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Orji CO
Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 53, Nigeria

Ogba RC
Department of Science Laboratory Technology, Faculty of Science, Federal Polytechnic Ohodo, P.M.B. 01801, Enugu State, Nigeria

Emeruwa AP
Department of Microbiology and Parasitology, David Umahi Federal University of Health Science, Uburu, P.M.B. 211, Ebonyi State, Nigeria

Peter IU
Department of Public Health, Faculty of Health Technology and Engineering, Federal University of Allied Health Sciences, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria

Uzoeto HO
Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University of Allied Health Sciences, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria

Agumah BN
Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 53, Nigeria

Correspondence
Peter IU
Department of Public Health, Faculty of Health Technology and Engineering, Federal University of Allied Health Sciences, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria

Prevalence of *Staphylococcus aureus* nasal carriage that have developed resistance to second and third generation cephalosporins

Orji CO, Ogba RC, Emeruwa AP, Peter IU, Uzoeto HO and Agumah BN

Abstract
Cephalosporins have recently been used as a last resort therapy for complex bacterial infections. Unfortunately, there has been a global increase in cephalosporin-resistant strains to this drug class. This strain has become a major issue with tolerance and persistence now recognized as potential reasons for cephalosporin treatment failure. This study was aimed at determining the prevalence of *S. aureus* nasal carriage resistance to 2nd and 3rd generation Cephalosporin circulating Ishieke metropolis.
A total of sixty (60) nasal swab samples were collected from students that reside around selected five off-campus hostel. The samples were analyzed using Standard microbiological culture and Staphaurex™ Latex Agglutination Test for identification of *S. aureus.* Antibiotic resistance profile of *S. aureus* was performed using Kirby-bauer Disk diffusion method. The result of the study revealed a total of 38 (63.3%) positive *S. aureus* nasal carriage. The high carriage rate of positive samples was found in hostel HD (9/12, 75%), followed by hostel HB (8/12, 66.7%), hostel HE (8/12, 66.7%), hostel HA (7/12, 58.3%) and hostel HC (6/12, 50%) with the least carriage rate of *S. aureus.* The overall resistance proportion of *S. aureus* nasal carriage 57.3% and 42. 7% was recorded for 2nd and 3rd generation cephalosporin respectively. *S. aureus* nasal carriage resistance to 2nd generation cephalosporin were as follows: Cefoxitin resistance 100%, cefuroxime 55.6%-100%, cefotetan 50.0%-100%. 3rd generation cephalosporin were as follows: Ceftazidime 0-28.5%, Cefotaxime 50-71.4%, Ceftriaxone 50-100%.
Our findings revealed a high level of resistance to most of the tested 2nd and 3rd Generation cephalosporin antibiotics except Ceftazidime. However, the low resistance rate to ceftazidime in our study substantiates the judicious use of the drug in treating *S. aureus* infection. In keeping with *S. aureus* prevention and containment methods, there is a need for novel approaches to prevent AMR to fourth-generation cephalosporins and other antibiotic classes. Combination therapy and the availability of novel anti-*S. aureus* drugs will be critical in combating AMR caused by *S. aureus*.

Keywords: *Staphylococcus aureus,* 2nd and 3rd generation, cephalosporin, resistance

Introduction
Staphylococcus aureus is of one common opportunistic human pathogen that is present in many bodily parts, including the nasal cavity, skin, upper respiratory tract and perineum (Congdon *et al.,* 2023) [1]. About 20% to 40% of the general population is colonized by *S. aureus*, and 20% to 60% are sporadic carriers. (Congdon *et al.,* 2023; Ullah *et al.,* 2022) [1, 2]. However, they cause myriad of detrimental infections when they invade the internal tissues or enter the bloodstream. *S. aureus* is a significant pathogen implicated in the community and hospital-acquired infection. It can cause a wide range of infectious diseases, including mild infections of the skin and soft tissues, infections of the bones and joints, infective endocarditis, cardiovascular disorders, osteomyelitis, bacteremia, and deadly pneumonia in both healthy individuals and those with underlying medical conditions. The bacteria is also implicated in *Staphyloccocal* food poisoning (Congdon *et al.,* 2023; Ezeh *et al.,* 2023; Peter *et al.,* 2022a; Peter *et al.,* 2022b; Ed-Dra *et al.,* 2018) [1, 3, 4, 5, 6].
The nasal carriage of *Staphylococcus aureus* varies according on the population under study, the sampling strategy, and the identification methods utilized (Congdon *et al.,* 2023) [1]. A higher risk of infection has been demonstrated in earlier investigations for asymptomatic *S. aureus* carriers (Kluytmans *et al.,* 1997; von Eiff *et al.,* 2001) [7, 8] and can serve as a reservoir of transmittance via direct contact or through fomites (Lu *et al.,* 2005; Jaradat *et al.,* 2020) [9, 10]. *S. aureus* is challenging to eradicate owing to its high propensity for developing drug resistance (Foster, 2017) [11].

S. aureus utilizes various mechanisms of resistance including: production of β-lactamase enzymes to deactivate beta-lactam antibiotics, efflux pump for extruding of antibiotics such as tetracyclines (Peter *et al.*, 2022b) [5], reduced accumulation of macrolides antibiotics (Congdon *et al.*, 2023; Ezeh *et al.*, 2023) [1, 5], production of aminoglycosides modifying enzymes to inactivate aminoglycoside antibiotics, alteration of DNA gyrase and topoisomerase IV expression of floroquinolones antibiotics, and expression of *Mec*A genes which alters penicillin binding proteins (Congdon *et al.*, 2023; Peter *et al.*, 2022b) [1, 5]. In recent years, *S. aureus* has developed resistance to a variety of antibiotic, particularly the beta-lactam class (Congdon *et al.*, 2023; Peter *et al.*, 2022b) [1, 5] including cephalosporins. Few studies on the prevalence of resistance of *S. aureus* to different antibiotics in Nigeria has reported increase resistance to second and third generation cephalosporin (Ezeh *et al.*, 2023; Peter *et al.*, 2022b; Adesoji *et al.*, 2019; Moses *et al.*, 2017) [3, 5, 12, 13].

In Nigeria, cephalosporin are one of the frequently prescribe drug for treatment of *S. aureus* infection but due to drug misuse, self-medication, lack of trained medical personnel, and poverty, the efficacy of this drug may be eroded in most health care and community settings. Thus, it is important to ascertain the prevalence of resistance of *S. aureus* to second and third generation cephalosporin, which are commonly used drug, in Abakaliki, Nigeria. This will assist in enhancing available treatment choices and educating the public on the threat posed by the rise of multiple antibiotic-resistant strains of the disease as well as potential treatment failure causes.

Materials and Methods

Study area

The research was carried out at a selected off-campus hostel in Ishieke, located at latitude 6.385° N and longitude 8.027° E in Ebonyi State, Nigeria. The majority of the residents of the Ishieke community are Igbo rice, salt producers, and students (studying at the prestigious Ebonyi State University where the permanent site of the Campus is located). The student population in the Ishieke community is over 1000 people inhabiting and spreading across various locations in the community.

Collection of Sample and *S. aureus* nasal carriage Identification: Prior to sample collection, consent were obtained from the students involved in the study. A total of sixty (60) nasal swab samples were collected from students that reside around selected five off-campus hostels namely HA, HB, HC, HD, and HE at Ishieke. All swabbed samples were aseptically placed in a tubes containing 5 ml of Staph broth (Merck Co., Germany) and incubated at 35 °C for 24 hours. After overnight incubation, the turbid broth cultures were subsequently grown overnight at 35 °C by streaking onto mannitol salt agar (MSA) plates (BD Difco) (Edemekong *et al.*, 2022) [14]. All colonies with golden-yellow pigmentation were re-streak on Brain-heart Infusion agar (Merck Co., Germany) and incubated overnight. After 24hours, the isolates were subjected to Coagulase/protein A testing using the Staphaurex™ Latex Agglutination Test (bioMérieux, France) according to the manufacturer's instructions

Antibiotic Resistance testing of 2nd and 3rd Generation Cephalosporin

Antibiotic resistance testing was carried out using the Kirby Bauer disk diffusion method on sterilized Mueller-Hinton agar (Merck Co., Germany) in compliance with Clinical and Laboratory Standards Institute (CLSI, 2021) [15]. The test isolate's suspension was produced using 0.5 McFarland standards and seeded on solidified Mueller-Hinton agar. For 5 minutes, the plates were allowed to pre-diffuse. Thereafter, the following 2nd generation cephalosporin antibiotic [Cefuroxime (30 µg), Cefoxitin (30 µg), Cefotetan (30 µg)] and third generation cephalosporin [Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg)], was impregnated at equidistant from each other on the inoculated Mueller-Hinton (MH) agar plates and incubated at 37 °C for 24 hours (Ilang *et al.*, 2023; Ekuma *et al.*, 2023) [16, 17]. The widths of zones of inhibition were measured after an overnight incubation, and the results were interpreted using Clinical and Laboratory Standards Institute standards (CLSI, 2021) [15].

Results

The results show the distribution of *Staphylococcus aureus* (*S. aureus*) isolated from nasal swabs of students in different hostels (HA to HE) in Ishieke location. A total of 60 nasal swabs were collected, and 38 (63.3%) were positive for *S. aureus*, while 22 (36.7%) were negative. The highest number of positive samples was found in hostel HA (7/12, 58.3%), followed by hostel HB (8/12, 66.7%), hostel HC (6/12, 50%), hostel HD (9/12, 75%), and hostel HE (8/12, 66.7%) as presented in Figure 1. *S. aureus* nasal carriage resistance to 2nd generation cephalosporin were as follows: Cefoxitin resistance 100%, cefuroxime 55.6%-100%, cefotetan 50.0%-100%. 3rd generation cephalosporin were as follows: Ceftazidime 0-28.5%, Cefotaxime 50-71.4%, Ceftriaxone 50-100% as shown in Figure 2. Figure 3 shows that the total resistance fraction of *S. aureus* nasal carriage to second and third generation cephalosporins recording 57.3% and 42.7% respectively.

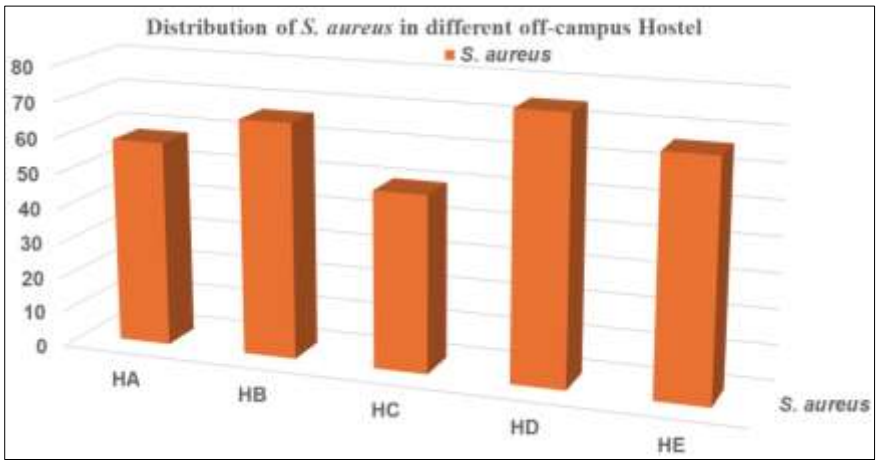

Fig 1: 3-D column chart show the distribution of *Staphylococcus aureus* isolate from nasal swabs of students in different hostels

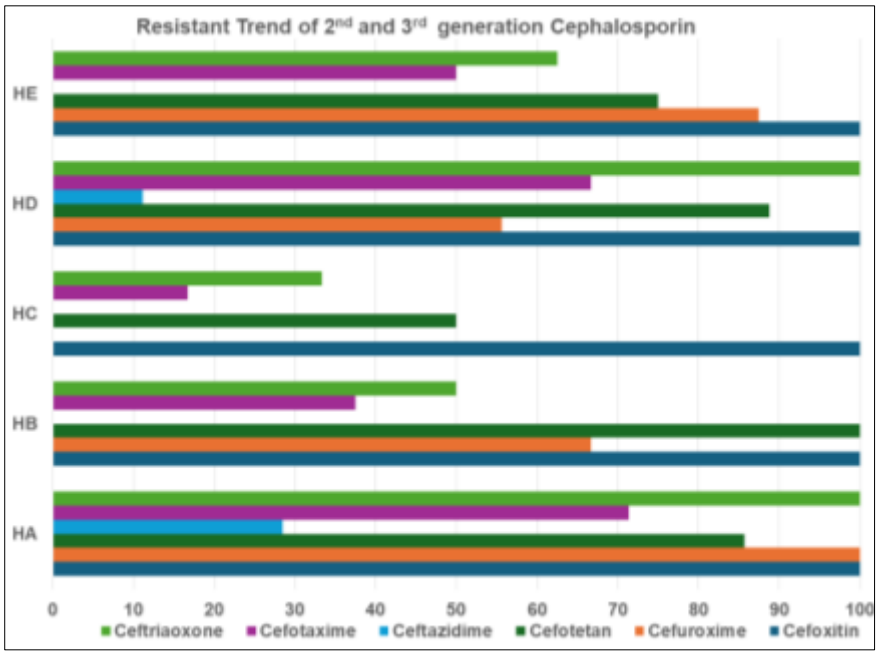

Fig 2: Chart presentation of *S. aureus* nasal carriage resistance to 2nd and 3rd Generation Cephalosporin Antibiotic

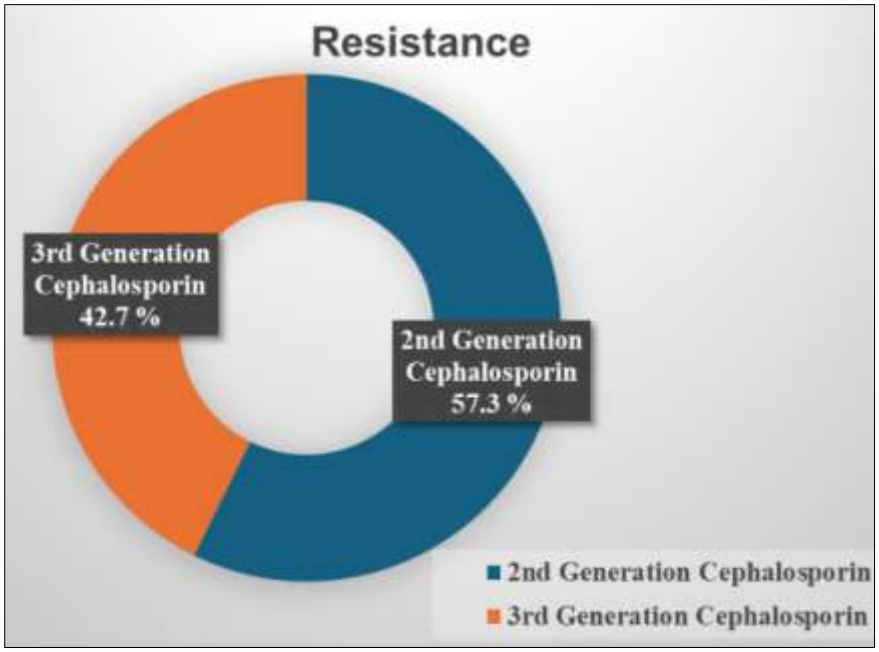

Fig 3: Overall resistance proportion of *S. aureus* nasal carriage

Discussion

Of the 60 nasal swab samples collected from students in different off-campus hostels, 38 (63.3%) were positive for *S. aureus*. The high prevalence of *S. aureus* nasal carriage in our study was not in accordance with earlier investigation amongst university students were nasal carriage rates ranging from 10-33% was reported (Stancu *et al.*, 2020; Morita *et al.*, 2007) [18, 19] while in University of Hartford students 21% was reported (Congdon *et al.*, 2023) [1]. Comparably, high prevalence of *S. aureus* nasal carriage 69.8% and 80.3% has been reported among atopic dermatitis and healthcare workers (80.3%) (Rukan *et al.*, 2021; Blicharz *et al.*, 2020) [20, 21]. These groups are either at a higher risk for *S. aureus* colonization due to compromised immune systems, which enhances *S. aureus* proliferation.

The high prevalence reported in our may stem from numerous factors, such as identification procedures geographic region, demographic variable of the studied population, body site sampled used, and sample size of the study, which may contribute to the considerable range in *S. aureus* colonization rates reported between studies. Consequently, the high prevalence of *S. aureus* found in our study, implies that the clinical significance of these bacteria colonizing the nasal niche should not be underestimate due to *S. aureus* capacity to serve as opportunistic pathogens (Heilmann *et al.*, 2019) [22], reservoirs for dissemination of antibiotic resistance for pathogenic organisms (i.e., *Staphylococcus aureus*) (Xu *et al.*, 2018) [23], or conversely, some of the non-pathogenic Coagulase Negative S *Staphylococcus aureus* (CoNS) could benefit human health by potentially serving as antagonists for *S. aureus* colonization (Sakr *et al.*, 2018) [24].

S. aureus resistance to 2nd and 3rd generation cephalosporin ranges from 50-100%. A high rate of resistance to cephalosporin has been published by few authors (Ezeh *et al.*, 2023; Buonsenso *et al.*, 2023; Chelkeba and Melaku, 2021; Uribe-García *et al.*, 2020; Mohammadi *et al.*, 2020; Ariom *et al.*, 2019; Deyno *et al.*, 2017) [3, 25, 26, 27, 28, 29, 30] which strongly indicates an increased level of resistance to this antibiotic group due to a larger exposure rate to the sub-lethal doses of the antimicrobial agents.

The resistance rate reported in our study has been reported by several researchers; Islam *et al.* (2018) [31] in Bangladesh reported that *S. aureus* showed 83.0% resistance to cefotaxime. Another study reported the prevalence of *S. aureus* resistance to ceftriaxone 34% (95% CI: 25%, 43%) and to cefoxitin 27% (95% CI: 6%, 54%) (Deyno *et al.*, 2017) [30]. Another researcher in Cameroon revealed that MRSA strains displayed high resistance to cefoxitin (100%) (Kenge *et al.*, 2017) [32]. In India 78.5% resistant to cefotaxime (Vidhani *et al.*, (2001) [33] was reported. Earlier study reported that 60% of MSSA bloodstream isolates were resistant to ceftriaxone (Pickering *et al.*, 2014) [34]. In Rivers State Nigeria, The isolates showed an overall 100% resistance to Ceftazidime and Cefuroxime (Onyeka *et al.*, 2021) [35]. In Malaysia, *S. aureus* showed 37.8% (191/505) resistance to ceftriaxone (Abu El Aish *et al.*, 2023) [36]. Another study showed 23.4% (11/47) and 34% (16/47) resistance to ceftriaxone and ceftazidime, respectively (Gashe *et al.*, 2018) [37] while in Busan, South Korea, the *S. aureus* isolates were highly resistant to cefotetan 69.5% (Yoon *et al.*, 2022) [38].

However, our result differs with other studies conducted in different areas which reported the susceptibility of the strains towards the second and third-generation cephalosporins (Aruna *et al.*, 2017; Breurec *et al.*, 2016; Masood and Aslam, 2010; Nkang *et al.*, 2009) [39, 40, 41, 42]. The results of this study suggest that, even within the same country or continent the resistance rate of *S. aureus* can differ between different regions over time.

Cephalosporins, like other beta-lactams, bind to the bacterial penicillin-binding proteins (PBPs). These correspond to the D-ala-D-ala trans-, carboxy- and endo-peptidases responsible for catalysing the cross-linking of newly formed peptidoglycan. Resistance arises when the PBPs-and particularly the transpeptidases-are modified, or when they are protected by beta-lactamases or 'permeability barriers'. Target-mediated cephalosporin resistance can involve either reduced affinity of an existing PBP component, or the acquisition of a supplementary beta-lactam-insensitive PBP. Beta-lactamases are produced widely by bacteria and may be determined by chromosomal or plasmid DNA (Oke *et al.*, 2020; Ilang *et al.*, 2023; Adibe-Nwafor *et al.*, 2023) [43, 44, 45].

Cefoxitin is considered to be a marker of the MRSA phenotype, it is clear that the evolution of *S. aureus* can be traced back to the acquisition of the exogenous gene (*mec*A), which is part of the staphylococcal cassette chromosome mec (SCCmec) (types I-VII) and is controlled by MecI (a repressor) and MecR1 (a transducer) and represents the regulatory/signaling proteins of the blaZ system (Congdon *et al.*, 2023; Peter *et al.*, 2022c) [1, 46]. The mecA gene produces PBP2a, a peptidoglycan transpeptidase that can provide resistance to all β-lactam and other antibiotics (Peter *et al.*, 2022c; Li *et al.*, 2006) [46, 47]. Other isolates carrying a specific variation of SCCmec types II and III exhibit a wider range of resistance due to the presence of additional resistance genes (Peter *et al.*, 2022c) [46].

Second and third generation cephalosporin's resistance is the result of numerous circumstances. First, failing to prevent infections leads to recurring infections, which in turn allows resistant strains to infect healthy people. The second is improper usage of antibiotics by patients after they are prescribed and dispensed. Antibiotics are widely available without a valid prescription in the majority of Nigerian cities, which encourages widespread overuse of the drugs. The third issue can be non-standard practice and medical practitioners' abuse of antibiotics. The fourth reason can be unsanitary conditions in the hospital that enhance the spread and convergence of drug resistant strain. The last possible contributing aspect could be the shift toward empiric therapy caused by the absence of standard antibiotic susceptibility testing.

Conclusion

It is important to note that, the studied settings have a high rate of unchecked cephalosporin use and over-the-counter sales of antibiotics without proper prescription. The significant prevalence of cephalosporin resistance seen in this study could be attributed to the cumulative influence of these factors over time. However the low resistance rate to ceftazidime in our study substantiate the judicious use of the drug in treating *S. aureus* infection. Additionally, drugs from other antibiotic class needed to treat or prevent *S. aureus* infections should be made affordable for the treatment of *S. aureus* infection.

In keeping with *S. aureus* prevention and containment methods, there is a need for novel approaches to prevent AMR to fourth-generation cephalosporins and other antibiotic classes. Combination therapy and the availability

of novel anti-*S. aureus* drugs will be critical in combating AMR caused by *S. aureus*.

Conflict of Interest: Not available.

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