considered as a positive reaction. Prolonged incubation was avoided as it might result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was used.

1.8 **Coagulase test**

Principle:This test is used to differentiate *Staphylococcus aureus*(positive) from coagulase negative*Staphylococci.Stapylococcusaureus*produces two forms of coagulase: bound and fre e. Bound coagulase or clumping factor, is bound to the bacterial cell wall and reacts directly with fibrinogen. When a bacterial suspension is mixed with plasma, this enzyme causes altera tion in fibrinogen of the plasma to precipitate on the staphylococcal cells, causing the cells to clump. Free coagulase is produced extra-cellularly by the bacteria that causes the formation of a clot when *S. aureus* colonies are incubated with plasma.

Procedure: A drop of coagulase plasma was placed on a clean, dry glass slide. And a drop of distilled water or saline is placed next to the drop of plasma as a control. With a loop, a portion of the isolated colony was emulsified after placing it into each drop. The solution was mixed well and rocked gently for 5 to 10 seconds. A positive result shows macroscopic clumping in 10 seconds or less in coagulated plasma drop and no clumping in s aline or water drop while a negative result shows no clumping in both drop.

1.9 **H2S Production:**

Principle: TSI agar (triple sugar iron agar) is a differential media that can detect fermentation and hydrogen sulfide production. It is a rich medium containing a pH indicator, four protein sources or extracts, three sugars (testing for fermentation), iron and sulfur compounds (testing for the production of hydrogen sulfide gas). Aerobic growth takes place on the slant and the butt favours the anaerobic growth. If the sulfur compound is reduced, hydrogen sulfide will form and interact with the iron compound to form a black precipitate, which especially is visible in the butt. If there is no H2S formation, no change in the colour of the medium occurs and it remains orange.

Procedure: Tubes was poured with TSI agar such that each tube contained both a slant (on the top) and a butt (on the bottom) and inoculated with bacteria. The tubes were then observed to examine the development of black colour

2.0 **DNAse test:**

Principle: DNA hydrolysis test or Deoxyribonuclease (DNase) test is used to determine the ability of an organism to hydrolyse DNA and utilise it as a source of carbon and energy for growth. An agar medium; DNase agar is used

Procedure: DNase agar prepared and sterilized according to manufacturer specification was poured into a sterile petri dish and allowed to set close to the flame. A colony of organism was picked and inoculated on the agar in a straight line and incubated for 24hours. After the observation of the plate, the organism was flooded with hydrochloric acid and left for 5 minutes for it to be absorbed into the agar. Excess hydrochloric acid was decanted and the plate was examined. Positive result is indicated by a clear zone around the organism

This test is used to differentiate *Staphylococcus aureus* from other Staphylococci.

2.1 **Camp test**:

Procedure: An inoculating loop was used to pick a colony of a beta-lysin-producing *Staphylococcus aureus* from freshly sub cultured plate and streaked in a straight line across the centre of a blood agar plate. After which the test organism was streaked in a straight line perpendicular to the *S aureus* leaving a 1cm space. The plate was incubated for 24hours.

Positive result showed an enhanced haemolysis indicated by a arrow head shape zone at the junction of the two organism.

2.2 **Turbidity standard for inoculum preparation**

A $BaSO_4$ 0.5 McFarland standards may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standards.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes should be tightly sealed and stored in the dark at room temperature.
5. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer
6. The barium sulfate standards should be replaced or their densities verified monthly.