J Biochem Technol (2023) 14(3): 67-71
https://doi.org/10.51847/u7ox8LFO5P
ISSN: 0974-2328

Prevalence of Community-Acquired Methicillin-Resistant Staphylococcus aureus (CA-MRSA) in the Nasal Carriage of Delta State University Students

Felix Oghenemaro Enwa*, Kingsley Chukwuka Amaihunwa, Collins Ohwonigho Adjekuko, Samuel Binichukwu Onyolu

Received: 07 June 2023 / Received in revised form: 06 September 2023, Accepted: 10 September 2023, Published online: 26 September 2023

Abstract

The study aimed to look into the prevalence of community-acquired methicillin-resistant Staphylococcus aureus in the nasal carriage of Delta State University students. 100 nasal swabs (samples) were collected from the anterior nares of both male and female students of DELSU and were cultured on Mannitol salt Agar. Staphylococcus aureus species were isolated. A standard biochemical identification test was carried out to identify the organism. An antibiotic susceptibility test was carried out for Staphylococcus aureus using the Agar well diffusion method and the zone of inhibition was determined. The average readings were taken of the zone of inhibition and were compared with the Kirby Bauer Standard for Staphylococcus aureus. zones of inhibitions within the range (≤10) were used to determine the Methicillin-resistant Staphylococcus aureus (MRSA). Out of the 100 samples collected and cultured, 93 were Staph species 33(35.5%) were found to be Staphylococcus aureus while the remaining 60(64.5%) were other species of Staphylococcus aureus. Only the Staphaureus species was worked on and out of the 33(35.5%) Staphylococcus aureus, 23(69.7%) of the Staphylococcus aureus were found to be methicillin-resistant. In comparison, 10(30.3%) were found to be sensitive to Oxacillin disk which was used as a reference to methicillin antibiotics because it's closely related to methicillin. This study shows increased Staphylococcus aureus methicillin resistance, therefore suggesting the prevalence of CA-MRSA in the nasal cavity of DELSU Students and suggesting that more work has to be done and awareness campaigns on how to curtail the spread of methicillin-resistant Staphylococcus aureus.

Felix Oghenemaro Enwa*, Samuel Binichukwu Onyolu
Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Delta State University, Abraka, Nigeria.

Kingsley Chukwuka Amaihunwa
Department of Medical Laboratory Science, Faculty of Science, Delta State University Abraka, Nigeria.

Collins Ohwonigho Adjekuko
Department of Biological Science, Faculty of Science, University of Delta, Agbor, Nigeria.

***E-mail:** felixenwa@delsu.edu.ng

Keywords: Staphylococcus aureus, CA-MRSA, Sensitivity, Resistant

Introduction

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; Balaji *et al*., 2022). It is one of the most common bacteria that causes infection in the community and healthcare settings (Ajani *et al*., 2020).

Staphylococcus aureus is a Gram-positive bacteria and among the first identified human pathogens and the most important human pathogen (Shokouhi *et al*., 2017). They mostly colonize the nose, perinea, and damaged skin (Abdulrahman *et al*., 2022; Özüdoğru & Tosun, 2022). It is been said that about 20% and 60% of the human population is been colonized by these bacteria permanently and intermittently (Shokouhi *et al*., 2017). It is also good to note that, there has been a wild spread of Methicillin-resistant S. aureus on a global scale since the introduction of β-lactam antibiotics (Turner *et al*., 2019).

Methicillin-resistant Staphylococcus aureus (MRSA) is a gram-positive bacterium that is resistant to methicillin and other beta-lactam anti-microbials, often exhibiting resistance to broad-spectrum antibiotics used in large-scale hospital settings (Tuta *et al.*, 2019).

Penicillin-resistant strains were reported in 1945, followed by methicillin in 1959, but MRSA was discovered in 1961 (Shokouhi *et al*., 2017). MRSA is any strain of Staphylococcus aureus that has developed resistance to β-lactam antibiotics (Nasr *et al*., 2022; Shetty *et al*., 2022). The MRSA can develop resistance by incorporating a mecA gene into its chromosome at a specific site; the mecA gene encodes 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 sresistance to antibiotics has been developed by methicillin-resistant Staphylococcus aureus (Panigrahi *et al*., 2022; Valverde *et al*., 2022). This has posed many challenges to the health sectors, giving clinical microbiologists issues due to drug therapy problems being developed. This drug therapy

problem is because CA-MRSA develops cross-resistance to beta-lactam antibiotics due to the presence of mecA gene sequence known to generate transpeptidase Pb2a that helps to lower affinity to beta-lactam antibiotics (Alharbi, 2022). Since the main goal of pharmaceutical care is to solve drug therapy problems, that is why the study is based on the prevalence of CA-MRSA in the nasal cavity of students of DELSU to know the prevalence and also provide solutions 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 to which students in the community can be predisposed to CA-MRSA infection. It is important to reduce further resistance of microorganisms to chemotherapeutic drugs. The study is also important to ensure that the right and effective drug is given to patients at the right dose at the right time to prevent the CA-MRSA.

Materials and Methods

Reagents and Media

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

Sample Collection

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

Nose Swabs

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

Sterilization of Materials

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

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 100 ml of sterile water and incubated at 35-37°C for 24 hours. Thereafter, the pure strains obtained were stored in slants from which they were taken for identification.

Identification of Test Micro-Organism

After incubation, reflected light was observed on the plates, and all alpha-hemolytic colonies with a central depression and mucoid greyish appearance were suspected to be Staphylococcus.

Gram staining confirmed the presence of gram-positive lanceolate-cocci, which were then, isolated using optochin sensitivity and bile solubility tests.

- *Growth on Solid Media (Plate Cultures)*

  The pattern of growth of the organism on the plate cultures was examined to determine the similarity to those in the approved literature.

- *Growth on Differential Media*

  The growth pattern and color of the colonies on mannitol agar were observed to ascertain whether the collected organism was staphylococcus. It was specifically used to identify and isolate staphylococcus species.

Microscopic Examination

Microscopic examination of stained slides containing broth specimens was used to identify the organism.

Staining Reaction

The clinical isolates were stained using a gram staining technique developed by Christian Gram in 1884 (Cheesbrough, 2006).

Results and Discussion

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 **(Table 1)** and the others were other species of Staphylococcus. Just the Staphylococcus aureus were worked on.

Table 1. Identification Result for *Staphylococcus aureus* after 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*
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; GGlucose; -Negative; SSucrose; COG: Coagulase

Table 2. Susceptibility result of Gram-positive Staphylococcus aureus isolate 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
22.	9.5	Resistant

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

Table 3. Susceptibility & Resistance Pattern to Methicillin Antibiotics (OXACILLIN).

Organism	Oxacillin	
	Percentage Susceptibility	**Percentage Resistance**
S. aureus (33)	10 30%	23 69.7%

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

The main risk factors for the pathogenesis of this infection are nose colonization and Staphylococcus aureus can be transmitted into nares, through dirty hands or surfaces where it may survive up to months. In both the community and hospital settings, nasal transport plays an important role as a source of invasive disease (Von-Eiff *et al*., 2001).

From the above result in **Table 1**, 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 isolates, 33(35.5%) were found to be Staphylococcus aureus while the remaining 60(64.5%) were other species of Staphylococcus. Susceptibility testing results in **Tables 2 and 3** showed that 23(69.7%) out of the total 33 Staphylococcus aureus are 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 the result obtainedby (Garoy *et al*., 2019) whereby out of 83 Staphylococcus aureus isolates, 59(72%) were found to be methicillin-resistant. 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 settings. The difference in colonization of anterior nares by methicillin Staphylococcus aureus has been attributed to host factor such as host immunity, age, gender, and environmental factors (Brown *et al*., 2014).

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

Conclusion

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

Acknowledgments: None

Conflict of interest: None

Financial support: None

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

References

Abdulrahman, B. I., Alanazi, A. J., Alanazi, A. J., Idrees, F. F., Abuabah, A., El Mansy, I. T., AbuMostafa, A., & Alanazi, K. J. (2022). Natural therapeutic agents in the treatment of recurrent aphthous ulcer: A systematic review and meta-analysis. *Annals of Dental Specialty*, *10*(1), 78-86.

Ajani, T. A., Elikwu, C. J., Nwadike, V., Babatunde, T., Anaedobe, C. G., Shonekan, O., Okangba, C. C., Omeonu, A., Faluyi, B., Thompson, T. E., et al. (2020). Nasal carriage of methicillin resistant Staphylococcus aureus among medical students of a private institution in Ilishan-Remo, Ogun State, Nigeria. *African Journal of Clinical and Experimental Microbiology*, *21*(4), 311-317.

Alharbi, S. S. (2022). Diabetes mellitus as a risk factor for different types of cancers: A systematic review. *Clinical Cancer Investigation Journal*, *11*(4), 19-24.

Balaji, A., Jei, J. B., Murugesan, K., & Muthukumar, B. (2022). Rehabilitation of distal extension edentulous case with claspless extra-coronal attachments- A case report. *Annals of Dental Specialty*, *10*(2), 1-4.

Brown, A. F., Leech, J. M., Rogers, T. R., & McLoughlin, R. M. (2014). Staphylococcus aureus colonization: Modulation of host immune response and impact on human vaccine design. *Frontiers in Immunology*, 4, 507. doi:10.3389/fimmu.2013.00507

Carvalho, M. S. M., Andrade, D. F. R. D., Sousa, Á. F. L. D., Valle, A. R. M. D. C., Freitas, D. R. J., Nascimento, G. C., Andrade, D. D., & Watanabe, E. (2016). Nasal colonization with Staphylococcus aureus in nursing students: ground for monitoring. *Revista Brasileira De Enfermagem*, *69*, 1046-1051. doi:10.1590/0034-7167- 2016-0210

Cheesbrough, M. (2000). *District Laboratory practice in tropical countries. Part 2* (434). Cambridge University Press.

Efa, F., Alemu, Y., Beyene, G., Gudina, E. K., & Kebede, W. (2019). Methicillin-resistant Staphylococcus aureus carriage among medical students of Jimma University, Southwest Ethiopia. *Heliyon*, *5*(1), e01191. doi:10.1016/j.heliyon.2019.e01191

Garoy, E. Y., Gebreab, Y. B., Achila, O. O., Tekeste, D. G., Kesete, R., Ghirmay, R., Kiflay, R., & Tesfu, T. (2019). Methicillin-resistant Staphylococcus aureus (MRSA): Prevalence and antimicrobial sensitivity pattern among patients—a multicenter study in Asmara, Eritrea. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2019*, 1-9. doi:10.1155/2019/8321834

Mama, M., Manilal, A., Gezmu, T., Kidanewold, A., Gosa, F., & Gebresilasie, A. (2019). Prevalence and associated factors of urinary tract infections among diabetic patients in Arba Minch Hospital, Arba Minch province, South Ethiopia. *Turkish Journal of Urology*, *45*(1), 56-62.

Moremi, N., Claus, H., Vogel, U., & Mshana, S. E. (2019). The role of patients and healthcare workers Staphylococcus aureus nasal colonization in occurrence of surgical site infection among patients admitted in two centers in Tanzania. *Antimicrobial Resistance & Infection Control*, *8*, 1-7.

Nasr, A. M. A., Ahmed, Y. A. M., Gafar, A. A. M., Ahmed, S. A. D., Meshref, E. T., Ahamed, E. A. M., & Alanazi, W. A. S. (2022). Prevalence and risk factors of choriocarcinoma in Saudi Arabia: A Systematic Review. *Clinical Cancer Investigation Journal*, *11*(6), 4-8.

Oliveira, E., Carvalho, A., Ferreira, A., Moura, L., Valle, A., Freitas, D., & Moura, M. (2021). Colonization of methicillin-resistant Staphylococcus aureus among healthcare students: An integrative review. *Sao Paulo Medical Journal*, *139*(6), 607-614.

Özüdoğru, S., & Tosun, G. (2022). Evaluation of microleakage and fatigue behaviour of several fiber application techniques in composite restorations. *Annals of Dental Specialty*, *10*(2), 60-66.

Panigrahi, C., Yuwanati, M., & Priyadharshini, R. (2022). Determination of intracellular iron in oral squamous cell carcinoma using perl's Prussian blue iron stain. *Clinical Cancer Investigation Journal*, *11*(5), 7-10.

Sapkota, A. R., Coker, M. E., Rosenberg Goldstein, R. E., Atkinson, N. L., Sweet, S. J., Sopeju, P. O., Ojo, M. T., Otivhia, E., Ayepola, O. O., Olajuyigbe, O. O., et al. (2010). Self-medication with antibiotics for the treatment of menstrual symptoms in southwest Nigeria: A cross-sectional study. *BMC Public Health*, *10*, 1-10.

Shetty, B., Chauhan, R. S., Vishwas, P., Rathi, N., Krishnapriya, N., & Tirupathi, S. (2022). Antimicrobial efficacy of curcumin modified zinc oxide eugenol against endodontic pathogens. *Annals of Dental Specialty*, *10*(1), 47-51.

Shokouhi, S., Darazam, I., & Zamanian, M. (2017). Community-acquired methicillin-resistant Staphylococcus aureus carriage rate and antimicrobial susceptibility in a tertiary center, Iran. *Journal of Research in Medical Sciences*, *22*(1), 71. doi:10.4103/jrms.jrms_833_16

Singh, A. K., Agarwal, L., Kumar, A., Sengupta, C., & Singh, R. P. (2018). Prevalence of nasal colonization of methicillin-resistant Staphylococcus aureus among schoolchildren of Barabanki district, Uttar Pradesh, India. *Journal of Family Medicine and Primary Care*, *7*(1), 162-166. doi:10.4103/jfmpc.jfmpc_345_16

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., Holland, T. L., & Fowler Jr, V. G. (2019). Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *Nature Reviews Microbiology*, *17*(4), 203-218. doi:10.1038/s41579-018-0147-4

Tuta, K. E., Okesola, A. O., & Umeokonkwo, C. D. (2019). The prevalence and risk factors associated with nasal methicillin-resistant Staphylococcus aureus colonization among children in a tertiary hospital in Nigeria. *Ethiopian Journal of Health Sciences*, *29*(4). doi:10.4314/ejhs.v29i4.10

Valverde, L. F., Rosas-Nexticapa, M., Alvarez-Ramirez, M., Lopez-Ramos, M., & Mateu-Armand, V. (2022). Theoretical evaluation of interaction of some dibenzo derivatives on both androgen receptor and 5a-reductase enzyme. *Clinical Cancer Investigation Journal*, *11*(5), 11-16.

Von Eiff, C., Becker, K., Machka, K., Stammer, H., & Peters, G. (2001). Nasal carriage as a source of Staphylococcus aureus bacteremia. *New England Journal of Medicine*, *344*(1), 11-16.