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Characterization of Methicillin-Resistant *Staphylococcus aureus*from Adult Out-Patients Visiting Delta State University Teaching Hospital, Oghara

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Abstract

Staphylococcus aureus poses a cause of concern in various healthcare settings, causing a number of infections ranging from mild to severe. The evolution of new genetically distinct multi-drug resistant strains, such as the Methicillin-Resistant *Staphylococcus aureus*, has only amplified the problem. Having efficient data on the local antimicrobial susceptibility pattern of this pathogen is necessary for the selection of appropriate antibiotics for combating infections that may arise *from Staphylococcus aureus*. To characterize methicillin-resistant *staphylococcus aureus* among adult out-patients visiting Delta State University Teaching Hospital, for each sample obtained, *staphylococcus aureus* was first isolated, and the identity of the isolates was confirmed by carrying out Biochemical reactions. Briefly, a Mueller-Hinton agar plate was prepared, and the test organism was inoculated. Results were interpreted as resistant or susceptible. Out of 95 samples taken and tested, *Staphylococcus aureus* accounted for 25 (26.7%). Antibiotic susceptibility testing carried out using the Disk diffusion method showed that 68% of specimens of confirmed *Staphylococcus aureus* were resistant to Oxacillin. About 32% of isolates were sensitive to it. The findings of this study show that the resistant strain of *Staphylococcus aureus* is the predominant strain considered in the study population. This will be a guide in proper prescription to prevent further increase in bacteria resistance.

Keywords: *Staphylococcus aureus*, MRSA, Susceptibility, Out-patients

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Introduction

Staphylococcus aureus, a gram-positive microbe, poses a significant threat in clinical settings, causing a range of infections that are exacerbated by the emergence of multi-drug resistant forms, notably Methicillin-Resistant Staphylococcus aureus (MRSA) (Nicoară *et al*., 2023). This bacterium, though typically harmless on healthy skin, can lead to severe infections when reaching internal tissues or the bloodstream. With MRSA implicated in nosocomial infections, including life-threatening pneumonia and severe sepsis, understanding its impact and developing effective therapies is imperative (Moremi *et al*., 2019). In this study, we aim to delve into the inter-professional team's role in treating patients with Staphylococcus infections, focusing on the evaluation and treatment of these infections. Recognizing the escalating challenge posed by MRSA, which can cause infections ranging from skin-related issues to life-threatening conditions affecting the blood, bones, and gastrointestinal system, our objective is to contribute to the development of innovative strategies for combating these infections. Considering the broader context of microbial influence on human health, particularly the role of microbes in the human microbiome, our study aligns with the growing body of health research (Gherman *et al*., 2019). As microbes, including bacteria, play a dual role of both potential harm and benefit to humans, understanding their dynamics within the human microbiome becomes crucial (Oliveira *et al.*, 2021). In light of these considerations, our work seeks to contribute meaningful insights into tackling *Staphylococcus aureus* infections, with particular emphasis on the challenges caused by MRSA in healthcare settings.

Materials and Methods

Materials: 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.

Collecting of Clinical Specimens (Nasal Swabs)

The specimen was collected from May- June 2022. A total of 95 nasal swab samples of adult out-patients visiting Delta State University Teaching hospitals were collected. A sterile flexible swab stick was inserted into the nostrils of the subject and gently rubbed. It was labeled and transferred to the Pharmaceutical Microbiology laboratory in the Faculty of Pharmacy, Delta State University, for further investigations.

All materials and media to be used were sterilized in an autoclave at 121℃ for 15 minutes. The work area was also sterilized using cotton wool and disinfectant before each work session.

Preparation of Media

All media to be used were prepared and sterilized in accordance with the manufacturer's instructions.

Isolation of test Micro-organism and Preparation of the Sub-Cultures

Upon collection of samples, the nasal swab was inoculated on Mannitol salt agar. This was used to distinguish *Staphylococcus aureus* from bacteria 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 sterilized nutrient agar into sterile Bijou bottles and then kept in a 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 37℃ for 24 hours (Cheesebrough, 2010).

Confirmation of Identity of Test Micro-Organism

After incubation, the plates and test tubes (slants) 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 hours of growth (Motta *et al*., 2015).

Characterization of Methicillin-Resistant Staphylococcus aureus (MRSA)

To identify Methicillin-resistant *Staphylococcus aureus* (MRSA), Antibacterial susceptibility was carried out using the disk diffusion method. Mueller Hinton Agar was prepared according to the manufacturer's instructions, poured into different sets of Petri dishes, and allowed to solidify on the agar after cooling for some time. With the aid of a sterile swab stick, a 24-hour broth culture was collected and swabbed all over the surface of the gelled Mueller-Hinton agar (Cheesbrough, 2010; Rus *et al*., 2020).

With the aid of sterile forceps, the antibiotic disk containing Oxacillin was introduced into the plates and was left on the bench undisturbed for 30minsfor 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 millimeters. The average reading was taken as a zone of inhibition of the bacterial isolate in question (Appelbaum, 2012).

Isolates showing a minimum inhibitory concentration greater than 12mm were identified as Methicillin Resistant *Staphylococcus aureus* (MRSA) (Cheesbrough, 2010).

Statistical Analysis

The data obtained were evaluated using Statistical Package for Social Sciences, Version22 (SPSS22), and then, data was summarized using graphs, frequency tables, means, and standard deviations.

Results and Discussion

Out of 95 samples collected from adult out-patients visiting Delta State University Teaching Hospital (DELSUTH), Oghara, *Staphylococcus aureus* accounted for 25 (27.7%). The identity of the *Staphylococcus aureus* isolates was confirmed using various biochemical tests, as shown in **Table 1**.

Table 1. Biochemical Identification Test on the tested organisms.

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**;** MSA: Mannitol Salt Agar**;** S/C: Simon Citrate

Table 2. Susceptibility pattern of *Staphylococcus aureus* isolates to Oxacillin.

S/N	Oxacillin(mm)	Inference
1	11	Resistant
2	14	Susceptible
3	11	Resistant
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

For several decades, micro-organisms, particularly the pathogenic ones, have played a role in human health. One such Micro-organismis *Staphylococcus aureus*, particularly the drug-resistant strain Methicillin-resistant *Staphylococcus aureus* (MRSA), which is commonly implicated in infectious diseases such as otitis media, sinusitis, bronchitis, and meningitis, which have led to anumber of deaths especially in children and elderly Delcea and Siserman, (2020). This study focuses on MRSA characterization among adult out-patients visiting Delta State University Teaching Hospital (DELSUTH), Oghara, Delta State, a tertiary health care facility where human interaction is frequent, sharing antibiotic-resistant bacteria, antibiotic-resistant genes, and diseases.

A total of Ninety-Five (95) swabs were collected as nasal samples during the study period. Methicillin-Resistant Staphylococcus aureus (MRSA) and Methicillin-Sensitive *Staphylococcus aureus* (MSSA) proportions were 68% and 32%, respectively as deduced in **Table 2**. This study Resistance of micro-organisms to antibiotics can arise inseveral ways, such as Self-medication, resistance due to gene mutation, antibiotic inactivation, target modification, altered permeability, and bypass of the metabolic pathway by the micro-organism (Radu *et al*., 2023).

This study emphasizes the importance of antimicrobial susceptibility testing of microbial isolates obtained. The emergence of the methicillin-resistant strain of *Staphylococcus aureus* is a global health problem and specifically a daunting challenge in African countries like Nigeria, thus fast becoming a public health concern (Tuta *et al*., 2019).

Conclusion

The findings of this study have shown that the nasal cavity is associated with micro-organisms that inhabit the nostrils. These organisms are sources of potential pathogens and can cause serious infections. Micro-organisms are ubiquitous and can be found in the nostrils, with *Staphylococcus aureus* being one of the most prevalent organisms. It is recommended that antibiotics should not be abused but used based on prescription in order to avoid resistance.

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Conflict of interest: None

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Ethics statement: The study was conducted according to the guidelines of the Declaration of Helsinki.

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