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Characterization and Antibiotic Sensitivity of Methicillin Resistant *Staphylococcus aureus* from Skin of Hospital Staff

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Abstract: This study was undertaken to investigate the characterization and antibiotic sensitivity of Methicillin-resistant *Staphylococcus aureus* from hospital staff of Federal Teaching Hospital Abakaliki, (FETHA II). A total of forty (40) samples were collected from the hospital staff twenty (20) each for skin and Nasal swab and transported using Staurt Transport Medium (STM) to Microbiology laboratory unit of Ebonyi State University, Abakaliki for bacteriological analysis. A total of (45%) and (33%) *Staphylococcus aureus* were isolated from skin and nasal swab samples. Antibiogram was determined using Kiraby-Bauer disc diffusion method with commonly used antibiotics; ampicillin, ciprofloxacin, levofloxacin erythromicin, norfloxacin, ofloxacin, ceftriaxone, ceftaxime, gentamycin and clindamycin. Highest resistance to the isolate was recorded amongst ceftriaxone (100%), ampicilin (83.3%), clindamycin (61.5%) and ceftaxime (55.5%). Highest susceptibility amongst norfloxacin (100%), ofloxacin(94.0%) and clindamycin (88.0%) antibiotics respectively. This study reported a high colonization of MRSA bacteria amongst hospital staff. The use of antibiotics especially in clinical settings with/without proper prescription and safety measure intensify the development of resistance bacteria and therefore this study recommend vital detection of bacteria in hospital in order to sustain the gains of antimicrobial chemotherapy.

Key words: Antibiotics · Sensitivity · Bacteriological · Resistance and bacteria

INTRODUCTION

Staphylococcus aureus is an ubiquitous gram positive bacteria, non-motile present asymptomatically in humans as normal flora, causing abundant pathological conditions including skin infections, empyema, pneumonia, meningitis, Conjuctivitis, especially in neonates, arthritis, endocarditis and osteomyelitis amongst others and it remains one of the most important pathogens implicated in Hospital-acquired and community-acquired infection [1]. Although Staphylococcus aureus infections were historically treatable with common antibiotics, emergence of drug-resistant organisms is now a major concern. MRSA is a strain of *Staphylococcus aureus* that is resistant to methicillin or β-lactamase resistant to penicillin. Usually these strains of *Staphylococcus aureus* are resistant to more than one antibiotic hence, the infections due to this strain of MRSA are very difficult to treat [2]. The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins, often called enzymes (clumping factor) used for their identification, [3].

Methicillin resistant *Staphylococcus aureus* (MRSA) started making wave soon after the introduction of methicillin (a more potent drug than penicillin) into clinical medicine in the early 1960's. MRSA started making rounds in the health sector and became a global public health issue in 1961 when the first strain of *S. aureus* that resisted the actions of methicillin was reported in the UK, thus making it the first report of MRSA in the world [4, 5].

Methicillin-Resistant *S. aureus* (MRSA) was recognised as a nosocomial pathogen and now represents a substantial proportion of *S. aureus* infections in hospitalized (in-patients) and community (out-patient) settings [6, 7]. Risk factors of healthcare associated

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MRSA(HA -MRSA) are well defined and included hospitalization, surgery, dialysis, residence in a long- term care facility and use of indwelling catheters or other percutaneous medical devices [8, 9]. Factors that appear to facilitate transmission of Community association MRSA (CA-MRSA) include frequent skin-to-skin contact, crowding, compromised skin integrity, sharing of potentially contaminated items and lack of personal hygiene (Dancer, 2008).Hospital-acquired (HA- MRSA) and Community-acquired (CA-MRSA) possess resistance to β-Lactam antimicrobial agents, conferred by the Staphylococcal cassette *mec* element [10]. However, CA-MRSA strains are typically less resistant to non- β-lactam antimicrobial agents [11].

In spite of the availability of considerable number of effective antimicrobial chemotherapeutic agents, MRSA still remains an important and increasing cause of post-surgical wound infections [11]. Some invasive infections such as nosocomial bacteremia and septicemia (sepsis) (Myelotte and Tayara, 2000), acute endocarditis and osteomyelitis, pneumonia and other soft tissue infections (STIs) [12, 13], are also traceable to them. Methicillin, a beta-lactam drug was introduced for clinical use following the resistance of pathogenic bacteria such as *S. aureus* to penicillin. Though implicated in some nosocomial infections, strains of *S. aureus* that are multidrug resistant known as hospital-acquired MRSA (HA-MRSA) now occur in the hospital and these are now widespread especially in places where antibiotics are used for other clinical purposes such as in treatment and prophylaxis of patient, thus exposing hospital staff to risk of harboring ubiquitous strains of Methicillin- resistant *Staphylococcus aureus* (MRSA). Prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stay reflects the fact that critically ill patients have a greater chance of becoming colonized or infected which may be agent necessary for rapid dissemination to hospital Staff through close contact and poor hygiene practice by some health workers, Once hospitals become heavily contaminated with MRSA, eradication is almost impossible, [14]. The proportion of MRSA among *S. aureus* isolates varies from country to country with increase morbidity and mortality associated with serious life-threatening infection and compromising human health. Therefore, this study is concerned with the increasing risk of MRSA and characterization of MRSA from hospital Staff in order to provide important baseline information that will be useful in MRSA control as well as selection of antimicrobials for empiric therapy.

Aim : To characterize methicillin resistant *S. aureus* from skin of hospital staff and obtain the antibiotic sensitivity of the bacterium isolated.

Specific Objectives:

- To determine the characterization of methicillin resistant *S. aureus* compared to other strains.
- To determine the multiple antibiotic resistance index (mari) of this pathogen.
- To determine the relationship that exists between this pathogen and the normal skin flora of the infected skin.
- To determine the effect of damage caused by the methiciilin resistant *S. aureus* on the skin.

MATERIALS AND METHODS

Materials: The materials used includes the following: beaker, glass test tubes, disposable petri-dishes, measuring cylinder, forceps, glass slides, conical flasks, spatula, pasture pipettes, meter rule, swab sticks, Bijou bottles, wire loops, masking tapes, markers, aluminum foil and cotton wool.

Equipment: Equipments used are as follows:

Microscope (Olympus, Germany) weighing balance, incubator (mereck, Germany,) autoclave and Bunsen burner.

Media: The following media were used.

Nutrient agar, Mannitol salt agar, Peptone water broth and Mueller - Hinton agar.

Chemicals and Reagents: The following chemical were used:

Crystal violet, lugol's iodine, acetone / alcohol, safranine, Kovac's reagent (Fisher scientific company USA), immersion oil, hydrogen peroxide, distilled and normal saline.

Antibiotics: The following antibiotics were used:

Erythromycin (10 µg), Ceftriaxone (30 µg), Ampicillin (30 µg) Cifixime (5 µg), levofloxacin (5 µg) Gentamicin (10 µg), Norfloxacin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg) and Clindarnycin (10 µg). The afore-mentioned antibiotics were procured from Oxoid, UK.

Methods

Study Area: The study was conducted in Federal Teaching Hospital Abakaliki (FETHA II), Ebonyi State, Nigeria. Ebonyi state is located in the South East zone of Nigeria. It was caved out of Abia and Enugu state on the

1st October, 1996 by then the military head of State, Gen. Abacha. It's name is derived from the Eboniy Rivers. The capital is Abakaliki. According to 2006 population and housing census, Ebonyi State is made up of 1,064,156 males and 1,112,791 female. There is an estimated population of 2.3 million and a land mass of 5,935km in Ebonyi State. Approximately 80% of the population of Ebonyi State lives in rural area and are farmers [15]. Federal teaching Hospital is situated along Enugu-Abakaliki express way, Abakaliki (FETHA II). The study was carried out at FETHA II annex of the Federal Teaching Hospital Abakaliki, Ebonyi State.

Sample Collection: Clinical samples were collected from different sites of the individual bodies such as the skin swab, nose swab from Hospital staff of FETHA II hospital Abakaliki. The samples after collection were immediately transported to the applied microbiology laboratory unit of Ebonyi State University for bacteriological analysis.

Preparation of Culture Media: All media used was prepared in line with the manufacturer's instructions.

Preparation of Nutrient Agar: This was prepared according to manufacturer's instruction and autoclaved at 121°C for 15 minutes, the media was allowed to cool up to 45°C. Twenty millimeter of the nutrient agar was aseptically poured into petri dish. It was allowed to gel and labeled.

Preparation of Peptone Water Broth: Peptone water broth was prepared by dissolving 15g of the media into l00mls of water and heated to dissolve completely. 5mls of the medium was dispensed into test tubes and cover with cotton woof. It was then sterilized by autoclaved at 121°C for 15 minutes. After cooling, sterile swab sticks containing the samples was inserted into the medium in the test tubes and labeled.

Preparation of Mannitol Salt Agar: A 111g of mannitol salt agar base was dissolved in 1000ml of water, allowed to mix by heating on a Bunsen burner flame to dissolve completely. It was then sterilized by autoclaving at 121°C for 15 minutes. It was allowed to cool at 45°C before it was poured aseptically in 20ml volume petri-dishes. The medium was allowed to gel [16].

Preparation of Mueller Hinton Agar: A 38g of muller-hinton was dissolved in 1000ml of distilled water. Heated to boil and dissolve completely. It was sterilized by autoclaving at 121°C for 15 minutes [17].

Sample Analysis: Samples collected with sterile swabs were first inoculated into test tubes containing 5ml each of peptone water broth and incubated at 37°C for 24hours. This was by way of enrichments. A loopful of the inoculum from peptone water broth was streaked onto mannitol salt agar and incubated at 37°C for 24hrs. The appearance of golden yellow colour on mannitol salt agar indicates the presence of *Stophylococcus spp.*

These bacteria growth were sub-culture onto nutrient agar severally for proper purification in order to get pure culture. Finally the isolates were transferred to nutrient agar slants from there Gram staining and other biochemical test were carried out.

Identification and Characterization of Isolates

Gram Staining: This was done on smears prepared from all samples in different culture plates. The smear preparation was made on a clean glass-slides. It was allowed to air dry and heat fixed by passing it on a flame of a bursen burner. The heat fixed smears were covered with crystal violet stain for 60 seconds and washed off under a slow running tap. The smears were covered with lugol's iodine for 60seconds and washed off with a slow running tap. The smears were decolourised using acetone and washed within a few seconds with a clean running tap water. The smears were covered finally with safranine for 60seconds and washed off with clean water. The smears were blotted dry and viewed using x100 objective lens of microscope with oil immersion and results of observation were recorded [18].

Biochemical Tests

CatalaseTest

Principles: Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen one released if the organism is a catalase producers.

Test: Several colonies of the test organism were picked with a sterilized wire loop and emulsified in a drop of normal saline on a slide. A drop of 3% hydrogen peroxide was added and observed for immediate bubbles [19].

Coagulase Test

Principle: This coagulase test identify *staphylococcus aureus* that produces the enzyme coagulase as it causes plasma to clump by converting fibrinogen to fibrin.

Test: Drop of sterile water was added on two slides separately and a growth of the test bacteria was used to emulsify in each of the drops to obtain a suspension that

is thick. Loopful of sleep blood plasma was placed on one of the suspension and observed for immediate clumping for 10 sec [20].

Preparation of 0.5 Mcfarland Turbidity Equivalent Standards: Tubidity standards equivalent of 0.5 Mcfarland standards was prepared by adding 1.0 ml of concentrated tetraoxosulphate (vi) acid (H_2S0_4) to 99 ml of distilled water. Then 0.5g of dehydrated barium chloride ($Bacl_2H_20$) was dissolved in 50 ml of distilled water in a separate flask.

A volume of 0.6ml of barium chloride solution was introduced into tetraoxosulphates (vi) acid solution (99.4ml) in a separate flask. It was mixed well to obtain 0.5 Mcfarland tubidity equivalent standards. Some portions of the mixture were transferred to test tubes and stored at room temperature (28°C). This was used to compare the tubidity of the isolates [22].

Standardization of Test Isolates: All the test isolates undergo standardization before use by inoculating 5ml normal saline in sterile test tubes with loopful of a 24hrs culture of the test Isolates from nutrient agar slant. Dilutions using loopful of the test bacteria and sterile water were done in order to achieve microbial population of 10^5 colony forming unit per milliiiter (CFU/ml) by comparing it with 0.5 Mcfarland tubidity standards [23].

Antibiotic Susceptibility Pattern Using Disc Diffusion Method: Twenty milliliter each of muellller-Hintom agar was poured aseptically into sterile petri-dishes and flamed with a bursen burner flame to remove air bubbles, allowed to gel and labeled. A sterile swab stick was used to inoculate the pre-culture of the Isolates on the plate of Mueller Hinton agar medium. The surface of the medium was streaked evenly in the directions, rotating the plate to ensure even distribution. The bacteria suspension was allowed to diffused with the medium for 15 minutes. Then a sterile forceps was used to place appropriate antibiotics disc and allowed to stay for 30 minutes, the plates were inverted and incubated at 37°C for 24hrs. A meter rule was used to measure the inhibition zone diameter of the antibiotics in milliliter (mm). The results were recorded as susceptible or resistance.

RESULTS

A total of forty (40) sample were aseptically collected with twenty (20) each for Skin and Nose of Hospital staff of Federal Teaching Hospital Abakaliki (FETHA II) for bacteriologies! analysis.

Table 1: Morphological and Biochemical Characteristic of the *S. aureus* Isolates from Skin Hospital Staff in Federal Teaching Hospital Abakaliki (FETHA II).

S/N	Colony Characteristics	Gram Reaction	Catalase Test	Coagulase Test	Suspected Organisms
1	Yellow, creamy and smooth	+	+	+	*S. aureus*
2	Yellows, glistering, dot and creamy	+	+	+	*S. aureus*
3	Pink, moist and rough	+	-	-	
4	Deep yellow, creamy and smooth	+	+	+	*S. aureus*
5	Creamy, clustered, yellow dots	+	+	+	*S. aureus*
6	Cocci shape, opaque yellow and creamy	+	+	+	*S. aureus*
7	Creamy, yellow dots and smooth	+	+	+	*S. aureus*
8	Creamy, yellow and smooth	+	+	+	*S. aureus*
9	Cocci shape, yellow and creamy	+	+	+	*S. aureus*
10	Deep yellow, creamy and cocci	+	+	+	*S. aureus*
11	Yellow dots and creamy	+	+	+	*S. aureus*
12	Pink, large and rough	+	-	-	
13	Small dots, orange and clustered	+	+	+	*S. aureus*
14	Small pink, orange in orange	+	+	+	*S. aureus*
15	Glistering surface, dots entire edge	+	+	+	*S. aureus*
16	Cocci shape, opaque yellow and creamy	+	+	+	*S. aureus*
17	Elevated and deep yellow	+	+	+	*S. aureus*
18	Creamy, deep yellow and cocci-shape	+	+	+	*S. aureus*
19	Pink, small dots and entire edge	+	+	+	*S. aureus*
20	Yellow, dots and creamy	+	+	+	*S. aureus*

The percentage distribution of *Staphylococcus aureus* isolate from hospital staff of federal Teaching Hospital Abakaliki (FETHA II) are presented below.

Table 2: Percentage distribution of isolate in sample

Sample Source	No. Samples	No(%)of *S. aureus*	Total (%)
Skin	20	18(45.0)	78.0%
Nasal Swab	20	13.(33.0)	

Antibiotics resistance pattern of Methicillin-resistant *Staphylococcus aureus* isolated from hospital staff of Federal teaching Hospital Abakaliki (FETHA II) are presented below.

Table 3: Antibiotics resistance pattern of MRSA isolated from Skin swab

Antibiotics (µg)	Resistance (%)	Intermediate (%)	Susceptible (%)
Ampiciliin 30	15(83.3)	1(5.5)	2(11.0)
Ceftriaxone 30	18(100)	0(0.0)	0(0.0)
Cefotaxime 5	10(55.5)	2(11.0)	6(33.3)
Ciprofioxacin 5	3(17.0)	4(22,2)	11(61.0)
Clindamycin 10	2(11-1)	0(0.0)	16(88.0)
Erythromycin 10	8(44.0)	5(28.0)	5(28.0)
Gentamycin 10	0(0.0)	0(0.0)	18(100)
Levofloxacin 5	7(39.0)	3(17.0)	8(44.0)
Norfloxacin 10	0(0.0)	0(0.0)	18(100)
Ofloxacin 5	1(5.5)	0(0.0)	17(94.0)

R = Resistance, 1= Intermediate, S= Susceptible. Number of isolate= 18

Antibiotics resistance pattern of Methicillin-resistant *Staphylococcus aureus* isolated from hospital staff of Federal teaching Hospital Abakaiiki (FETHA II) are presented below.

Table 4: Antibiotics resistance pattern of MRSA isolated from Nasal swab

Antibiotics (µg)	Resistance (%)	Intermediate (%)	Susceptible (%)
Ampicillin 30	13(100)	0(0.0)	0(0.0)
Ceftriaxone 30	12(92.3)	1(7.7)	0(0.0)
Ceftaxime 5	13(100)	0(0.00)	0(0.0)
Ciprofioxacin 5	5(38.7)	2(15.4)	6(46.2)
Clindamycin 10	8(61.5)	1(7.7)	4(30.8)
Erythromycin 10	0(0.0)	0(0.0)	13(100)
Gentamycin 10	0(0.0)	0(0.0)	13(100)
Levofloxacin 5	4(30.8)	2(15.4)	7(53.8)
Norfloxacin 10	0(0.0)	0(0.0)	13(100)
Ofloxacin 5	3(23.0)	0 (0.0)	10(76.0)

R = Resistance, I = intermediate, S= Susceptible, Number of isolate = 13

DISCUSSION

The emergence and spread of microbes that are resistant to cheap and effective first-choice drags has become a major occurrences. Out of forty (40) samples investigated for *Staphylococcus aureus,* 18(45.0%) from skin and 13(33.0%) from nasal swab were identified as *S. aureus* representing a total of (78.0%) of the isolate. In the present work, methicillin resistant *S. aureus* carriage rates varies among different hospital staff and it is lower than those found in studies conducted in Gaborone hospital staff, Botswana recorded about (35.8%) [24], Vaidivia hospital staff, Chile, at (34.9%) [25] and comparable to those of another study conducted in Chile at (27.5%) [26], a study in Nepal recorded 25% [27] and a study in Nairaobi hospital, Kenya at (18.3%) [28]. Different records among different countries and hospitals may be explained by microbiological methods (from sampling techniques to culture media) and local infection control standards. In view of the above Table 1 shows the biochemical characterization and colonial morphology of the *S. aureus* isolated from hospital staff of Federal Teaching Hospital. Abakaliki, (FETHA II). Table 2 shows percentage distribution of *S. aureus* in sample. Table 3 shows antibiotics resistance pattern of MRSA isolated from Skin swab with highest resistance to ceftriaxone (100%), ampicillin (83.3%) ceftaxime (55.5%) and erythromycin (44.0%). Highest susceptibility of the isolate to gentamycin (100%), norfloxacin (100%), ofloxacin (94.0%), clindamycin (88.0%) and ciprofloxacin (61.0%) was recorded amongst the antibiotics. A higher rate of resistance was observed in this study as compared to a report on health-care workers at Jimma specialized hospital with gentamicin at (57.1%), erythromicin (64.7%) and clindamycin at 72.4% [28]. Table 4 shows antibiotics resistance pattern of MRSA isolated from Nasal swab with highest resistance to ceftriaxone (92.3%), ampicillin (100%) ceftaxime (100%) and clindamycin (61.5%). Highest susceptibility of the isolate to gentamycin (100%), norfloxacin (100%), ofloxacin (76.0%), erythromycin (100%) and ciprofloxacin (46.0%) was recorded amongst the antibiotics respectively. In this present study, resistance observed amongst penicillin derived antibiotics is as a result of acquisation of horizontal genetic material (β-lactamase enzyme) coded by plasmid which hydrolyses penicillins drugs and increased consumption of aminoglycosides and aminopenicillins by hospital staff. The relationship between antibiotic use and resistance is complex; a major driving factor for antibiotic resistance is antibiotic use/abuse both within medicine and veterinary medicine. These commonly used antibiotics resistance may be due to the mutation or strain gene transfer, over use of antibiotic and perhaps, the lack of standardized antimicrobial susceptibility testing prior to the prescription of environment conducive for bacterial resistant strains and improper use of antibiotics increases the risk of the disseminating antibiotic resistant bacteria [28].

The MRSA isolates were multidrug - resistant and there is a need for the adoption, development and enforcement of adequate control policies in the hospital wards/departments where there are no effective MRSA control.

CONCLUSION AND RECOMMENDATION

Conclusion: As methicillin-resistant *Staphylococcus aureus* (MRSA) from intensive care units and blood cultures (clinical samples) have become increasingly resistant to greater numbers of antibiotics leading to reduced number of effective antimicrobial agents such as methicillin. The discovery of this research work will enhance better treatment of MRSA infection with these antibiotics. Hence, more research work is needed in order to combat side effects of multiple-drug-resistance *S. aureus* against skin infections.

Recommendation: As an ongoing concern for public health workers, this study recommends that there is need to characterized and identify this bacteria and usher a new therapeutic target in developing new drugs that will combat side effects of multiple drug resistance when used against MRSA. Further research is recommended on the molecular study of the gene encoding resistance in clinical settings.

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