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Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among medical students in Delta State University Teaching Hospital, Oghara

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Abstract

Methicillin-resistant Staphylococcus aureus presents with a broad-spectrum severity. Some reports have outlined the potential risk of medical students being part of the dissemination of the spread of methicillin-resistant Staphylococcus aureus in hospital setting. The objectives of this study are to determine the prevalence of nasal carriage of MRSA among medical students in Delta state university teaching hospital, Oghara DELSUTH, investigate antibiotic susceptibility patterns, and explore the frequency and distribution of MRSA in DELSUTH. A case study of 65 students in 400level to 600 levels, 28 males and 35 females, ethical approval was taken from the hospital's research and ethics committee before the commencement of collection of samples. Nasal swabs were collected from each student and cultured for isolation of Staphylococcus aureus; biochemical tests were carried out for identification. The prevalence of Staphylococcus aureus from the collected samples from the students was 38.5%. Recording the highest in females which was 32.3% and males 6.2%. After antibiotics susceptibility testing with oxacillin it was shown that 70.3% of the isolated sample was resistant to oxacillin. The prevalence of MRSA was 26.15%. The prevalence of MRSA in males was 0% and 33.9% in females. The overall prevalence rate was high which indicates the need for an antimicrobial stewardship program to reduce the carriage and transmission of MRSA by medical students. Staphylococcus aureus.

Keywords: Medical students; MRSA; Susceptibility

1. Introduction

The gram-positive bacteria Staphylococcus aureus is one of the most common causes of hospital and community-acquired infections and it can cause infections ranging from skin to septic shock (Man wu, et al., 2019). It is found mostly around nasal and skin flora in about 50% of the population (Akerele et al., 2015). Staphylococcus aureus has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious, Staphylococcus aureus can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections. Originally these clinical manifestations were treated with beta-lactam antibiotics, although the nature of treatment solely depends on the type of infection; complicated and uncomplicated. (Steven et al., 2015).

Staphylococcus aureus is a leading source of illness and mortality in both the community and clinical settings around the world. Its virulence factors and resistance to drugs used in therapy increase its ability to cause disease, as

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demonstrated by the emergence of methicillin-resistant Staphylococcus aureus (Shuaibu et al., 2020). In 1960 Methicillin-resistant Staphylococcus aureus was first observed, after the discovery the introduction of second-generation beta-lactam antibiotics into use in clinical practice (Catriona, et al., 2017). Methicillin-resistant Staphylococcus aureus strains have been clonally spreading in hospitals around the world since the early 1980s. Methicillin-resistant *Staphylococcus aureus* epidemiology varies a lot over the world and there are even significant disparities at the regional level (Michael et al., 2007).

In Africa, there is a lack of information on epidemiology and molecular characterization of Staphylococcus aureus strains, unlike in other countries in Asia, Europe, and North America. Studies have shown a high incidence of Staphylococcus aureus, this shows there would be a high prevalence of MRSA in Africa, however, little information is known about this trend in Africa including Nigeria (Osahon et al., 2020). Between 1996 and 1997, the prevalences of MRSA, determined in 8 African countries were relatively high in Nigeria, Kenya, and Cameroon. The isolates showed different susceptibilities to different drugs but were all sensitive to vancomycin (Ghebremedhin et al., 2009). Methicillin-resistant *Staphylococcus aureus* strains have gained a gene that makes them resistant to almost all beta-lactam drugs (Anna 2016).

The successful use of therapeutic agents is greatly compromised by the development of resistance to the agent first employed as seen in the case of beta-lactams in the treatment of Staphylococcus aureus infections. This could be a result of a wide range of factors like physiological and or biochemical mechanisms. Some methods of resistance are explained below; Genetic jugglery- random mutations of the genes encoding the enzymes have given rise to modified catalysts with increasingly extended spectra of resistance. Intrinsic resistance- refers to the existence of genes in bacterial genomes that could generate a resistance phenotype that is proto- or quasi- resistance (Julian and Dorothy 2010). Staphylococcus aureus acquires a gene called the mecA gene, which encodes for a new protein called PBP2a which belongs to the group of enzymes essential for constructing the bacterial cell wall, which results in resistance to methicillin. Methicillin and other beta-lactams are resistant due to PBP2a, which has a very low affinity for beta-lactam antibiotics (Kenneth et al., 2019). It is therefore very difficult to treat because of this resistance to many antibiotics available. Transmission occurs majorly through contact. That is the touch of contaminated objects or even humans. It has also been said that 2% of the population carries MRSA but may not be infected (Sabrina 2021). Resistance to other antibiotics is also widespread, particularly in MRSA found in hospitals. Community-associated MRSA is also common, that is those originating from outside the hospital (Anna 2016). Other groups of antibiotics resistant to Methicillin-resistant Staphylococcus aureus are chloramphenicol, aminoglycoside, tetracyclines, and fluoroquinolones (Dardi and Sadhana 2015). *Staphylococcus aureus* infections have different clinical manifestations which include; skin and soft tissue infections, endocarditis, pleuropulmonary and device-related infections, etc. (Steven et al., 2015).

In 1960 Methicillin-resistant *Staphylococcus aureus* was first observed, after the discovery the introduction of second-generation beta-lactam antibiotics into use in clinical practice (Catriona, et al., 2017). MRSA could be hospital-associated HA-MRSA or community-associated CA-MRSA. Community-associated MRSA is recovered from people who have not been hospitalized or had a medical procedure during the past year. While hospital-associated MRSA is recovered from people who have had a long hospital stay. Most nosocomial infections have been directed to be caused by MRSA strains. Community-associated and hospital-associated can be differentiated through the possession of unique drug resistance patterns and molecular characteristics (Haiying et al., 2018).

Hospital-acquired MRSA strains have large staphylococcal chromosomal cassette mec belonging to type I, II, or III. These cassettes all contain the mecA gene and they are often resistant to even other chemotherapeutic drugs that are not beta-lactams. These hospital-acquired MRSA also carries the genes for the Panton-Valentine leukocidin (PVL). While community-acquired MRSA carries a smaller amount of mec belonging to type IV or V (Michael and Robert 2010). Staphylococcus aureus is known for its ability to produce virulence factors, but the community-associated virulent factor is poorly understood. A comparison of hospital-associated and community-associated virulent factors could help in distinguishing them. According to a study community-associated MRSA has more susceptibility to antibiotics when compared to hospital-associated MRSA (Haiying et al., 2018).

2. Material and methods

Sterile swab stick, normal saline, syringe, test tubes, test tube racks, culture media (Nutrient agar, Sabouraud Dextrose agar, mannitol salt agar, MacConkey agar, Cetrimide agar, peptone water, nutrient broth, miu agar, Urease broth base), sterile water, microscope, incubator, autoclave, refrigerator, beam balance, measuring cylinder, beaker, wire loop, glass holder, Bunsen burner, EDTA bottle. The various reagents and equipment used for this work were obtained from the laboratories of Pharmaceutical Microbiology Department of the Faculty of Pharmacy Delta State University, Abraka (DELSU).

2.1. Collection of Clinical Specimens (Nasal Swab)

Specimen collection was carried out from May 2022 to June 2022; a total of 145 Nasal swab sample of all 400 level to 600 level clinical medical students at Delta State University Teaching hospital (DELSUTH), Oghara was collected.

A sterile flexible swab stick was inserted in the patients nose and was then quickly and firmly rubbed in the area to obtain a good sample The sterile swab sticks were labelled and transferred to microbiological investigation in the pharmaceutical microbiology laboratory, Faculty of Pharmacy, Delta State University, Abraka for further laboratory investigations.

2.2. Sterilization of Materials

Glassware's such a test tubes, beakers, measuring cylinder were wrapped in foil paper and sterilized in an autoclave at 121 C for 15 minutes. Corn borers were sterilized by cleaning with cotton wool soaked with 99% methanol and flames over a Bunsen burner. The work area was also sterilized by cleaning it with cotton wool soaked in disinfectant before each work is carried out. Media used in the research was sterilized by autoclaving at 121 C for 15 minutes and inoculating wire loops were sterilized by heating to redness using the Bunsen burner before each use.

2.3. Preparation of Media

The various media for incubation of the microbial were prepared according to the manufacturer's instruction and were sterilised by autoclaving at 121 C for 15 minutes

2.4. Isolation of test Micro-organism and Preparation of Sub-cultures

Upon collection of samples, the nasal swab was inoculated on Mannitol salt agar. This was used to distinguish *Staphylococcus aureus* from bacterial on the basis of colony morphology. The emerging Staphylococcus aureus colonies on the Mannitol salt agar were preserved on Slants. These slants were made by pouring sterilised nutrient agar into sterile Bijou bottles and then kept in slant position (the bottle laying at an angle resulting in a large surface area for spreading a culture) till they got solidified in an aseptic environment. After solidification the Staphylococcus aureus isolates were inoculated into the agar slant and incubated at 37C for 24 hours. (Cheesebrough, 2010).

2.5. Confirmation of Identity of Test Microorganism

After incubation, the plates and test tubes were observed, and each bacteria colony was identified based on information in the literature.

The identification of Staphylococcus aureus was based on morphological characteristics, and biochemical tests were carried out on the isolates after 24 h of growth.

2.5.1. Gram Staining

Gram staining was obtained using a flamed wire loop. A thin smear of each pure 24-hour old culture was prepared to clean grease-free by cleaning the slide with cotton wool soaked in alcohol. The smear was allowed to dry, after which it was heat fixed and oxidised by passing the slide over the flame.

Crystal violet was added and allowed to stay for one (1) minute; it was drained off the slide with water. Subsequently, mordant iodine was added for about one (1) min and was rinsed off with alcohol until it decolourised. Finally, Safranin was added for about one (1) min and rinsed off with water. The slide was then allowed to dry at a slanting position, mounted on a microscope and viewed under oil immersion. (Cheesbrough, 2006).

2.5.2. Biochemical Reactions

Biochemical tests are performed on different bacteria for their identification based on their biochemical activities towards different biochemical compounds. Biochemical tests are one of the traditional methods for identifying microorganisms, usually performed with phenotypic identification. (Cheesbrough, 2006).

2.6. Characterization of Methicillin Resistant *Staphylococcus aureus* (MRSA)

To identify Methicillin Resistant Staphylococcus aureus (MRSA) Antibacterial susceptibility was carried out using disk diffusion method. Mueller Hinton Agar was prepared according to the manufacturer's instruction and poured into different sets of Petri-dishes and was allowed to solidify on the agar after cooling for some time. With the aid of a sterile

swab stick, a 24hr broth culture was collected and swabbed all over the surface of the gelled Mueller-Hinton agar. (Cheesbrough, 2006).

With the aid of a sterile forceps, antibiotic disk containing oxacillin was introduced into the plates and was left on the bench undisturbed for 30mins for pre-diffusion of the drug to occur and then it was incubated at 37C for 24hrs. The resulting zone of inhibition was then measured with a ruler calibrated in millimetre. The average reading was taken as zone of inhibition of the bacterial isolate in question.

Isolates showing a minimum inhibitory concentration greater than 12mm was identified as Methicillin Resistant Staphylococcus aureus (MRSA). (Cheesbrough, 2006).

2.7. Statical Analysis

The data obtained were evaluated using Statistical Package for Social Sciences, Version 22 (SPSS 22) and then, data was summarized using graphs, frequency tables, means and standard deviations.

3. Results

3.1. Identification of Staphylococcus aureus isolates

The identification tests carried out on collected nasal swabs in Table 3.1 showed that the isolated Staphylococcus aureus presented a characteristic change in color (fermentation) of mannitol salt agar from pink to yellow. Which distinguished it from other Staphylococcus species. Other identification tests was carried out which included citrate test, indole test, catalase test, coagulase test and gram staining. Staphylococcus aureus has the ability to utilize citrate as a source of energy, therefore all samples tested positive to citrate test. In fermentation test the sugars used were lactose, glucose and sucrose. Staphylococcus aureus ferment sugars and therefore all sugars fermentation test was positive. Coagulase test is used to differentiate Staphylococcus aureus which produces the enzyme “coagulase” therefore the isolated Staphylococcus aureus all tested positive to coagulase test. Catalase test demonstrates presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide. It tests whether a gram positive cocci is a Staphylococci or a Streptococci.

Table 1 General Results of *Staphylococcus aureus* Identification

S/N	Identification Tests	Results
1.	Staining Reactions	They appear as dark purple gram-positive cocci
2.	Motility	Non-Motile
3.	Shape	Cocci
3.	Biochemical Reactions	
I.	Coagulase	Positive
II.	Catalase	Positive
III.	Urease	Positive
IV	Oxidase	Negative

Table 2 Biochemical test on Staphylococcus isolates

S/N	Shape	Motility	G/S	S/C	Catalase	Oxidase	Indole	Coagulase	MSA
1.	Cocci	-	+	+	+	-	-	+	+
2.	Cocci	-	+	+	+	-	-	+	+
3.	Cocci	-	+	+	+	-	-	+	+
4.	Cocci	-	+	+	+	-	-	+	+
5.	Cocci	-	+	+	+	-	-	+	+
6.	Cocci	-	+	+	+	-	-	+	+
7.	Cocci	-	+	+	+	-	-	+	+
8.	Cocci	-	+	+	+	-	-	+	+
9.	Cocci	-	+	+	+	-	-	+	+
10.	Cocci	-	+	+	+	-	-	+	+
11.	Cocci	-	+	+	+	-	-	+	+
12.	Cocci	-	+	+	+	-	-	+	+
13.	Cocci	-	+	+	+	-	-	+	+
14.	Cocci	-	+	+	+	-	-	+	+
15.	Cocci	-	+	+	+	-	-	+	+
16.	Cocci	-	+	+	+	-	-	+	+
17.	Cocci	-	+	+	+	-	-	+	+
18.	Cocci	-	+	+	+	-	-	+	+
19.	Cocci	-	+	+	+	-	-	+	+
20.	Cocci	-	+	+	+	-	-	+	+
21.	Cocci	-	+	+	+	-	-	+	+
22.	Cocci	-	+	+	+	-	-	+	+
23.	Cocci	-	+	+	+	-	-	+	+
24.	Cocci	-	+	+	+	-	-	+	+
25.	Cocci	-	+	+	+	-	-	+	+

KEYS: ISO: Isolates +: Positive-: Negative**;** G/S:Gram staining **;** S/C: Simon Citrate; MSA: Mannitol Salt Agar

3.2. Result on Susceptibility

Result showing Susceptibility of Staphylococcus aureus isolates to Oxacillin. Out of 25 Staphylococcus aureus isolates, 17 isolates exhibited methicillin resistance (68%) (MRSA), and 8 isolates were methicillin susceptible (32%), as shown in table 3.

Table 3 Susceptibility of Staphylococcus aureus isolates to Oxacillin

S/N	OXACILLIN (mm)	INFERENCE
1.	11	Resistant
2.	10	Resistant
3.	14	Susceptible
4.	10	Resistant
5.	9.5	Resistant
6.	8	Resistant
7.	11	Resistant
8.	9	Resistant
9.	24	Susceptible
10.	11	Resistant
11.	24	Susceptible
12.	10	Resistant
13.	21	Susceptible
14.	22	Susceptible
15.	20	Susceptible
16.	12.5	Susceptible
17.	11	Resistant
18.	10.5	Resistant
19.	11.5	Resistant
20.	8	Resistant
21.	9	Resistant
22.	11	Resistant
23.	14	Susceptible
24.	11	Resistant
25.	9.5	Resistant

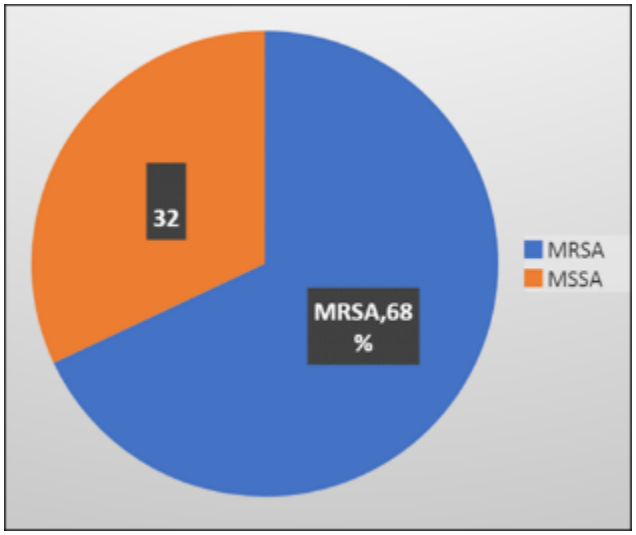

Figure 1 Prevalence of methicillin resistant *Staphylococcus aureus* Among Male and Female population

From table 3 the prevalence of MRSA in females is 45.95%.

Table 4 The prevalence of MRSA among male and female medical students

Gender	participants	Isolated S. aureus	Isolated MRSA	% Of isolated MRSA
Male	28	4	0	0
Female	37	21	17	45.95
Total	65	25	17	26.15

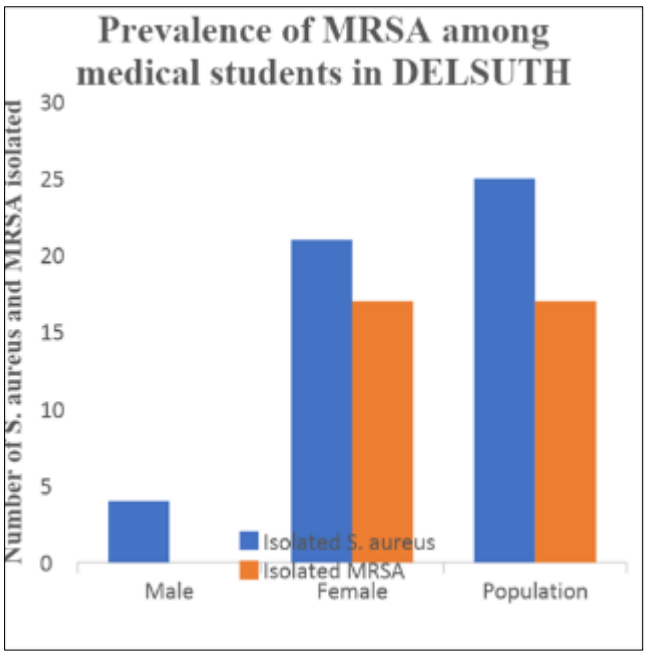

Figure 2 Graph showing prevalence of MRSA among medical students in DELSUTH

4. Discussion

The anterior nare is heavily colonized predominantly with Staphylococcus aureus. Staphylococcus aureus is a well-known pathogen with an alarming increasing level of resistance to many available antimicrobial agents. Nasal Staphylococcus aureus has been implicated in infections like skin and soft tissue infections and bacteremia. (Akerele et al., 2015).

Methicillin-resistant Staphylococcus aureus is a serious public health challenge. It causes infections both in the community and in hospitals. Healthcare workers including medical students can be a route to the spread of this infection especially when infection control practices are not adhered to. The emergence of multidrug-resistant MRSA strains with unique properties has raised public health concerns. The acquisition of MRSA by medical students is often a result of long hospital visits and poor hygiene practices. A total of 65 medical students were studied; 28 males and 37 females.

The prevalence of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus in the nasal carriage was 38.5% and 26.15%. In this study prevalence of nasal carriage Staphylococcus aureus, MRSA was significantly higher in females than in males which were 32.3% and 6.2% respectively. This result can be compared to a study carried out by Nagi et al., in 2018 which was 26.2% in females and 20.2% in males. The relationship between sex and Staphylococcus aureus infection has not been fully understood yet. In a study prevalence of nasal Staphylococcus aureus strains among medical students was 33%. Differences in prevalence may be due to different sampling methods and culture techniques (Yasman et 2021). Nasal carriage strain of Methicillin-resistant Staphylococcus aureus in medical students in Delta state university, teaching hospital was higher in females than males which were 45.95% in females and 0% in males respectively.

The total prevalence rate of Methicillin-resistant Staphylococcus aureus among medical students from 400 level to 600 level in Delta state university teaching hospital, Oghara is therefore 26.15%. Feyissa reported 30.5% as the prevalence of MRSA In teaching hospitals in Ethiopia. However, in comparison with a study carried out in a Taiwanese university and a university teaching hospital in Tanzania was 21.9% and 21% respectively. The overall prevalence is high compared to other studies carried out in Saudi Arabia, and Nepal which were 6.7% and 4%. Although in Nigeria some teaching hospitals have recorded a prevalence of MRSA which was 43.5%, 28.6%, and 34.7% in Jos, Kano, and Ilorin respectively. This was higher in inpatients. The high incidence of MRSA infection in the hospital studied may not be unconnected with the location of the hospital. But can be connected to poor hygiene practices, nonadherence to or lack of relevant antibiotic policy.

Antimicrobial resistance and sensitivity were recorded, the drugs used was oxacillin. The resistance to oxacillin was 70.3%, which shows a big resistance to methicillin like drugs. It can be said that there is a high prevalence of Methicillin resistant Staphylococcus aureus among medical students in Delta state university teaching hospital, Oghara.

5. Conclusion

Antibiotic resistance is a growing public health crisis worldwide. However, the epidemiology of this resistance in some regions like Nigeria is poorly understood. This also applies to MRSA. MRSA among medical students in Nigeria has a poor recording so the statistics are therefore unclear or not available. The study has shown a high prevalence of nasal carriage of Staphylococcus aureus and MRSA with high rates of resistance to commonly available and used antimicrobials among students in the hospital.

Therefore, routine screenings of all clinical Staphylococcus aureus in the entire hospital is needed, to get clearer and more accurate information on the spread of MRSA in the hospital as a whole to create measures to curb the spread. With these findings, awareness should be raised among medical students of the need to adhere to standard infection prevention and control practices to limit the spread of MRSA in the hospital.

Recommendation

This study has shown the prevalence of Methicillin-resistant Staphylococcus aureus at Delta state university teaching hospital, Oghara. The prevalence obtained from this study is very high and it calls for attention from the hospital. The students may have encountered the organism before coming to the hospital or after coming to the hospital, therefore continuous cleanliness and the dangers of the misuse of antibiotics cannot be overemphasized. The high rates of prevalence make it important for a survey to be conducted to understand the student's knowledge of epidemiology and the spread of MRSA in a hospital setting.

In this study females had higher nasal carriage of Staphylococcus aureus than men, although the relationship between sex and the infection of Staphylococcus aureus is not clear this variation may be due to sampling methods and culturing techniques. Not enough demographical parameters were taken in this research like age, religion, rate of antibiotic use, etc to give clearer and more accurate information on the spread of this resistant strain. It is recommended the hospital carry out studies and research to determine the cause and rate of spread.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Research and Ethical Committee, Delta State University Teaching Hospital (DELSUTH), Oghara.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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