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Prevalence of Community Acquired Methicillin Resistant Staphylococcus Aureus (Ca-Mrsa) In The Nasal Cavity of Delta State University Students.

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ABSTRACT

INTRODUCTION Staphylococcus aureus (SA) is a Gram positive, opportunistic bacterium that frequently colonizes the oral cavity, nasal cavity, and skin of the healthy people. This can cause a variety of localized and invasive problems ranging from superficial skin infections to life threatening Pneumonia and bloodstream infections

OBJECTIVE: The study is aimed at investigating the prevalence of community acquired methicillin resistant Staphylococcus aureus in the nasal cavity of Delta State University students

METHOD:100 nasal swabs(samples) were collected from the anterior nares of both male and female students of DELSU using Sterile swab sticks and was cultured on mannitol salt Agar .After culturing on mannitol salt Agar, all the Staphylococcus aureus specie were isolated.Standard identification test was carried out by performing Gram staining test and other biochemical test like coagulase test, Catalase test and Fermentation test were further used to determine the identity of the organism. Antibiotic susceptibility test was carried out for Staphylococcus aureus using the Agar well diffusion method.The zone of inhibition in millimeters was measured with a meter rule. The average readings were taken to be the zone of inhibition and were compared with KirbyBauer Standard for Staphylococcus aureus. zones of inhibitions within the range(≤10)were used to determine the Methicillin resistant Staphylococcus aureus(MRSA).

RESULT: Out of the 100 samples collected and cultured on the mannitol salt agar, 93 were staph species why 33(35.5%) were found to be Staphylococcus aureus why the remaining 60(64.5%) were other specie of Staphylococcus aureus .Only the staph aureus specie were worked on and out of the 33(35.5%) Staphylococcus aureus, 23(69.7%) of the Staphylococcus aureus were found to be methicillin resistant why 10(30.3%) were found to be sensitive to Oxacillin disk which was used as a reference to methicillin antibiotics because its closely related to methicillin.

CONCLUSION: The result from this study shows increased Staphylococcus aureusresistance to methicillin(Oxacillin),therefore community acquired methicillin resistant Staphylococcus aureus is prevalent in the nasal cavity of DELSU Students and therefore suggest that more work has to be done as an individual and even in the clinical setting and in the community to curtail the spread of methicillin resistant Staphylococcus aureus.

I. INTRODUCTION

Background of study

Staphylococcus aureus is considered to be a persistent member of the human endogenous micro-biota and has historically been associated with important and serious cases of infection(Oliveira, et al., 2021).It is one of the most common bacterial that causes infection in the community and healthcare settings(Ajani et al., 2020).

Staphylococcus aureus is a Gram-positive, round-shaped, catalase- and coagulase-positive bacterium.Among the first identified human pathogens are staphylococcus spp (species) and the most important human pathogen in this genus**(**Shokouhi, Darazam&Zamanian, 2017).They mostly colonize the nose, perinea, and damaged skins. About 20% and 60% of the population are colonized permanently and intermittently by these bacteria, respectively(Shokouhi, 2017). Since the introduction ofβ-lactam antibiotics, the spread of methicillin-resistant S. aureus (MRSA) has increased on a global scale (Jevons et al., 1963; Humphreys H. et al., 2009).

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 523-530 www.ijprajournal.com ISSN: 2456-4494

Methicillin resistance Staphylococcus aureus MRSA is a gram positive bacterium resistant to methicillin (which is a member of the penicillin family) and many other beta-lactam anti-microbialssuch as penicillins, cephalosporins and macrolides. Methicillin-resistant Staphylococcus aureus strains are usually resistant to several groups of broad spectrum antibiotics that are used in large scale in the hospital (Kemi, 2019).

After pennicilli was introduced in 1940s, the penicillin-resistant strains were gradually reported in 1945. In 1959 methicillin was introduced; however, themethicillin-resistant S. aureus (MRSA) were discovered not quite long after its administration in 1961 (Shokouhi, 2017). Methicillin-resistant Staphylococcus aureusis any strain of Staphylococcus aureus that has developed resistance to beta-lactam antibiotics is referred to as Staphylococcus aureus. Methicillin-resistant Staph aureus can develop resistance by incorporating a mecA gene into its chromosome at a specific site; mecAgeneencodes an alternative penicillin-binding protein with low affinity for semi-synthetic penicillin, such as methicillin, nafcillin, and oxacillin agents (Mama et al., 2019).

In recent time resistance to antibiotics has been developed by methicillin resistant Staphylococcus aureus.This has posed a lot of challenges to the health sectors giving clinical microbiologist issue as a result of drug therapy problems being developed.This drug therapy problem is due to the fact that CA-MRSA develop cross resistance to beta-lactam antibiotics due to the presence of mecA gene sequence known to generate transpeptidasePb2a that helps to lower affinity to betalactam antibiotics(Shahkaramiet al.,2014).Since the main goal of pharmaceutical care is to solve drug therapy problem, that is why the study is based on the prevalence of CA-MRSA in the nasal cavity of student of DELSU to know the prevalence and also provide solution to drug therapy problem

Significance of Study

Knowing the prevalence of MRSA in the nasal cavity of students of DELSU is important to know the extent at which students in the community can be predisposed to CA-MRSA infection .It is important to reduce further resistance of microorganism to chemotherapeutic drugs .The study is also important ensure that the right and effective drug is given to patients at the right dose at the right time to prevent the CA-MRSA.

II. MATERIALS AND METHODS

Materials

Reagents and Media

Media used include; nutrient Agar, nutrient broth, peptonewater Mueller Hinton agar, mannitol salt agar, glucose, sucrose &lactose. Reagent includekovac's reagent, phenol red, 3% hydrogen peroxide, alcohol, Gram's reagent.

Apparatus

Autoclave, incubator, test tubes, wireloop, conical flask and beaker, cottonwool, aluminum foil, Analytical balance, microscopeslide, cover glass, petri-dishes ,bijou bottles, pipette, spirit lamp.

Sample collection

This study was conducted from 15th of March 2022, to 1st of May 2022 on 93 gram positive isolate after culturing the samples on mannitol salt agar. The samples were gotten from the nasal cavity of student of Delta State University. 100 samples were collected and analyzed.

Nose swabs: The nasal samples were obtained with a sterile swab sticks, which were gently lead into the inner area and robbed over the anterior nares of both nostrils.

Sterilization of Materials

The sterilization of glasswares such as bijou bottles, McCartney bottles, conical flasks, measuring cylinders, beakers and test-tubes after washing with detergent solution was carried out in hot air oven for 20 minutes. All culture media were sterilized by autoclaving at 121 C for 15minutes.

Isolation of Staph. aureus from nasal swab samples

Immediately after being delivered at the microbiology laboratory, the swab was streaked on a petri dish surface containing 11.1g of solidified mannitol salt agar dissolved in 100ml of sterile water and incubated at 35-37°C for 24hours.Thereafter, the pure strains obtained were stored in slants from which they were taken for identification.

The slide was then allowed to dry at a slanting position, mounted on a microscope and viewed under oil immersion. (Cheesbrough, 2006).

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 523-530 www.ijprajournal.com ISSN: 2456-4494

BIOCHEMICAL REACTIONS
All biochemical tests were carried out as descrised by Oghenemaro et al 2018.

Antibiotics Sensitivity Testing: Antibacterial susceptibility was carried out using the agar well 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 0.2ml of a 24hr broth culture was collected and swabbed all over the surface of the gelled Mueller-Hinton agar using a sterile glass rod .with the aid of a sterile 6mm corkborer, two well were made on the different Mueller-Hinton agar plate, allowing at least 30mm between adjacent wells and between peripheral wells and the edges of the petri-dish. Fixed volumes (0.1ml) of the Oxacillin were then introduced into each of the wells in the plate after dissolving 500mgoxacillin in 10ml of water. The plates were allowed on the bench undisturbed for 40mins for pre-diffusion of the drug to occur and then it was incubated at 37C for 24hrs. The resulting zone of inhibition was the measured with a ruler calibrated in millimeter. The average reading was taken to be zone of inhibition of the bacterial isolate in question and the zone of inhibition less than or equal to 10 was use to determine the MRSA (Cheesbrouhg, 2006).

III. RESULTS

Identification of Test Micro-Organisms

Out of 100 samples collected from delta state university students 93 isolates were obtained after culturing on mannitol salt agar.33 accounted for Staphylococcus aureus and the others were other species of Staphylococcus aureus. Just the Staphylococcus aureus were walked on.

Motility test results showed that Staphylococcus aureus is non-motile. Catalase test results showed that they are catalase positive and fermentation test result showed they ferment glucose to lactic acid. Gram staining reaction test results showed that they are gram positive showing a lancet shaped cocci (elongated cocci with a slightly pointed outer curvature), they were seen as pairs of cocci (diplococci) but they also occur singly and in short chains.

Table 3.1: General Results of Identification.

	Identification Tests	**Results**
1	Motility Tests	They are non- motile
2	Staining Reactions	They appear as dark purple gram positive coccioccurring in pairs, singly or short chains.
3	Biochemical Reactions Coagulase Indole Sugar Fermentation Citrate urease Catalase	Coagulase positive Negative (They lack Indole) Show a yellow colour with bubbles in a tube (they ferment glucose to lactic acid). Citrate positive Urease positive Positive

Table 3.2: Identification Result for Staphylococcus aureusafter culturing on mannitol salt Agar

S/N	MSA	Shape,G	Catalase	Citrate	Indole	Urease	COG	G	L	S	Organism
1	+	+COCCI	+	+	-	+	+	+	-	+	S.aureus
2	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
3	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
4	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
5	+	+COCCI	+	+	-	+	+	+	-	+	S.aureus
6	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
7	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
8	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
9	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
10	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
11	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
12	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus

International Journal of Pharmaceutical Research and Applications
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13	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
14	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
15	+	+COCCI	+	+	-	+	+	+	-	+	S.aureus
16	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
17	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
18	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
19	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
20	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
21	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
22	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
23	+	+COCCI	+	+	-	+	+	+	-	+	S.aureus
24	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
25	+	+COCCI	+	+	-	+	+	+	-	+	S.aureus
26	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
27	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
28	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
29	+	+COCCI	+	+	-	+	+	+	-	+	S.aureus
30	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
31	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
32	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus
33	+	+COCCI	+	+	-	+	+	+	+	+	S.aureus

KEYS
GS: Gram stain
L: Lactose
MSA: Manitor Salt Agar + Positive
G Glucose - Negative
S Sucrose
COG: Coagulase

TABLE 3.3:Susceptibility result of Gram-positiveStaphylococcus aureusisolate to Oxacillin

S/N	OXACILLIN (mm)	INFERENCE
1.	9	Resistant
2.	8	Resistant
3.	9	Resistant
4.	7	Resistant
5.	9.5	Resistant
6.	8	Resistant
7.	10	Resistant
8.	9	Resistant
9.	24	Susceptible
10.	13	Susceptible
11.	24	Susceptible
12.	10	Resistant
13.	21	Susceptible
14.	9	Resistant
15.	20	Susceptible
16.	9.5	Resistant
17.	9	Resistant
18.	13.5	Susceptible
19.	8.5	Resistant
20.	8	Resistant
21.	9	Resistant

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 523-530 www.ijprajournal.com ISSN: 2456-4494

22.	9.5	Resistant
23.	10	Resistant
24.	8	Resistant
25.	12.5	Susceptible
26.	14	Susceptible
27.	15	Susceptible
28.	9.5	Resistance
29.	12	Susceptible
30.	10	Resistant
31.	9	Resistant
32.	8	Resistant
33.	5	Resistant

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KEY: **S**-Susceptibility R-Resistance

Table 3.4: Susceptibility& Resistance Pattern For To Methicillin Antibiotics(OXACILLIN)

ORGANISM	OXACILLIN	
	PERCENTAGE SUSCEPTIBILITY	PERCENTAGE RESISTANCE
S. aureus (33)	10 30%	23 69.7%

IV. DISCUSSION AND CONCLUSION

Discussion

In the study, site of sample collection was the Nares. A good reason for choosing the Nares is that it is one of the main site of colonization for Staphylococcus aureus, whose prevalence reaches an average of 40% in the adult population. (Efa, et. al., 2019; Moremi et. al., 2019; Carvalhoet. al., 2016).

Nasal colonization is important risk factors in the pathogenesis of this infection and Staphylococcus aureus can be transmitted to the nares by contaminated hands and from surface where it can survive for months. Nasal carriage has a crucial function as a source of invasive infection in both community and hospital setting (Von-Eiff et al., 2001).

From the above result in chapter 3, out of the 100 samples that were collected with a sterile swab from the nostrils of delta state university students and cultured on Mannitol salt agar, 93 isolates were obtained. Out of the 93 isolate, 33(35.5%) were found to be Staphylococcus aureus while the remaining 60(64.5%) were other species of staphylococcus. Susceptibility testing result showed that 23(69.7%) out of the total 33 Staphylococcus aureus to be MRSA due to the low susceptibility to oxacillin .Just 10(30.3%) were sensitive to oxacillin showing an increase in the prevalence of methicillin resistant Staphylococcus aureus(MRSA).The result of the prevalence of methicillin resistance in this study was found to be almost similar to result gotten by (Garoy et. al., 2019) whereby out 83 Staphylococcus aureus isolate, 59(72%) were found to be methicillin resistance. Also, in a similar study conducted by (Singh et al., 2018) on the prevalence of nasal colonization of methicillin resistance Staphylococcus aureus among school children of Barabanki district Uttar Pradesch, India, out of 300 children 140(46.67) were found to be nasal carriage for Staphylococcus aureus among which MRSA was found to be 23 (7.67%)

From the above result, we can now see that MRSA is becoming prevalent both in community settings as well as hospital setting. The difference in colonization of anterior nares by methicillin Staphylococcus aureus have been attributed to the host factor such as host immunity, age, gender, and environmental factors (Garcia-Rodriguez and Freesnadillo -Martinez 2002)

The high prevalence to methicillin resistance in the present study demonstrates the urgent need for proper management of antibiotic use. Nigeria has a high rate of antibiotic misuse as well as high prevalence of self-medication use (Sapkota et al., 2010).This could also be a reason for the increased methicillin in this study

International Journal of Pharmaceutical Research and Applications
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Conclusion

The low susceptibility of Staphylococcus aureus to methicillin(oxacillin) shows that community acquired methicillin Staphylococcus aureus is prevalent in the community and it suggest the need for proper use of methicillin antibiotic in treatment of CA-MRSA infections. Also, our result from this study shows that young adult(delta state students), even if many are asymptomatic to CA-MRSA are common carriers of this resistant Staphylococcus aureus strain.

Healthy people particular those living together or having close physical contact with each other are also predisposed to this resistant strain of Staphylococcus aureus

Most reports in the literature refer to athletes of team sports, individuals doing military service, prison inmates, intravenous drug users, homeless people, and children in day care centers as having a higher risk of developing CA-MRSA infections. The typical presentation would be a young athlete with abscess and cellulites, with contributing factors such as physical contact, skin lesions, and sharing of contaminated equipment (Carvalho et al., 2022).

The result from this study, showing increase prevalence of this CA-MRSA, therefore suggest that more work has to be done as an individual and even in the clinical setting and in the community to curtail the spread of methicillin resistant Staphylococcus aureus due to increased prevalence in this study.

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