

Biochemical Characterization and Antibiotic Susceptibility Profile of *Staphylococcus Aureus* Isolated From Patients Attending Barau Dikko Specialist Hospital, Kaduna State, Nigeria

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Abstract: The research work titled Biochemical Characterization and Antibiotic Susceptibility Profile of *Staphylococcus aureus* isolated from Patients Attending Barau Dikko Specialist Hospital, Kaduna State, Nigeria was carried out. A total of 40 samples were screened and biochemically characterized using standard procedures, out of which 34 tested positive. The percentage prevalence of *Staphylococcus aureus* was found to be 85%, where males and females recorded prevalence of 37.5% and 47.5% respectively. The antimicrobial susceptibility of the isolated bacterium showed that the isolates are highly sensitive to Ciprofloxacin, Norfloxacin, Streptomycin, Rifampicin, Erythromycin and Chloramphenicol. Whereas, Levofloxacin, Amoxicillin and Gentamicin recorded moderate activity against the isolates. While, the isolates were found to be resistant to Ampiclox.

Keywords: Antibiotic, Bacteria, Biochemical, Isolation, *Staphylococcus*, Susceptibility.

I. INTRODUCTION

Staphylococcus aureus (literally the “golden cluster seed” or “the seed gold” and also known as “golden staph” and Oro Staphira) is a facultative anaerobic, gram-positive coccus and is the most common cause of staphylococcal infections. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence from skin, soft tissue, respiratory, bone joint, endovascular to wound infections. It shows golden coloration when grown on Mannitol Salt Agar, often with hemolysis when grown on blood agar plates. *Staphylococcus aureus* is a catalase positive, so is able to convert hydrogen peroxide (H₂O₂) to water (H₂O) and Oxygen (O₂), which makes the catalase test useful to distinguish Staphylococci from Enterococci and Streptococci. A small percentage of *S. aureus* is primary coagulase-positive that causes clot formation, whereas most other *Staphylococcus species* are coagulase-negative (Ryan and Ray, 2004).

It is frequently part of the skin flora found in the nose and on skin. Approximately, 15% of normal healthy adults are persistent nasopharyngeal carriers of *Staphylococcus aureus*, with a higher incidence reported for hospitalized patients, medical personnel, persons with eczematous skin disease, and those who regularly use needles either illicitly (e.g. drug abusers) or for medical reasons (patients with insulin-dependent diabetes, patients receiving allergy injections, and those undergoing hemodialysis) (Murray *et al.*, 2002).

Because Staphylococci are found on the skin and in nasopharynx, shedding of the bacteria is common and is responsible for many hospital-acquired infections. Staphylococci are susceptible to high temperatures, as well as to disinfectants and antiseptic solutions, however, the organisms can survive on dry surface for long periods. The organism can be transferred to a susceptible person through direct contact or through contact with fomites. Therefore, medical personnel must use proper hand washing techniques to prevent the transfer of Staphylococci from themselves to patients or among patients (Kloos and Lambe, 1991; and Murray *et al.*, 2002).

About 20% of the human populations are long-term carrier of *Staphylococcus aureus*. The carotenoid pigment staphyloxanthin is responsible for its characteristics golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbes evade death by reaction oxygen species used by the host immune system. Staphylococci which lack the pigment are more easily killed by host defenses. *S. aureus* is a genus comprising of aerobic, facultative anaerobic, catalase positive, gram positive cocci ranging from 0.5–1mm in diameter. The cocci are mainly arranged in grapelike clusters, but some when examine in pathological specimens, may occur as single cells or pairs of cells. The organisms are non-sporing, non-motile and usually non-capsulated (Clauditz, 2006; and Greenwood *et al.*, 2002).

Currently, 31 species have been placed in the genus staphylococcus, but most important human pathogens are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, and *Staphylococcus hominis*, close relatives that are common skin flora, and *Staphylococcus saprophyticus*. Of these, *Staphylococcus aureus* is considered the most serious pathogen (Talaro and Talaro, 2002). Staphylococci are ubiquitous organisms present on the skin and mucous membranes and their introduction through breaks in the skin occurs frequently. However the number of organisms required establishing an infection (infections dose) is generally large unless a foreign body is present in the wound (e.g. dirt, a splinter) (Murray *et al.*, 2002).

Based on growth and physiological characteristics, *Staphylococcus aureus* grows in large, round, opaque colonies at an optimum temperature of 37°C, though it can grow anywhere between 10°C and 46°C. The growth of the species is enhanced in the presence of oxygen (O₂) and carbon dioxide (CO₂). They can digest proteins and liquids and ferment sugars (Talaro and Talaro, 2002).

The treatment of choice for *Staphylococcus aureus* infection is penicillin, but in most countries, penicillin resistant is extremely common and first line therapy is most commonly penicillinase-resistant penicillin (for example, Oxacillin or Flucloxacillin). Combination therapy with gentamicin may be used to treat serious infection like endocarditis. The duration of treatment depends on the site of infection and on severity. Staphylococci have demonstrated the remarkable ability to develop resistant to most antibiotics (Murray *et al.*, 2002). Patients who are found to carry resistant strain of *Staphylococcus aureus* may be required to undergo “eradication therapy” which may include antiseptic washers and shampoos (such as chlorohexidine) and application of topical antibiotic ointment such as mupirocin or neomycin to the anterior nares of the nose (Kloos and Lambe, 1991).

Staphylococci are resistant to dry heat condition and high salt concentration (7.5 - 10%) and are well suited to their ecological niche, which is the skin. *Staphylococcus aureus* is present in the nose of 30% of healthy people. It causes infection most commonly at sites of lowered host resistance, for instance in damage skin or mucous membranes. Organisms are spread from these sites into the environment by handkerchief, clothing and dust consisting of cloth fibres (Greenwood *et al.*, 2002).

Staphylococci contain antigenic polysaccharides as well as other substances important in cell wall structure. Peptidoglycan, a polysaccharide polymer containing linked sub units, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acid or exposure to lysozyme. It is important in the pathogenesis of the infection (Brooks *et al.*, 2004; and Johnson *et al.*, 2001).

Teichoic acid, which are polymers of glycerol or ribitol phosphate are linked to the peptidoglycan and can be antigenic. Some *Staphylococcus aureus* have capsules which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present (Brooks *et al.*, 2004).

Staphylococcus aureus is the first most common important causative agent of bacterial skin infections and other respiratory tract complications as well as variety of nosocomial infections. The research focuses on the laboratory identification and determination of the antimicrobial sensitivity profile of the bacterium (Johnson, 2001).

Despite advances in recent decades in management as well as use of new and effective antimicrobials people with bacterial skin infections, Staphylococcal Scalded-Skin Syndrome (SSSS), Toxic Shock Syndrome (TSS) and respiratory diseases, still incur significant morbidity and mortality in Tropical countries like Nigeria. *Staphylococcus aureus* was found to be a very important culprit of these complications which need to be extensively studied in order to determine the most effective antimicrobials that can be used to treat infections caused by the bacterium.

Microbial resistance to antimicrobials is a matter of great importance, especially if sensitive strains are supplanted/replaced by the resistant ones, then a valuable drug may become useless (Laurence and Bennette, 1993). Therefore, this survey aimed at the determination of the antimicrobial sensitivity pattern of the *Staphylococcus aureus* with emphasis on the determination of the most effective drugs that can be used as first-line drugs of choice in the treatment of the infections caused by the bacterium as well as determination of the possible emergence of resistance to the previously active drugs by the bacterium. Also, due to rapid increase in the development of the Staphylococcal diseases, this research becomes an imperative tool in the medical and paramedical fields.

This research work is aimed to determine the antimicrobial susceptibility profile of *Staphylococcus aureus* isolates with view to determine the prevalence; isolate and biochemically characterize pure cultures of *Staphylococcus aureus*; and subject the isolates to the antibiotic sensitivity test.

II. MATERIALS AND METHODS

A total of 40 samples (27 Skin and 13 Nasal swabs) were collected for the *Staphylococcus aureus* screening at the Skin Clinic, Barau Dikko Specialist Hospital, Kaduna. The samples were collected using sterile swab sticks. Consent forms were distributed to the patients in the skin clinics, the forms were filled and returned before samples were collected. All samples collected were transported to the Microbiology Laboratory, Kaduna State University, Kaduna and were cultured immediately.

Mannitol Salt Agar (MSA) was prepared based on the manufacturers' instructions by weighing 108g of the powdered medium and suspended in 1litre of distilled water and sterilized using an autoclave (Equitron Your Quality of Assurance MODEL[®], USA) for 15 minutes at 121°C. The sampling swab sticks were aseptically inoculated on Mannitol Salt Agar and incubated at 37°C for 24 hours under aerobic condition.

The incubated plates were examined for the appearance of the golden yellow colonies on Mannitol Salt Agar. Suspected colonies were Gram stained and examined microscopically for typical staphylococci. Each colony was sub-cultured into Nutrient Agar in preparation for Catalase, Coagulase and Antibiotics Sensitivity Tests as adopted by Brook *et al.*, (2004).

1. BIOCHEMICAL CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATES:

All isolates suspected to be *Staphylococcus aureus* on the basis of characteristic reaction on Mannitol Salt Agar and microscopic observations were subjected to catalase and coagulase test as described by Brooks *et al.*, (2004).

Catalase Test:

An inoculum from the growth colonies of the test organism was removed from a sub-cultured Mannitol Salt Agar plate using a flamed-sterilized wire loop and placed in a drop of 3% hydrogen peroxide (H₂O₂) on a clean glass slide. Bubbling (release of oxygen) of the mixture indicated a positive test which confirmed the presence of *Staphylococcus aureus* as adopted by Cheesbrough, (2000).

Coagulase Test:

A clean glass slide was divided into two partitions with the aid of grease free pencil. Two drops of physiological saline was added about 2cm apart on the glass slide. One colony of each isolates was carefully emulsified in each drop of the saline and a loopful of citrated human plasma was dispensed onto in the bacterial suspension on one side of the slide and mixed using a wire loop. The slide was gently held for about a minute. Clumping of the plasma cells indicated coagulase positivity confirming *Staphylococcus aureus* (Cheesbrough, 2000).

2. ANTIBIOTIC SENSITIVITY TESTING

Murray *et al.*, (2002) described the antimicrobial susceptibility pattern of *Staphylococcus aureus* to be determined using Kirby-Bauer method. The antimicrobial discs were dispensed onto the surface of the Nutrient Agar plates already inoculated with the test organisms by streaking using a flamed-sterilized wire loop. With the use of a sterile forceps as a dispenser, each disc (OPTUDISC[®], USA) was mounted and pressed down to ensure complete contact with the Agar surface. All plates were allowed for 20 – 30 minutes for pre-diffusion and later incubated at 37°C for 24 hours. Then, the average zones of growth inhibition of *Staphylococcus aureus* were measured with the aid of a meter rule in millimeters. The zones of inhibition were determined using the recommendations of National Committee for Clinical Laboratory Standards (NCCLS, 2004).

III. RESULTS

The results obtained from this study showed that; out of forty (40) samples screened, 34 (85%) were found to be *Staphylococcus aureus* isolates. These were identified based on the preliminary cultural appearance, morphological characteristics, Gram reactions, Catalase and Coagulase tests.

Table I shows the different antimicrobials used for the study and their corresponding concentrations. The results from the study of the antimicrobial susceptibility of *Staphylococcus aureus* shows the percentage prevalence of the test organism for the samples collected in relation to gender in the study area as shown in table II. Table III shows the antibiotic susceptibility profile of the positive isolates. Fig 1 shows a bar-chart showing antimicrobial sensitivity of the isolated *Staphylococcus aureus*. Fig 2 shows a column chart showing antimicrobial resistance and sensitivity on *Staphylococcus aureus*.

TABLE I: ANTIBIOTICS USED AND THEIR VARIOUS CONCENTRATIONS

S/N	ANTIBIOTICS	ABREVIATION	Potency (µg)
1	Ciprofloxacin	CPX	10
2	Norfloxacin	NB	10
3	Gentamicin	CN	10
4	Amoxicillin	AML	20
5	Streptomycin	S	30
6	Rifampicin	RD	20
7	Erythromycin	E	30
8	Chloramphenicol	CH	30
9	Ampiclox	APX	20
10	Levofloxacin	LEV	20

(NCCLS, 2004)

TABLE II: PERCENTAGE PREVALENCE OF STAPHYLOCOCCUS AUREUS BASED ON GENDER

Sex	No. of samples	Skin	Nostril	No. Positive	(%) Prevalence
Male	19	13	6	15	37.5
Female	21	14	7	19	47.5
Total	40	27	13	34	85

TABLE III: ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *STAPHYLOCOCCUS AUREUS* ISOLATES.

ANTIBIOTICS	RESISTANCE	INTERMEDIATE	SENSITIVE
Ciprofloxacin	0 (0%)	0 (0%)	34 (100%)
Norfloxacin	0 (0%)	0 (0%)	34 (100%)
Gentamicin	11 (32.55%)	6 (17.64%)	17 (50.0%)
Streptomycin	0 (0%)	0 (0%)	34 (100%)
Rifampicin	0 (0%)	0 (0%)	34 (100%)
Amoxicillin	13 (38.23%)	3 (8.82%)	18 (52.94%)
Erythromycin	0 (0%)	0 (0%)	34 (100%)
Chloramphenicol	0 (0%)	0 (0%)	34 (100%)
Ampiclox	19 (55.88%)	1 (2.94%)	14 (41.17%)
Levofloxacin	4 (11.76%)	10 (29.41%)	20 (58.82%)

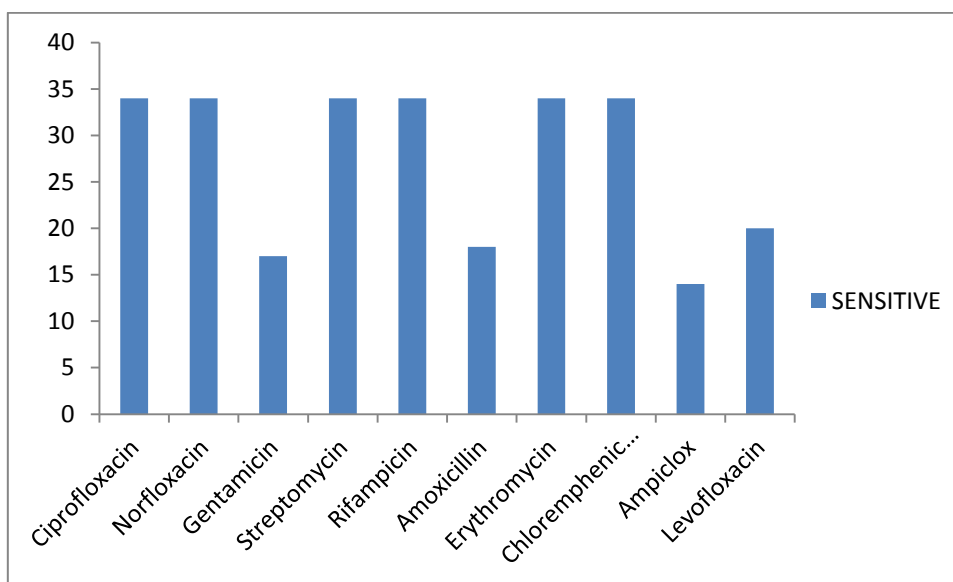


FIG 1: A BAR-CHART SHOWING ANTIMICROBIAL SENSITIVITY OF THE ISOLATED *STAPHYLOCOCCUS AUREUS*

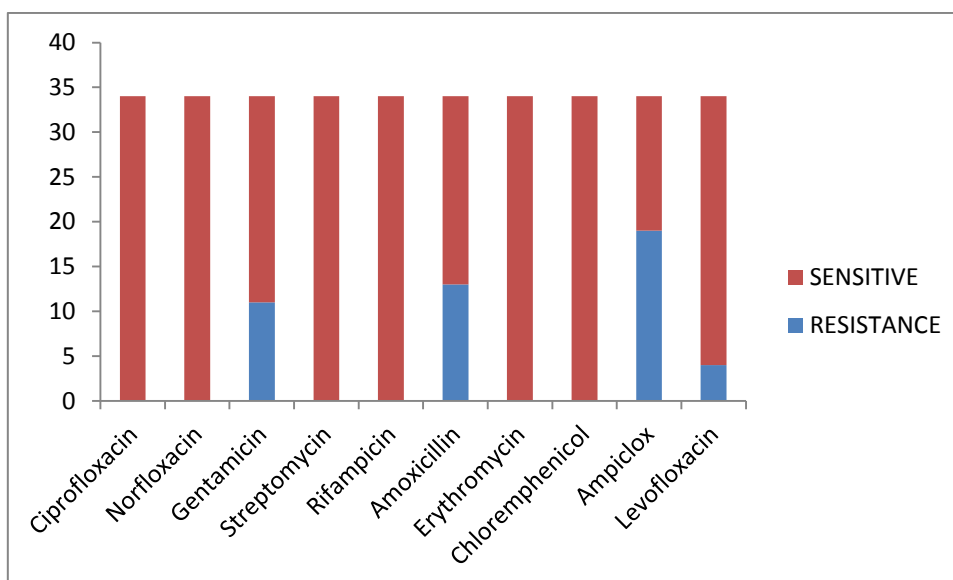


FIG. 2: A COLUMN CHART SHOWING ANTIMICROBIAL RESISTANCE AND SENSITIVITY ON *STAPHYLOCOCCUS AUREUS*

IV. DISCUSSIONS AND RECOMMENDATIONS

Based on the findings of the research study, it was found that, from the percentage prevalence of both males and females, there is higher percentage prevalence in females (47.5%) than males (37.5%). The overall prevalence of *Staphylococcus aureus* in the study area was found to be 85% as recorded in table II. This deviates from the findings of Chigbu and Ezeronye (2009), who reported that in non-hospital environment, the prevalence was 28% and the low prevalence can be as a result of several factors such as environmental conditions.

The percentage susceptibility of the ten (10) antimicrobials used for sensitivity testing as determined in this study; Ciprofloxacin (100%), Norfloxacin (100%), Streptomycin (100%), Rifampicin (100%), Erythromycin (100%), and Chloramphenicol (100%) recorded high activity against the isolates, followed by Levofloxacin (58.82%), Amoxicillin (52.9%), Gentamicin (50%), and the isolates recorded percentage susceptibility to Ampiclox (41.17%) as illustrated in Table III. This concurs with the findings of Murray *et al.*, (2002) who reported that, Erythromycin is effective against gram-positive organism especially staphylococci. Also, Johnson, (2001) and Murray, (2002) recommended the use of gentamicin in the treatment of staphylococcal endocarditis.

Amongst the ten (10) antimicrobials used for sensitivity testing, Ciprofloxacin, Norfloxacin, Streptomycin, Rifampicin, Erythromycin and Chloramphenicol show appreciable mean zones of growth inhibition and followed by Levofloxacin, Amoxicillin, and Gentamicin. Therefore, they are drugs of choice for treating Staphylococcal infections. Ampiclox recorded the lowest mean zones of growth inhibition against the tested organisms indicating a relative resistance. This conformed to the findings of Murray, (2002) who reported that the treatment of choice for *Staphylococcus aureus* infection is penicillin, but in most countries, penicillin resistant is extremely common due to the increase occurrence of penicillinase- producing staphylococcal species. World Health Organization (WHO, 2002) also reported that, many pathogens develop resistance to previously active antimicrobials. As a microbial population is exposed to an antibiotic, more susceptible organisms will succumb leaving behind only those resistant to the agent. These organisms can either pass on their resistance genes to their offspring by replication or to their related bacteria through conjugation, whereby plasmids carrying genes “jump from one organism to another” (WHO, 2002).

The findings as recorded in this study appeared to be contrary to the report in which glycopeptides antibiotics such as Vancomycin was reported as the only drug to which methicillin-resistant *Staphylococcus aureus* is susceptible to (Mehta *et al.*, 1998; and Saeed, 2007). Ciprofloxacin according to previous studies had always been a drug of choice with 100% but in the case of this study, it appears that Streptomycin, Chloramphenicol, Norfloxacin, Erythromycin and Rifampicin recorded highest percentage susceptibility of 100%, therefore, these drugs are first-line drugs of choice that can be used for treating Staphylococcal infections. Ciprofloxacin, as stated by Maxheal pharmaceuticals, is an agent with potent activity against bacteria including *Pseudomonas aeruginosa*, enterobacteriaceae and *S. aureus*. Its bacterial spectrum is wider than that of Aminoglycosides, third generation cephalosporins and other fluoroquinolones (Saeed, 2007).

A comparative of this work with some other previous work gave an explanation that, the discrepancies (resistance and susceptibility) of *Staphylococcus aureus* to Ciprofloxacin and other drugs (antimicrobials) can arise considering factors such as the type of in vitro system used, phenotypic and genotypic heterogeneity of bacteria tested, method of drug administration and duration of the experiments (Marchbank *et al.*, 1993). Although, *Staphylococcus aureus* from clinical specimens showed higher susceptibility to individual antibiotic when compared with others, higher percentage of multi drug resistance is also obtained (Majunder *et al.*, 2009).

Chigbu and Ezeronye (2003) reported that the nasal reservoir which appears to be a major source of dissemination is the reservoir from where infection usually arises. Selective treatment of the anterior nerve with topically applied antimicrobial agents do not only temporarily depresses the proliferation of nasal *Staphylococcus* but markedly reduce the frequency with which *Staphylococcus aureus* can be isolated from skin and the air around the treated carrier.

V. CONCLUSION

Based on the findings of the research study, *Staphylococcus aureus* was isolated from the study area, with overall prevalence of 85%, with highest prevalence of 47.5% for females and least prevalence of 37.5% for males. This may be due to the fact that the bacterium is a normal commensal found on the surface of human skin, which may become

pathogenic at certain threshold or in immunocompromised individuals. Also, socioeconomic status may be one of the predisposing factors, since majority of males in the study area are working class compared to most of the females who are housewives and students. The isolates of *Staphylococcus aureus* were found to be highly sensitive to Ciprofloxacin, Norfloxacin, Erythromycin, Streptomycin, Rifampicin and Chloramphenicol. While, Levofloxacin, Amoxicillin, Gentamicin and Ampiclox were found to be inactive against the isolated organisms. Therefore, it is concluded that all drugs that are active against the test organism can be used as first-line drugs of choice for the treatment of skin and nasal infections caused by *Staphylococcus aureus*, while those that showed relative inactivity should be discouraged in the treatment of infections caused by the bacterium. The findings of this research study will pave a way for the early detection of the emergence antibiotic resistance by *Staphylococcus aureus*, and can help clinicians and doctors on the right choice of antimicrobials to be prescribed to the patients suffering from staphylococcal infections. The research can be used by policy makers towards public enlightenment on the increase prevalence and dangers associated with *Staphylococcus aureus* diseases, as well as preventive measures. Researchers can also use the findings for further studies, since *Staphylococcus aureus* is a common normal flora of the body, and one of the predominant bacterial species that are associated with human skin, clothing and other fomites, which needs to be studied intensively in order to determine a proper treatment for the diseases caused by the bacterium, as well as proper preventive measures.

VI. RECOMMENDATIONS

1. Regular researches have to be maintained to determine antibiotic resistant *Staphylococcus* as soon as it is in existence.
2. Doctors and other health workers should have detailed information of recent researches carried out on this organism to help in administration of drugs.
3. Different routes of sanitary measures have to be improved so as to minimize the microbial load of the body which eventually results to the cause of these infections.
4. Proper hygiene needs to be adopted seriously in health institutions like clinics and hospitals, since these organisms are highly nosocomial that can spread from reservoirs to healthy persons.
5. Improvement of broad spectrum antibiotics in order to increase synergic means of treatment over infections caused from these sources of isolates.
6. Thorough checking has to become mandatory for individuals that are immunocompromised patients since these organisms (*Staphylococcus aureus*) tend to cause infection in them.

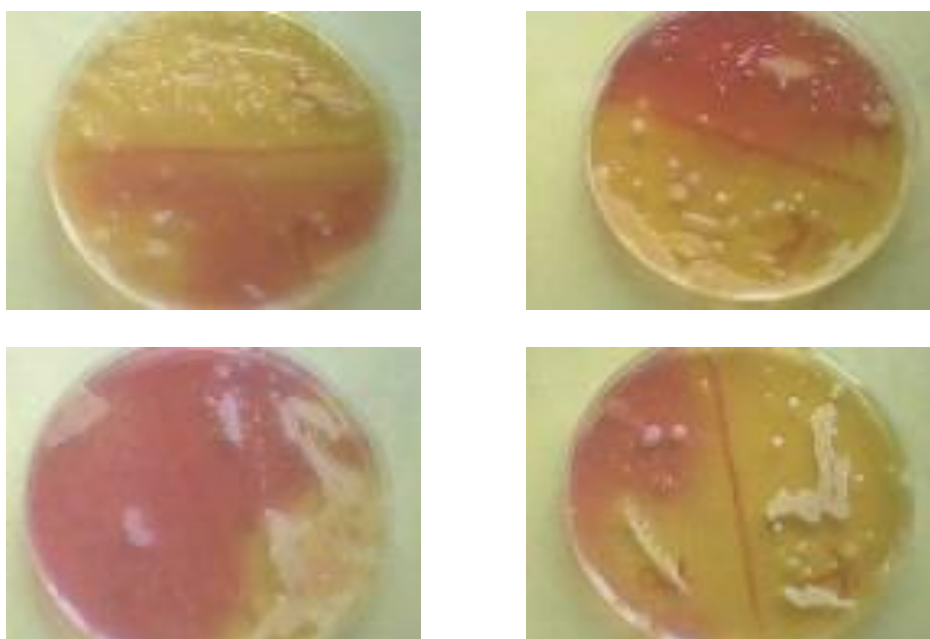


Plate 1. Cultural appearance of *Staphylococcus aureus* colonies on Mannitol Salt Agar

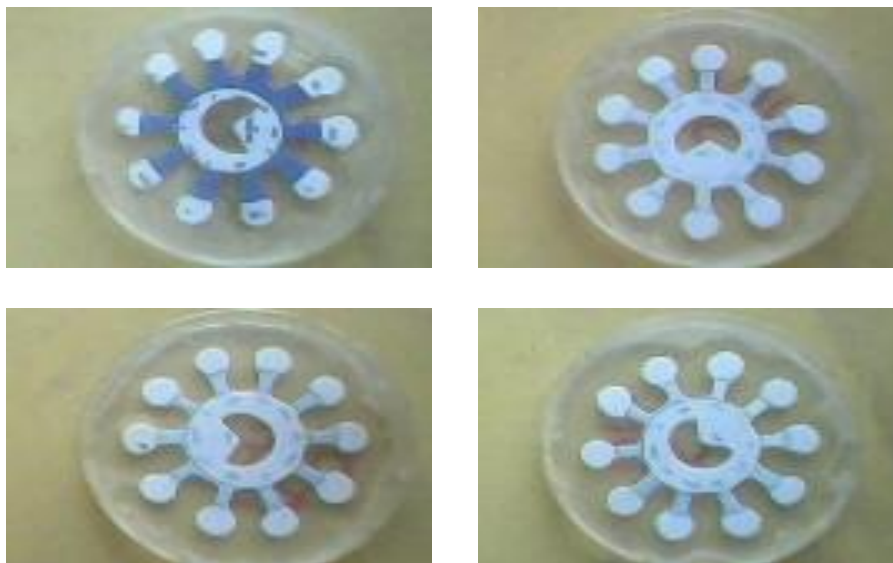


Plate 2. Antimicrobials showing zones of inhibition against *Staphylococcus aureus*

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International Journal of Novel Research in Life Sciences

Vol. 2, Issue 6, pp: (1-9), Month: November-December 2015, Available at: www.noveltyjournals.com

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