www.iqresearchjournal.com
ISSN: 2790-4296(Online)

IQRJ: Volume 001, Issue 001, Jan 2022
Original Research Article

Drug Susceptibility Profile Of Staphylococcus Aureus Strains Isolated From Endo-Cervical Swab Specimens In Saint John The Baptist Catholic Health Centre Ndop.

Asakizi Augustine Nji1

Kesmonds International University. Ministry of Higher education, University of Bamenda, North West of Cameroon, Department of Biological Sciences

Email address:
asji2000@yahoo.com

To cite this article:

Authors: Asakizi Augustine Nji1. Paper Title: Drug Susceptibility Profile Of Staphylococcus Aureus Strains Isolated From Endo-Cervical Swab Specimens In Saint John The Baptist Catholic Health Centre Ndop.
IQ Research Journal of IQ res. j. (2022)1(2): pp 01-23. Vol. 001, Issue 002, 02-2022, pp. 0918-0941

Received: 26 02, 2022; **Accepted**: 26 02, 2022; **Published**: 27 02, 2022

Keyword

Drug, Susceptibility, Profile, Staphylococcus, Aureus Strains Endo-Cervical Swab, Specimens, Ndop.

Received:
26 02, 2022

Accepted:
26 02, 2022

Published:
27 02, 2022

Abstract

Empirical antimicrobial therapy for endo-cervical Staphylococcus aureus infections in the community where Saint John the Baptist Catholic Health Centre Ndop is located, is of major importance since it has helped to obtain medications of choice, deduce appropriate prescriptions and a standard antibiotic profile for Staphylococcus aureus in endo-cervical swab specimens in Ndop area and Njokitunjia Division at large.

This study was a prospective and analytical study on women who consulted in Saint John the Baptist Catholic Health Center Ndop with one or more signs and symptoms of urinogenital (vaginal and or endocervical) infections. Adopting an open ended quantative aproach, we aimed at determining antimicrobial susceptibility profile for Staphylococcus aureus from endocervical swab culture. Fifty eight (58) women who were both in patient and out patient were subjected to this study. The study precisely consisted of collection of endocervical swab specimen, identification and isolation of S. aureus strains in cultures, and conduction of in-vitro antimicrobial susceptibility testing by the Disc Diffusion Technique.

In this study, it was found that the S. aureus strains showed 100% susceptibility to vancomycin, Gentamicin (91.4%), Augmentin (89.7%), ciprofloxacin (86.2%) and streptomycin (82.7%) which were highly effective against the 58 isolates of S. aureus tested. Percentages of susceptibility were low (below 50%) for ampicillin, tetracycline, chloramphenicol, erythromycin, and penicillin.

This study challenges the health body as a whole to implement adequate means of epidemiological surveillance antibiotic sensitivity of bacterial strains isolated in laboratories.

1- INTRODUCTION

Antimicrobial chemotherapy involves the use of chemicals in treatment of microbial infections. During the last 25 years, chemotherapeutic research was largely centred around antimicrobial substance of microbial origin called antibiotics. These substances are isolated from filtrates of media in which their receptive molds have grown and some have subsequently been synthesized. Recently, chemical modification of molecules by biosynthesis has been a prominent new drug development method. Due to the increase in occurrence of fungal infections and resistant bacterial strains over the last decade, (Shadomy et al., 1985), efforts have been made in the production of new antimicrobial agents which result in development in the clinical laboratory for selection and monitoring of antimicrobial chemotherapy. Efforts are now being made to standardise laboratory testing with these agents.

The emergence and spread of antimicrobial susceptibility constitutes a major relieve in modern medicines. Resistance to antibiotics limits the success of antimicrobial agents in the therapy and prevention of infectious diseases. Yet society should be aware of the fact that many accomplishments of modern medicine have only been possible because of the availability of a protective antibiotic umbrella. However, continuous positive selection of resistant bacteria clones, whether pathogenic, commensal or even environmental bacteria, will modify the population structure of microbial communities, leading to accelerated evolutionary trends with unpredictable consequences for human health. Whatever the type of infection, treatment is based on the administration of antibiotics empirically (therapy based on a particular geographic or epidemiologic region), or guided by the results of endo-cervical cytology examination (Collee G. al, 2003). Knowledge of empirical treatment failures becomes increasingly disturbing. The same is true for the frequency of bacterial resistance to antibiotics. The emergence and spread of resistance mechanisms acquired in the bacterial species now limit the indications of a number of first-line antibiotics.

The most common organism implicated in endo-cervical infections (80–85%) is *staphylococcus aureus*, (Nicolle LE, 2008). Endo-cervical infections are associated with high morbidity and long term complications like suppurative abscess, pyogenic infection and fatal septicaemia. It also causes febrile illness, which often remain undiagnosed (Jawetz et al., 1991).

Monitoring of this resistance of some bacteria is needed to verify the validity protocols for first-line therapy and to suggest possible measures to control this evolution trend towards inactivity of antibiotics. The multi-resistant strains, have increased (Moustapha T, 2005).Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of

infections that potentially have a bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis.

The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of antimicrobial drugs in many countries and regions not forgetting areas like Ndop where this study was carried. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically. All these reasons motivated my study entitled: Antibiotics Susceptibility Profile of Staphylococcus Aureus isolated from endo-cervical swab specimens.

Antibiotic susceptibility testing is one of the biggest problems in the treatment of most infections, so un-precised determination may lead to improper treatment which will lead to long term effects such as drug toxicity, drug resistence such as methicillin resistant *Staphyloccus aureus (*MRSA). A rise in bacterial resistance to antibiotics complicates treatment of infections. In general, up to 95 % of cases with severe symptoms are treated without bacteriological investigation (Raz et al; 2005). Following the increase resistance of many drugs to this infection, there is need to carry out this research hence knowing the drugs that is susceptible will help in effective treatment of patients in this geographical area and also to come out with an empirical therapy. Due to the increase in occurrence of infections due to *Staphylococcus aureus* stains and Methicilin Resistant *Staphylococcus aureus* (MRSA) strains over the last decade (Shadomy et al; 1985), efforts have been made in the production of new antimicrobial agents which result in development in the clinical laboratory for selection and monitoring of antimicrobial chemotherapy. Efforts are now being made to standardise laboratory testing with antibiotics. Thus

empirical antimicrobial therapy for endocervical *Staphylococcus aureus* infections in the community where Saint John the Baptist Catholic Health Centre Ndop, North West Region, is located is of major importance since it will go a long way to obtain medications of choice, appropriate prescriptions and a standard antibiotic profile for *Staphylococcus aureus* in endo-cervical swab specimens in Ndop area and Njokitunjia Division at large.

2-LITERATURE REVIEW

2.1 Antimicrobial chemotherapy

Antibiotic sensitivity is the susceptibility of bacteria to antibiotics. Ideal antibiotic therapy is based on determination of the aetiological agent and its relevant antibiotic sensitivity. Antimicrobial chemotherapy involves the use of chemicals in treatment of microbial infections. During the last 25 years, chemotherapeutic research was largely centred on antimicrobial substance of microbial origin called antibiotics. These substances are isolated from filtrates of media in which their receptive molds have grown and some have subsequently been synthesized. Recently, chemical modification of molecules by biosynthesis has been a prominent new drug development method. Due to the increase in occurrence of fungal infections and resistant bacterial strains over the last decade, (Shadomy et al, 1985), efforts have been made in the production of new antimicrobial agents which result in development in the clinical laboratory for selection and monitoring of antimicrobial chemotherapy. Efforts are now being made to standardise laboratory testing with these agents (Chawla R et al; 2010).

An ideal antimicrobial agent exhibits selective toxicity, meaning that a drug is harmful to a pathogenic microbe without harmful effects to the host. Thus, selective toxicity may be a function of a specific receptor required for drug attachment and it depends on inhibition of biochemical events essential to microbes but not to the host (Fritsche T.R, 2005). Mechanism of action of these agents against micro organisms include: inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis and inhibition of nucleic acid synthesis by the micro organisms (Cooper R.A, 2003).

Empiric treatment is often started before laboratory microbiological reports are available when treatment should not be delayed due to the seriousness of the disease. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to resist or inactivate the antibiotic. Some antibiotics actually kill the bacteria (bactericidal), whereas others merely prevent the bacteria from multiplying (bacteriostatic) so that the host's immune system can overcome them. Müeller-Hinton agar is most frequently used in this antibiotic susceptibility test.

The goal of antimicrobial susceptibility testing is to predict the in-vivo success or failure of antibiotic therapy. Tests are performed in vitro, and measure the growth response of an isolated organism to a

particular drug or drugs. The tests are performed under standardized conditions so that the results are reproducible. The test results should be used to guide antibiotic choice. The results of antimicrobial susceptibility testing should be combined with clinical information and experience when selecting the most appropriate antibiotic for your patient.

2.2 -Resistance to antimicrobial agents

Antibody mediated immunity (humoral) and cell-mediated immunity (cellular immunity), play little or no host immunity to fungal antigens as it is the reverse of this statement to protozoan antigens. This then demands the need for antimicrobial chemotherapy. Microbes can exhibit resistance to drugs in many ways:

3 Micro organisms produce enzymes that destroy active drugs. For example, staphylococci pathogens produce β-lactamase that makes it to resist penicillin drugs.

4 Micro organisms change their permeability to drugs. For example, streptococci have a natural permeability barrier to amino glycoside drugs (any of a group of bacterial antibiotics, derived from various species of streptomyces, that interfere with the function of bacterial ribosomes. They include gentamicin, streptomycin).

5 Micro organisms alter structural targets for some drugs. This is noticed in chromosomal resistance to amino glycosides by alteration of the specific protein in 30 “s” subunit of bacterial ribosome.

6 Some micro organisms alter the metabolic partway that by passes the reaction inhibited by the drug.

7 Some microbes alter enzymes that can still perform their metabolic functions. However, limitation of drug resistance may be minimised in the following ways:

-Maintenance of sufficiently high levels of drugs in tissues so as to inhibit both the original population and first step mutants.

-Simultaneously, administration of two drugs (synergism) that do not give cross resistance, with the other being able to delay emergence of mutant resistance to the drug. For example, Rifampin and isoniazid in the treatment of tuberculosis.

-Avoid exposure of microbe to particularly valuable drug by restricting its use, especially in hospitals and in animal feeds (G. M. Matar et al; 2005).

2.3Antimicrobial activity in-vitro

Antimicrobial activity is measured in-vitro, in order to determine the potency of an antimicrobial agent in solution, its concentration in body fluids and in determining sensitivity if a given micro organism to know concentration of the drug. Thus, susceptibility tests with antimicrobial agents are performed to provide reliable data which permits selection of suitable agents for use in treatment of human infections. This data may be quantitative

(determination of minimum inhibitory concentration: MIC) or qualitative, that is prediction to probable clinical responses and is needed to explain apparent therapeutic failures and problems associated with selected or secondary resistance. Methods for performing susceptibility testing include various methods:

2.3.1 Quantitative Methods:

In these tests, the minimum amount of antibiotic that inhibits the visible growth of an isolate or minimum inhibitory concentration (MIC) is determined. Bacterial isolate is subjected to various dilutions of antibiotics. The highest dilution of antibiotic that has inhibited the growth of bacteria is considered as MIC. These tests can be performed on broth or agar.

2.3.1.1 Broth dilution methods:

2.3.1.1.1Macrobroth dilution tests:

A serial two-fold dilution of antibiotic are made in test tubes from 0 to maximum concentration that is achieved in vivo without toxic effect on patient. The inoculums density of bacterial isolate to be tested is standardized with 0.5 McFarland turbidity standard. The suspension should have a final inoculums of 5 x 105 cfu/ml. 1ml of bacterial suspension is added to rows of antibiotic solution and incubated at 37oC overnight. The lowest concentration of antibiotic that completely inhibits visual growth of bacteria (no turbidity) is recorded as MIC.

2.3.1.1.2 -Micro broth dilution tests:

A polystyrene tray containing 80 wells is filled with small volumes of serial two-fold dilutions of different antibiotics. The inoculum suspension and standardization is done according to McFarland standard. The bacterial inoculum is then inoculated into the wells and incubated at 37oC overnight. MIC is determined as in macro broth dilution test.

2.3.1.2- Agar dilution method:

A serial two-fold dilution of the antibiotic is prepared in Mueller-Hinton agar. The bacterial inoculum is standardized according to McFarland standard. Using calibrated loops a volume of 0.001-0.002 ml is inoculated on the surface of agar and incubated at 37oC overnight. The lowest concentration of antibiotic that inhibits visible growth on surface of agar is taken as MIC.

2.3.2-Qualitative Methods:

With the introduction of a variety of antimicrobials it has become necessary to perform the antimicrobial susceptibility test as a routine. For this, antimicrobial contained in a reservoir is allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST (Antimicrobial Susceptibility Testing) is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries,

it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. These tests categorize a bacterial isolate as sensitive, intermediate or resistant to a particular antibiotic.

2.3.2.1- Disc diffusion test:

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc. For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, World Health Organization (WHO) recommends the National Committee for Clinical Laboratory Standards. (NCCLS) modified Kirby-Bauer disc diffusion technique (Bauer. A; 1966).

Using the Interpretative Chart, interpret the zones sizes of each antimicrobial in reference to the measured diameter of zones of inhibition, reporting the organism as 'Resistant', 'Intermediate/Moderately susceptible', and 'Susceptible' according to the Kirby Bauer Chart.

A pathogen reported as 'resistant' implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site of infection.

A pathogen reported as intermediately susceptible suggests that the infection it has caused is likely to respond to treatment when the drug is used in larger doses than normal or when the drug is concentrated at the site of infection, e.g. in the urinary tract. Consideration should be given to using other drugs that may provide more optimal therapy.

A pathogen reported as susceptible suggests that the infection it has caused is likely to respond to treatment when the drug to which it is susceptible is used in normal recommended doses and administered by an appropriate route. The raw data are in the form of a zone size .

2.4 Activity and measurable qualities of antibacterial agent

Antibacterial agents possess varying specific activity and measurable qualities with respect to the different body tissues and their related pathogenic infections.

All beta-lactamase drugs as bacitracin, cephalospropins,cycloserine, penicillin and vancomycin are selective inhibitors of bacterial cell wall synthesis. The action mode here consists ofbinding of the drug to cell receptors as penicillin

binding proteins and some transpeptidaton enzymes. Inhibition of the transpeptidase enzyme by penicillins and cephalosporins may be due to a structural similarity of these drugs and the transpeptidation reaction involves loss of a D-alanine from the pentapeptide. Insusceptibility to penicillin is in part determined by the organism's production of penicillin destroying enzymes (β-lactamases). Bacitracin, vancomycin, ristocetin and novobiocin inhibit early steps in the biosynthesis of the peptidoglycan (Jawetz et al., 1991).

Antibiotics as chloramphenicol, tetracyclines, aminoglycosides, erythromycins and lincomycins act by inhibiting protein synthesis in bacteria. The concept of their mode of action is based on the subunit of each type of ribosome, their chemical composition and their functional specificities are sufficiently different to explain why antimicrobial drugs can inhibit protein synthesis in bacterial ribosomes without having a major effect on mammalian ribosomes (Jawetz et al., 1991).

Antimicrobials as rifampin, quinolones, pyrimethanine, sulfonamides and trimethoprim act by inhibiting bacterial nucleic acid synthesis. Rifampin inhibits bacterial growth by binding strongly to the DNA-dependent RNA polymerase of bacteria. All quinolones and fluroquinolones inhibit microbial DNA-synthesis by blocking DNA gyrase.

Sulphonamide is involved in synthesis of folic acid (precursor to synthesis of nucleic acids) thus inhibits dihydropteroate synthetase thereby preventing further growth of bacterial cell. Many bacteria that synthesize folic acid are consequently susceptible to action by sulfonamides (Jawetz et al., 1991). Trimethoprim inhibits dihydrofolic acid reductase 50,000 times (Jawetz et al., 1991) more efficiently in bacteria than in mammalian cells. Pyrimethamine plus sulphonamide is the current treatment of choice in toxoplasmosis and some protozoal infections (Jawetz et al.1991).

2.5 Factors Influencing Antimicrobial Susceptibility Testing

i) pH

The pH of each batch of Müeller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

-Macerate a sufficient amount of agar to submerge the tip of a pH electrode.

-Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.

-Use a properly calibrated surface electrode.

ii) Moisture

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

iii) Effects of Thymidine or Thymine

Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Müeller-Hinton agar that is as low in thymidine content as possible should be used. To evaluate a new lot of Müeller Hinton agar, Enterococcus faecalis ATCC 29212, or alternatively, E. faecalis ATCC 33186, should be tested with trimethoprim/sulfamethoxazole disks. Satisfactory media will provide essentially clear, distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

iv) Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with P. aeruginosa strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems.

Performance tests with each lot of Müeller-Hinton agar must conform to the control limits.

2.6- STAPHYLOCOCCI

2.6.1 General concepts: Staphylococci are Gram positive coccal organism of the genus Staphylococcus. The main species of medical importance include; *Staphylococcus aureus*, *S. saprophyticus*and Staphylococcus *epidermidis.*The staphylococci are divided in two groups based on the presence or absence of the enzyme coagulase. This enzyme converts fibrinogen into fibrin, causing blood plasma to clot. The species called S. aureus is coagulase-positive while S. epidermidis (and other "non-pathogens") is coagulase-negative. Typically, staphylococci are opportunistic pathogens or saprophytes.

2.6.2 Distinctive properties:

Staphylococci are Gram-positive cocci usually arranged in clusters like a bunch of grapes. This appearance is due to the fact that staphylococci divide along two separate planes. The morphologically similar streptococci can be differentiated from staphylococci by testing for the enzyme catalase; staphylococci possess this enzyme while streptococci do not (Cheesebrough. M; 1985).

Staphylococci possess both group specific and type specific antigens: 90% of S. aureus isolates have protein A. This substance is capable of binding the Fc portion of immunoglobulin IgG. This property helps the bacterium escape the potentially lethal effects of

immunoglobulin action and also serves as the basis for some serological tests (coagglutination). Toxins produced by S. aureus include: hemolysins, leukocidins, enterotoxin, exfoliative toxin and toxic shock syndrome (TSS) toxin. Extracellular enzymes produced by S. aureus include: coagulase, fibrinolysin, DNAse, lipases and hyaluronidase.

2.6.7 Pathogenesis:

S. aureus causes boils, styes, pustules, impetigo, infections of wounds (cross-infections), ulcers and burns, osteomyelitis, mastitis, septicaemia, meningitis, pneumonia and pleural empyema. Also, toxic food-poisoning (rapid onset, no fever), toxic shock syndrome and toxic skin exfoliation.*S. aureus* is carried in the nose and on the skin of many healthy people. Coagulase negative strains of Staphylococcus are generally non-invasive. Under certain conditions, however, they may cause severe disease (e.g. S. epidermidis and subacute endocarditis). *S. aureus* is a common cause of boils, sties and skin infections. Serious (life-threatening) infections (pneumonia, deep abscesses, meningitis) may occur in debilitated persons (Daniyan S.Y et al; 2010). *S. aureus* is the common cause of bacteria vaginosis and cervicitis which can lead to infertility, ectopic pregnancy, abortion and many other complications of the reproductive health.

S. aureus is the most common cause of Gram-positive bacteremia, most commonly involving hospital strains of the organism. *S. aureus* is also responsible for scalded skin syndrome and toxic shock syndrome. It is the most common cause of food poisoning. Symptoms occur only a few hours following ingestion of preformed enterotoxin but large amounts of toxin are required.*S. saprophyticus*and *S. aureus* are the common cause of urinary tract infections in young women and occasional nonspecific urethritis in men.

2.6.8 Epidemiology and prevention:

S. aureus is a frequent colonizer of skin and mucosa. High carrier rates (up to 80%) are the rules among hospital patients and staff. The principle localization of colonization in these persons is theanterior nasal mucosa area, from where the bacteria can spread to hands or with dust into the air and be transmitted to susceptible persons. S. aureus is frequently the causal pathogen in nosocomial infections(Eyoh et al; 2013). Certain strains are known to cause hospital epidemics. Identification of the epidemic strain requires differentiation of relevant infection isolatesfrom other ubiquitous strains. Lysotyping can be used for this purpose, although use of molecular methods to identify genomic DNA “fingerprints” is now becoming more common. The most important preventive measure in hospitals iswashing the hands thoroughly before medical and nursing procedures. Intranasal application of antibiotics (mupirocin) is a method of reducing bacterial counts in carriers.

2.6.9 Morphology:

Staphylococci are Gram positive cocci of uniformsize, occurring characteristically in groups but alsosingly and in pairs. They arenon-motile and non capsulate.

2.6.10 Laboratory diagnosi :

Generally, a Gram stain of exudate from a lesion can demonstrate the characteristic Gram-positive cocci arranged in clusters as observed under the microscope.

As concerns culturing, Staphylococci grow well aerobically and in a carbon dioxide enriched atmosphere. Most strains also grow anaerobically, but less well. Temperature range for growth is 10–42 ºC, with an optimum of 35–37 ºC. *S. aureus* produces yellow to cream or occasionally white 1–2 mm in diameter colonies after overnight incubation when cultured on blood agar and or chocolate agar(Cheesebrough. M; 1985). On MacConkey agar, smaller (0.1–0.5 mm) lactose fermenting colonies are produced after overnight incubation. Mannitol salt agar is a selective medium for culturing Staphylococci.

As concerns biochemical tests, staphylococci ferments sucrose and mannitol, it is DNase positive , coagulase positive and catalase positive.

2.6.11Test kits to identify *S. aureus:*

Latex agglutination test kits are available toidentify *S. aureus* based on the detection ofclumping factor and, or, protein A (latex particles aresensitized with fibrinogen and immunoglobulin G). Pastorex Staph Plus test detects all strains ofS. aureus, including up to 95% methicillin-resistant*S. aureus* strains (MRSA).

2.6.12 Antimicrobial susceptibility:

Antibiotics with activity against *S. aureus* include; penicillins, vancomycin**,** macrolides, cephalosporins and fusidic acid. Most strains of *S. aureus* (particularly hospital strains) are resistant to penicillin due to the production of plasmid-coded *beta*-lactamase. MRSA (methicillin resistant S. aureus) are strains are resistant to methicillin (Eyoh et al; 2013) and related penicillins and are particularly difficult to treat because they are also resistant to most other common antibiotics. MRSA strains cause hospital-acquired infections, particularly wound infections and septicaemia (Daniyan S.Y et al; 2010).

3- MATERIALS AND METHOD

3.1-Research design

The study put in place was prospective and analytical study since it involved empirical susceptibility testing. This study lasted from February 2018 to October 2018, and was carried out in the Saint John the Baptist Health Centre Ndop .

Study area

The study was carried out in Saint John the Baptist Catholic Health Centre in Ndop, Ngoketunjia division, situated in North West Region of Cameroun. Bamuka where Saint John the Baptist Health Centre is located is a second degree referral hospital in Ndop. It is a

public health institution located in the heart of Ndop . Bamuka is the administrative environment. This town enjoys both rainy and dry seasons. It has good water supply and roads, educational and health facilities are also available. The people are mostly farmers.

3.4 Study population

It was a hospital base study carried out in Saint John the Baptist Catholic Health Center Ndop involving patients receiving medical care and those found with urinogenital infections due to *S. aureus*. The study population consisted of *S. aureus* isolates from endocervical swab cultures (on selective media of Mannitol salt agar) during the study period.

3.5 Sample Size

This study was based on *S. aureus* isolates from the patients visiting the Saint John the Baptist Health Centre Ndop Laboratory for antimicrobial sensitivity test within this period.

3.6 Sampling procedure

All *S. aureus* stains isolated from endocervical swab cultures during our study period were systematically collected and were been subjected to antimicrobial susceptibility testing.

3.7 Ethical Clearance

An administrative permission from the school was issued to the student and that obtained from the hospital.

3.8- Data collection by Administration of a questionnaire.

Detailed information relevant to the study was collected from each patient using a questionnaire (Appendix- 3). Such data included the age, province of origin, place of residence, religion, ethnicity, marital status, occupation and that of spouse, underlying clinical conditions, type of antibiotics often taken, whether they practice drug abuse, buy antibiotics from the street stores without prescription by a medical doctor, duration of use of a particular antibiotics.

3.9 Processing of specimens

3.9.1) Collection of endocervical swab specimen

After answering the questions, the procedure of collection of high vaginal (cervical) swab specimens was explained to the individuals concerned and the need for the test explained. They were then taken to a private section where the sample was collected, following the procedure described below:

- The participant was asked to remove her pants and lie with her back on collection bed with her legs open.
- With gloves on, a sterile speculum was inserted in the vagina and opened
- A sterile cotton wool swab (two that is one for the research student another for laboratory diagnoses) was inserted into the vagina and specimen collected at the walls of the vagina.

- The pubic area was swabbed with 70% ethanol after which a sterile speculum was used to dilate the vagina.
- The walls of the endocervical canal were then gently scrapped with two sterile swab sticks; one at a time, using a rotatory motion.
- Two swabs were collected per patient. One swab specimen to be used for wet preparation and direct Gram staining while the other swab was used for culturing on Mannitol salt agar which is a selective medium for *S. aureus*.
- The speculum was removed and the participant was asked to dress up.
- To avoid contamination, the swab was immediately taken to the bacteriology unit or laboratory for analysis.

3.9.2) Wet Smear Preparation and Microscopy

- Normal saline (0.85% sodium chloride) was dropped on a grease free slide
- The swab was mixed in saline
- The swabs was removed and sample cover with the cover slip
- The slide was examined directly using a light microscope starting with 10 X objective followed be the 40X objective with the iris diaphragm closed to reduce the amount of light transmitted

3.9.3) Gram staining and microscopy

- The sample was spread on a well labeled glass slide free from grease, air dried in a safe area free from dust and ants.
- The slide was stained using the Gram staining procedure as described by (Cheesbrough M ; 1985)

1. The slide was covered with crystal violet for 30-60 second
2. The stain was washed off the slide with clean tap water.
3. The water was tipped off and the smear cover with Lugol's iodine (mordent) for 30-60 second
4. The lugol's iodine was washed with tap water off the smear and decolorized rapidly with acetone and it was washed immediately with tap water. Considering the flammable nature of acetone alcohol, this step was carried out away from any opened flame
5. .The smear was covered with dilute carbol Fuchsin for 2 minutes
6. The stain was washed with tap water, the back of the slide wiped and placed on a rack for the smear to dry, immersion oil was dropped on the stained smear
7. The smear was examined microscopically under oil immersion objective (100x

objection) and the results reported as positive indicating the presences of yeast cells or negative indicating the absence of yeast cells (20 fields and above were observed)

8. The stained smear appeared as follows under the microscope

    - Gram positive bacterial stained dark purple. These Gram positive cocci organisms seen in clusters or groups were identified as *S. aureus.*
    - Gram negative bacterial stained red.

3.9.4) Testing the pH sample: The pH of each sample collected was determined by touching the swab specimen used for direct examination with a strip of pH paper and then recording the appropriate pH.

3.10 Culture and isolation

3.10.1) Preparation of cultural media

Cultural media preparation included the following steps.

- Mannitol salt agar (used for isolation of S. aureus) and Müeller-Hinton agar (used for sensitivity test) were prepared from a commercially available dehydrated base according to the manufacturer's instructions.

-Immediately after autoclaving at 121ºC for 15 minutes, they were allowed to cool (45 to 50°C water bath).

- The freshly prepared were dispense aseptically and allowed to cool into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.

-The agar medium was allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).

-Cultural media plates were used after been sufficiently cooled or the next day depending on the number specimens collected. Also Müeller-Hinton Agarwere used for sensitivity depending on the isolated strains of *S. aureus* after every two days.

-A representative sample of each batch of plates was examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

3.10.2) Culturing of specimens:

The second swab specimen got from each patient not used was cultured on Mannitol salt agar which is a selective media for culturing of *S. aureus*. The dried surface of Mannitol salt agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. The sets of Mannitol salt agar media plates were incubated at 37°C for up to 48 hours.

3.11 EXAMINATION OF CULTURES

-The cultural characteristics (macroscopy) of various colonies showing significant growth on the agar media used were studied. Reading of the cultural plates was done by studying the macroscopic growth characteristics such as colour, edge, odour, shape, consistency, opacity, size, haemolysis, elevation.

3.12 - Invitro Antimicrobial susceptibility testing (Disk diffusion method).

The antimicrobial susceptibility testing was carried out, using the disk diffusion technique of Bauer (Bauer et. al., 1966), modified and standardized by the (NCCLS) National Community for Clinical Laboratory Standard (Lalitha, 2005).

The antibacterial susceptibility testing on*S. aureus* was carried out on Mueller Hinton agar using industrially or commercially manufactured antibiotic discs (ABTEK- BIOLOGICAL Ltd LIVERPOOL, UK) with the following drug contents: Penicillin(10µg) ,Erythromycin(15µg), Chloramphenicol(10µg), Tetracycline (30µg), Ampicillin(30µg), Streptomycin (10µg),Ciprofloxacin(5µg), Augmentin (10 µg), Gentamicin(10µg), Vancomycin(30 µg). The antibacterial testing was carried out on fresh isolates of *S. aureus* on Mueller Hinton agar at 37°C for 24 hours.

3.13 Disc diffusion techniques

Disc diffusion techniques was used to test routinely for antimicrobial susceptibility. A disc of blotting paper was impregnated with a known volume and appropriate concentration of an antimicrobial, and this was placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffused from the disc into the medium and the growth of the test organism was inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial were inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grew up to edge of the disc.

3.13) Standardization of the inoculum

The inocula were prepared from pure isolates grown on nutrient agar at 37°C for 24 hours and or on Mannitol salt agar. Five pure colonies of each strain on the 24 hours old cultural plates were randomly selected, touched and inoculated into 5 ml of sterile 0.85% normal saline in bijoux bottles. The turbidity of the cell suspension was adjusted to match that of a 0.5Mc Farland Barium Sulphate standard containing approximately 1x 10^6 cells/ml of the inoculum (Bauer. A; 1966). A sterile cotton swab was dipped into the standardised respective microbial suspension; drained off and then used to inoculate the dry surface of the Mueller Hinton agar plate. The antibacterial discs were each aseptically placed on the inoculated plate using a sterile forcep and then incubated at 37°C for 24 hours.

After incubation at 35 °C for 24 hours, zone sizes were measured and interpreted usingNCCLS standards since World Health Organization (WHO)

recommends the National Committee for Clinical Laboratory Standards. (NCCLS) modified Kirby-Bauer disc diffusion technique (Bauer. A; 1966) for clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories.These are derived from the correlation which exists between zone sizes and MICs (Minimium Inhibitory Concentrations). An approximately linear relationship exists between log MIC as measured by the dilution test, and the inhibition zone diameter in the diffusion test. A regression line expressing this relationship can be obtained by testing a large number of strains by both techniques. This has been done and enables zone diameter sizes to be correlated to MIC values in the NCCLS modified Kirby-Bauer technique.

3.14 Procedures of antimicrobial susceptibility testing.

-Using a sterile wire loop, 3- 5 colonies well-isolated colonies of similar appearance to the test organism were emulsified in 3–4 ml of sterile physiological saline or nutrient broth.

-In a good light, the turbidity of the suspension was matched to the turbidity standard (mix the standard immediately before use).

-Using a sterile swab, a plate of Mueller Hinton agar was inoculated. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The swab was streaked evenly over the surface of then medium in three directions, rotating the plate approximately 60° to ensure even distribution.

-With the Petri dish lid in place, allow 3–5 minutes (*no longer than 15 minutes*) for the surface of the agar to dry.

-Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, the appropriate antimicrobial discs were placed, evenly distributed on the inoculated plate. The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.

- Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35 °C for16–18 h (temperatures over 35 °C invalidate results for oxacillin).

-After overnight incubation, the control and test plates were examined to ensure that growth is confluent or near confluent. Using a ruler on the underside of the plate, the diameter of each zone of inhibition in millimeters were measured noting that the endpoint of inhibition is where growth starts.

3.14.1) Reading Plates and Interpretation of zone sizes

Using the Interpretative Chart, the zones sizes of each antimicrobial was interpreted and reported as Resistant, Intermediate/Moderately susceptible, Susceptible.

A pathogen reported as ‘resistant’ implies that the infection it has caused will not respond to treatment

with the drug to which it is resistant irrespective of dose or site of infection.

A pathogen reported as intermediately susceptible suggests that the infection it has caused is likely to respond to treatment when the drug is used in larger doses than normal or when the drug is concentrated at the site of infection.

4-DATA PRESENTATION AND RESULTS

4.1 - Identification of respondents

A total of 58 positive culture respondents were isolated using the Mannitol salt agar. The participants are predominantly in the age groups18-50years.

In the antibacterial susceptibility testing, there was 100% susceptibility to vancomycin. Gentamicin (91.4%), Augmentin (89.7%), ciprofloxacin (86.2%) and streptomycin (82.7%) were also highly effective against the 58 isolates of *S. aureus* tested. Percentages of susceptibility were low (below 50%) for ampicillin, tetracycline, chloramphenicol, erythromycin, and penicillin (Table I).

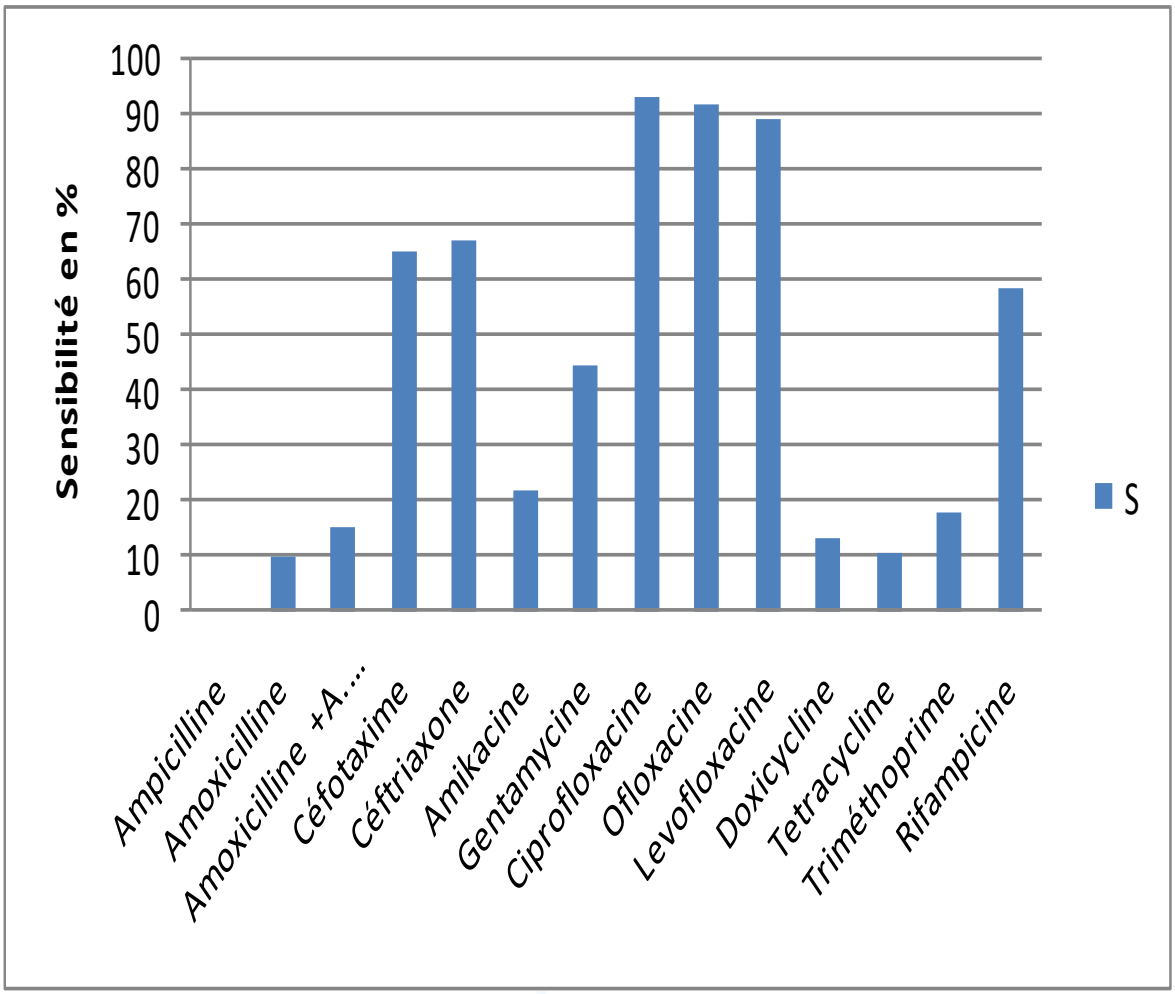
IQ RESEARCH JOURNAL

Table 1: Distribution of 113Staphylococcus aureuesaccording to antibiotic susceptibility.

Parameters / Antibiotics	SENSITIVE	INTERMEDIATE	RESISTANCE	Total (%)
vancomycin	58(100 %)	00(0 %)	00(0 %)	58(100%)
Gentamicin	53(91.4 %)	03(5.2 %)	02(3.4 %)	58(100%)
Augmentin	52(89.7 %)	04(6.9%)	0 2(3.4%)	58(100%)
Ciprofloxacin	50(86.2 %)	05(8.6 %)	03(5.2 %)	58(100%)

Streptomycin	48(82.7 %)	05(8.6%)	05(8.6 %)	58(100%)
Ampicillin	10(17.2%)	00(0%)	48(82.8%)	58(100%)
Tetracycline	08(13.8%)	08(13.8 %)	42(72.4 %)	58(100%
Chloramphenicol	07(12.1 %)	10(17.2 %)	41(70.0 %)	58(100%)
Erythromycin	7(12.1 %)	11(18.9 %)	40(68.9 %)	58(100%)
Penicillin	00(0%)	00(0%)	58(100%)	58(100%)

Figure-2: Chart distribution of 58 *Staphylococcus aureus* according to antibiotic susceptibility.



J O U R N A L

S = susceptible I = moderate susceptibility R = resistant

4.3 DISCUSSION

In this study, the result of the anti-bacterial susceptibility showed that vancomycin, gentamicin, augmentin, ciprofloxacin and streptomycin were highly effective (>80% sensitivity) against. *S. aureus*strains. High resistance was recorder forerythromycin (68.9%), chloramphenicol (70.0%), Tetracycline(72.4%), Ampicillin (82.8%) and penicillin (100%).

These results are similar to the findings of in Brazil (Sattar et al.; 2005) in Islamabad (Groppo et al.; 2005)where *S. aureus*was also reported to be highly resistant to penicillin, ampicillin and erythromycin. Findings in Kuwait show 100% susceptibility to

glycopeptides, 84% resistance to gentamycin and 96% resistance to erythromycin by isolates of *S. aureus* (Al Sweich et al.; 2005). Studies carried out inYaoundé, Cameroon showed high resistance to penicillin followed by ampicillin to *Staphylococcus aureus (*(Eyoh et al ;2003) which are almost similar to this our study carried out in Ndop.Traditional medicine and drug abuse may greatly contribute to drug resistance development in this environment.

These results obtained in Ndop contradicts the findings in Dschang town, Cameroon (Asakizi et al; 2007), where *S.aureus* was reported to be highly sensitive to amoxycillin – clavulanic acid (87.7%) and Rifampicin (81.3%), and instead are in agreement with resistant to penicillin as in our studies.

Changing patterns of Staphylococcus aureus resistance to drugs continues to pose a problem to health care providers globally despite the development of new antibiotics. These high rates of resistance have been attributed to factors such as misuse of these drugs by patients, unskilled practitioners among others . Increased period of hospitalization, high morbidity and increased treatment cost has been associated to disease severity due to drug resistance.

Susceptibility to gentamicin might be due to the route of administration which hinder its frequent misuse while the high sensitivity observed in ciprofloxacin and others, has been attributed to the fact that it is a relatively an expensive drug, therefore less available for abuse .

4.4 Conclusion

At the end of our prospective study fifty eight (58) *S. aureus* isolated from endo-cervical swab samples and were subjected to antimicrobial susceptibility in-vitro testing. The monitoring of these developments is necessary to verify the validity of protocols to the first-line treatment. The irregularity of the sensitivity of the bacteria to antibiotics coupled with the involvement of this species in most infections imposes a periodic reassessment of their sensitivity to antibiotics.

The variability of the susceptibility of *S. aureus* from region to region is a pointer to the fact that a rational and successful therapy of female genital microbial infection warrants the results of microbial analysis. In this study vancomycin, gentamycin, Augmentin, Ciprofloxacin, and Streptomycin were highly effective against *S. aureus* strains.

4.5- **Recommendations**

At the end of this study, we make the following recommendations:

i) Laboratory technicians:

-Always follow good laboratory practice;

-Be more supportive in the work;

-Repeat as often as possible similar studies to monitor levels of bacterial resistance.

ii) Prescribers:

-Avoid systematic prescription of a type or family of ATB;

-Ask the possible susceptibility testing before considering antibiotic therapy;

iii) At the direction of the Saint John the Baptist Health Centre in Ndop:

-Provide laboratory reagents and consumables sufficient quality and quantity to enable it to fulfill its role of regional reference laboratory;

-Building an archive room of the laboratory;

-The extension of the results to all medical and paramedical personnel.

iv) Ministry of Health:

-Implement adequate means of epidemiological surveillance antibiotic sensitivity of bacterial strains isolated in laboratories;

-Finance study to study the phenotypes of resistance to study the evolution of mechanisms of resistance of bacteria isolated.

v) Populations:

-Avoid self medication.

References

[1] -A. N. Asakizi, C N Fusi Ngwa, V K Payne (2007). Antibiotic resistance in Staphylococcus aureus involved in vaginitis: Case study of Dschang Town, Cameroon. Cameroon Journal of Experimental Biology Vol. 3 (1) 2007: pp. 20-25.

[2] -Andrews J. (2005). Standardize method for disc susceptibility testing Antimicrobial Chemotherapy. BSAC Standardize method development centre. City hospital NHS trust. Dudley road. Birmingam B187QH.

[3] - Bauer. A. W; Kirby, Qnim; sherrie J. C., Turck. M; (1966). Antibiotics susceptibility testing by a standardized single disk method American Journal of clinical pathology 45:493-6.

[4] - Cooper R.A., The contribution of microbial virulence to wound infection, In: White RJ,ed. The Silver Book. Dinton, Salisbury, UK: Quay Books, 2003;

[5] - Chawla R, Sahoo U, Arora A, Sharma PC, Vijayaraj R, Acta Pol. Pharm. Drug Res., 2010,67(1), 55-61.

[6] -Collee G, Duguid P, Fraser G, Marmian P. *Mackey and MacCartney's practical medical microbiology*, 14th ed., vol.II. Singapore: Churchill Livingstone Publishers. Longman; 2003.

[7] -Cheesbrough, M. (1985). Medical laboratory manual for tropical countries vol II. English Launguage Book Society (ELBS). Tropical Health Technology. Better worths. 420p.

[8] -Daniyan S.Y , Galadima, M. 1Ijah, U.J.J, Odama, L.E (2010). In-vitro Susceptibility of Oxacilin, Vancomycin and Teicoplaninagainst Clinical strains of *Staphylococci.*Department of Microbiology, Federal Univerity OF Technology P.M.B. 65, Minna, Nigeria

[9] -Eyoh Agnes B., Michel Toukam 1, Hortense Gonsu 1, Charles Fokunang 1, Julius Atashili2, Emilia E. Lyonga1, 2 George Ikomey 1, 2, Bertha Mukwele2,Martha -Mesembe2, Nguefack Tsague1, Henshaw Mandi3, Marie Claire Okomo Assoumou (2013). Antibiotics Susceptibility Profile of Staphylococcus Aureus Isolated from the Anterior Nares of Hospital Personnel in Yaounde, Cameroon. Center for the study and control of communicable diseases (CSCCD). Faculty of Medicine and Biomedical Sciences, University of Yaounde 1.

[10] - Fritsche T.R., Moet G., Sader H.S. Geographic occurence and resistance patterns among pathogens isolated from the SENTRY antimicrobial surveillance program 2004, 423, 43rd IDSA, San Francisco, 6-9 oct. 2005;

[11] - Jawetz; Menich; Adelbergs; (1991). Medical microbiology. 19th ed. Apppelletion and Lange Medical Book, Prentrice-Hall international Inc, New-York. P, 1 – 400.

[12] - Nicolle LE. *UrolClin North Am..,* 2008, *35* (1): 1–12.

[13] - Shadomy. S., Espinel Ingroff A., Cartwright. R. Y.,(1985). Laboratory studies with antifungal agents: Susceptibility test and Bioassays, In E.H. Lenette; A.Balows; W. J. Hauster. Jr., Beek. H. J. Shadomy (eds). Manual of Clinical microbiology. ASM PressWashington D.C: 991-999.

Appendix-1

Table 2**:** Interpretative break point for *S. aureus* strains.

Antimicrobials	Disc potency	Zone diameter of growth inhibition (mm).		
		Resistant (<)	Intermediate	Sensitive(>)
Amikacin	10µg	18	-	24
Ampicillin	10µg	24	-	35
Bactracin	10 Unit	17	-	22
Cephalothin	30µg	25	-	37
Chloramphenicol	10µg	17	-	18
Clindamycin	2µg	23	-	29

Erythromycin	15µg	23	-	30
Gentamycin	10µg	19	-	27
Kanamycin	30µg	19	-	26
Methicillin	5µg	17	-	22
Neomycin	30µg	18	-	26
Augmentin	10µg	18	-	24
Penicillin	10µg	26	-	37
Polymycin B	300 Units	7	-	13
Streptomycin	10µg	14	-	22
Sulfamethoxazole-trimethopim	25µg	24	-	32
Tetracycline	30µg	19	-	22
Tobramycin	10µg	19	-	29
Ciprofloxacin	1µg	17	-	19
Vancomycin	30µg	15	-	19

IQ RESEARCH JOURNAL