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Prevalence and antibiotic susceptibility profiles of methicillin-resistant *Staphylococcus aureus* isolates from apparently healthy school pupils and students in Enugu and Ebonyi States, Nigeria

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Abstract:

Background: Infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) remains a big health challenge around the world because it is associated with increase morbidity, mortality and healthcare costs. This study aimed to determine the prevalence and antibiotic susceptibility profiles of MRSA isolated from apparently healthy school pupils and students in Enugu and Ebonyi States, Nigeria.
Methodology: This was a descriptive cross-sectional study of apparently healthy pupils and students from the three Senatorial districts in each of Enugu and Ebonyi States, recruited by multi-stage random sampling technique. Urine and swab samples of nose, armpit and groin were collected from the participants and cultured for *Staphylococcus aureus* using standard microbiological methods. MRSA isolates were detected by the Kirby-Bauer disc diffusion method using cefoxitin 30µg disc. Statistical analysis of data was done using Chi-square and Fisher's exact tests, and binary logistic regression analysis to compare the prevalence of *S. aureus* and MRSA with respect to gender, schools, senatorial districts, and States. P value less than 0.5 was considered statistical significance.
Results: A total of 441 participants were recruited with 234 (53.1%) males and 207 (46.9%) females. Samples of 206 (46.7%) participants yielded *S. aureus,* and 140 (31.7%) were confirmed as MRSA. Gender distribution showed comparable *S. aureus* colonization rate between males (47.0%, n=110) and females (46.4%, n=96), while the prevalence of MRSA colonization was higher in females (35.3%, n=73) than males (28.6%, n=67). State-specific prevalence of MRSA colonization was 30.7% (n=71/231) in Enugu and 32.9% (n=69/210) in Ebonyi. There was no statistically significant association between MRSA colonization prevalence and sex (χ^2=2.89, p=0.089) or State (χ^2=0.46, p=0.50). However, prevalence of MRSA colonization was higher among secondary school students compared to primary school pupils, with borderline statistical significance (χ^2=3.84, p=0.050). The overall antibiotic resistance profile of MRSA isolates revealed resistance rates ranging from 15.1% for gentamicin to 97.9% for penicillin.
Conclusion: This study reported high prevalence of MRSA colonization among apparently healthy pupils and students in both Enugu and Ebonyi States, southeast Nigeria. Government should strengthen infection prevention efforts in schools and enforce surveillance policies for antimicrobial resistance in the States. Antibiotics such as ciprofloxacin and gentamicin may still be effective for therapy of MRSA infections in the region.

Keywords: Prevalence; Antibiotic susceptibility; MRSA; Colonization; Pupils; Students

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Prévalence et profils de sensibilité aux antibiotiques des isolats de *Staphylococcus aureus* résistant à la méthicilline chez des élèves et étudiants apparemment en bonne santé dans les États d'Enugu et d'Ebonyi, au Nigéria

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Résumé:

Contexte: L'infection à *Staphylococcus aureus* résistant à la méthicilline (SARM) demeure un problème de santé publique majeur à l'échelle mondiale, car elle est associée à une augmentation de la morbidité, de la mortalité et des coûts des soins de santé. Cette étude visait à déterminer la prévalence et les profils de sensibilité aux antibiotiques du SARM isolé chez des élèves et étudiants apparemment en bonne santé dans les États d'Enugu et d'Ebonyi, au Nigéria.
Méthodologie: Il s'agit d'une étude descriptive transversale menée auprès d'élèves et étudiants apparemment en bonne santé issus des trois districts sénatoriaux de chacun des États d'Enugu et d'Ebonyi, recrutés par échantillonnage aléatoire à plusieurs degrés. Des échantillons d'urine et des prélèvements nasaux, axillaires et inguinaux ont été collectés chez les participants et mis en culture pour la recherche de Staphylococcus aureus selon les méthodes microbiologiques standard. Les isolats de SARM ont été détectés par la méthode de diffusion sur disque de Kirby-Bauer, en utilisant un disque de céfoxitine 30µg. L'analyse statistique des données a été réalisée à l'aide des tests du χ^2 et de Fisher, ainsi que d'une régression logistique binaire, afin de comparer la prévalence de *S. aureus* et du SARM en fonction du sexe, de l'établissement scolaire, de la circonscription sénatoriale et de l'État. Un seuil de signification statistique de $p<0,05$ a été retenu.
Résultats: Au total, 441 participants ont été recrutés, dont 234 hommes (53,1%) et 207 femmes (46,9%). Des prélèvements ont révélé la présence de *S. aureus* chez 206 participants (46,7%), et 140 (31,7%) ont été confirmés comme porteurs du SARM. La répartition par sexe a montré un taux de colonisation par *S. aureus* comparable entre les hommes (47,0%, n=110) et les femmes (46,4%, n=96), tandis que la prévalence de la colonisation par le SARM était plus élevée chez les femmes (35,3%, n=73) que chez les hommes (28,6%, n=67). La prévalence de la colonisation par le SARM était de 30,7% (n=71/231) à Enugu et de 32,9% (n=69/210) à Ebonyi. Aucune association statistiquement significative n'a été observée entre la prévalence de la colonisation par le SARM et le sexe ($\chi^2=2,89$, $p=0,089$) ou l'État ($\chi^2=0,46$, $p=0,50$). Cependant, la prévalence de la colonisation par le SARM était plus élevée chez les élèves du secondaire que chez ceux du primaire, avec une signification statistique limite ($\chi^2=3,84$, $p=0,050$). Le profil global de résistance aux antibiotiques des isolats de SARM a révélé des taux de résistance allant de 15,1% pour la gentamicine à 97,9% pour la pénicilline.
Conclusion: Cette étude a mis en évidence une forte prévalence de la colonisation par le SARM chez des élèves et étudiants apparemment en bonne santé dans les États d'Enugu et d'Ebonyi, au sud-est du Nigéria. Les autorités devraient renforcer les efforts de prévention des infections dans les écoles et appliquer des politiques de surveillance de la résistance aux antimicrobiens dans ces États. Des antibiotiques comme la ciprofloxacine et la gentamicine peuvent encore être efficaces pour le traitement des infections à SARM dans la région.

Mots-clés: Prévalence; Sensibilité aux antibiotiques; SARM; Colonisation; Élèves; Étudiants

Introduction:

Staphylococcus aureus remains one of the most clinically important bacterial pathogens worldwide due to its remarkable capacity to colonize humans, cause a wide range of infections, and rapidly acquire antimicrobial resistance (1,2). The emergence and global spread of methicillin-resistant *S. aureus* (MRSA) have significantly altered the epidemiology and clinical management of *S. aureus* infections over the last five decades. Initially confined to hospital settings in the 1960s and 1970s, MRSA evolved into a major cause of healthcare-associated infections (HA-MRSA), particularly among hospitalized and immunocompromised individuals (3,4). However, a major epidemiological shift occurred in the mid-1990s when MRSA strains began to emerge in individuals with no prior healthcare exposure, giving rise to MRSA in settings outside healthcare (5-8).

Staphylococcus aureus commonly exists as a normal flora on the skin and in the nasal passages of healthy individuals, yet it possesses the capacity to cause infections ranging from minor skin conditions to serious systemic diseases (6-8). Its silent colonization makes it a major source of transmission within communities. This is especially heightened among children and adolescents, who often interact closely in crowded settings like schools, promoting its spread (9-12). Although it is not a typical cause of urinary tract infections, its presence in urine especially among apparently healthy individuals may indicate colonization or transient carriage with potential clinical and epidemiolo-

gical significance (13).

In Africa, MRSA epidemiology is complex and underreported, with significant regional variations. Studies across West, East, and Southern Africa consistently reveal increasing prevalence of MRSA in both hospitals and community settings, fueled by widespread antibiotic misuse, poor infection control practices, overcrowding, and limited surveillance systems (14, 15). Nigeria, in particular, bears a substantial burden of *S. aureus* infections and has reported high and rising MRSA prevalence across various States and population groups. Previous research from southeastern Nigeria shows that MRSA colonization is widespread among school children, adults, hospital outpatients, livestock handlers, and apparently healthy community members (16-17). This highlights the expanding reservoir of MRSA and the increasing risk of transmission within public institutions, including schools.

School environments represent one of the most important settings for MRSA transmission due to overcrowding, frequent skin-to-skin contact, limited access to hygiene facilities, and behavioral factors common among children and adolescents such as sharing of personal belongings (11,18-20). Several studies indicate that primary school pupils may be more vulnerable to MRSA colonization because of poor hygiene practices, increased physical interaction, and susceptibility to minor skin injuries that facilitate bacterial colonization (6). Gender-related differences also exist, with some research reporting higher MRSA colonization among females, possibly due to grooming habits, sharing of personal items, or cultural practices that enhance bacterial dissemination (21, 22). The occurrence of *S. aureus* in urine samples of asymptomatic individuals is increasingly recognized as an important public health concern (18,23). Such carriage may serve as a silent reservoir for transmission and may predispose individuals to future infections under favorable conditions (24,25). Hence, surveillance in this population is essential for timely detection and outbreak prevention.

The detection of MRSA in urine, groin, armpit anterior nares and wound samples from apparently healthy school pupils and students raises concerns about the hidden burden of antimicrobial resistance within the community. The southeastern region of Nigeria, particularly Enugu and Ebonyi States, has experienced rising incidences of antibiotic-resistant infections over the last decade. Despite this, data specifically focusing on urinary carriage among asymptomatic school populations, particularly in Enugu and Ebonyi States, remain limited. Generating such data is crucial for understanding local epidemiology and informing control strategies.

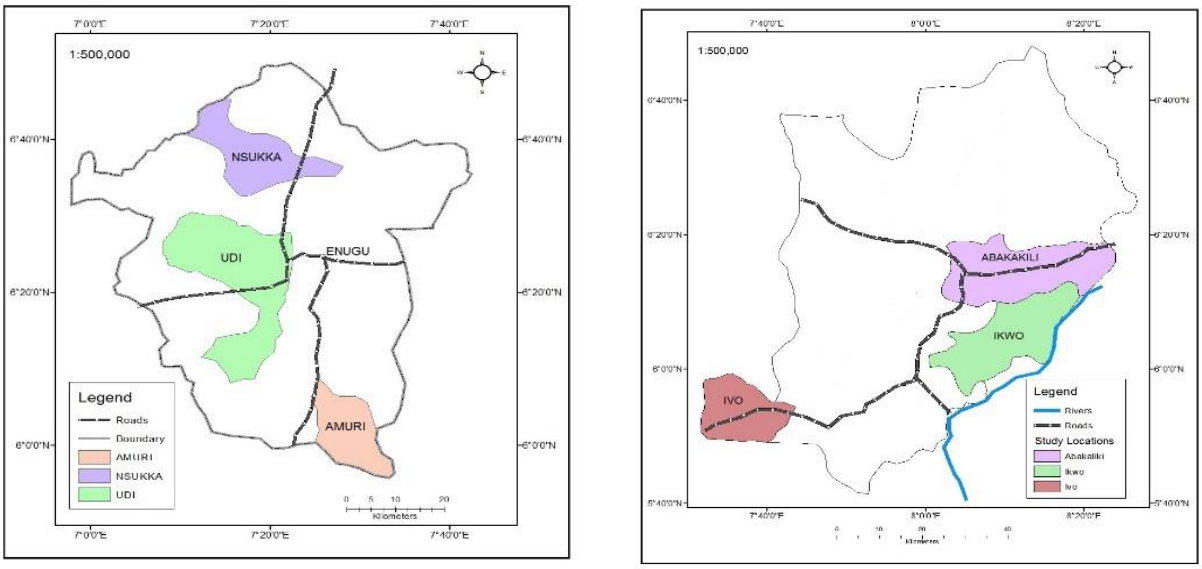
This study therefore aimed to determine the prevalence and antibiotic susceptibility patterns of methicillin-resistant *S. aureus* isolated from samples of apparently healthy school pupils and students in Enugu and Ebonyi States, Nigeria. The findings are expected to contribute to existing knowledge and support the development of effective infection prevention measures and rational antibiotic use policies.

Materials and method:

Study design and setting:

This was a descriptive cross-sectional study of apparently healthy selected pupils and students carried out in Enugu and Ebonyi States, located in the southeastern region of Nigeria (Fig 1a and b). Enugu State lies between 5°56′N–7°06′N and 6°53′E–7°55′E. It has a tropical climate with wet and dry seasons that support microbial persistence. The State is largely urban, especially in Enugu, with high population density and frequent interpersonal contact, facilitating *S. aureus* and MRSA transmission. Numerous schools and healthcare facilites, including the University of Nigeria Teaching Hospital, provide a suitable setting for studying bacterial colonization and antimicrobial resistance patterns (27).

Ebonyi State lies between 5°40′N–6°45′N and 7°30′E–8°30′E. It has a tropical climate with significant rainfall supporting agriculture. The State is predominantly rural, with Abakaliki as the main urban center. Farming and livestock rearing increase human–animal contact, influencing *S. aureus* transmission. Limited healthcare access and variable sanitation may affect resistance patterns. Facilities such as the Alex Ekwueme Federal University Teaching Hospital support healthcare delivery and research (28).



(a) (b)

Fig 1: Geographical Map of Enugu State (1a) and Ebonyi State (1b) showing the study locations in the three different senatorial study location zones (26)

Study participants and inclusion criteria:

For this study, participants were apparently healthy pupils and students aged 8-12 years and 12-18 years, respectively. Apparently healthy individuals were defined as participants who, upon brief clinical assessment, exhibited no signs of active infection (e. g. no fever ≥38°C, no pain or disability, abscesses, or nasal discharge), had no recent (≤3 months) hospitalization, surgery, or invasive medical procedures, no antibiotic use within the preceding 2–4 weeks, and no known immunocompromising conditions.

Eligibility was further supported by participant self-report and, where applicable, review of medical history. Participants were selected from representative schools (primary and secondary) from each of the three senatorial districts in both States i. e. three primary and three secondary schools from each State.

Ethical consideration:

Informed consent was obtained from the parents or legal guardians of all participants below 18 years of age after providing detailed information about the study objectives, procedures, potential risks, and benefits. In addition to parental/guardian consent, assent was obtained from adolescents aged 12–17 years after the study was explained in age-appropriate language. These participants were informed that their participation was voluntary and that they could decline participation or withdraw at any stage without penalty.

For younger children below 12 years of age, the study procedures were explained in simple and understandable terms, and verbal assent was obtained where appropriate before sample collection. Every fundamental study was done in line with the World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human subjects and identifiable human material or data (29).

Determination of sample size:

The sample size for the study was calculated using the single population proportion formula, $n = Z^2p(1-p)/d^2$ where, n = required sample size, Z=95% confidence level (=1.96), p = estimated prevalence of 45.6% (=0.456) from a previous study (8), and d=margin of error of 5% (0.05). Thus, applying these parameters, the minimum sample size required for this study was calculated as 381. However, a sample size of 441 was considered adequate to compensate for non-response or sample loss and to improve statistical power.

Sampling technique:

The study area covers two States (Enugu and Ebonyi) hence, a multistage sampling method was applied to systematically select participants in stages. Stage 1 involved selection of senatorial districts or local government areas (LGAs) within each State; stage 2 involved selection of schools (primary and secondary) within each selected LGA; and stage 3 involved selection of pupils and students within the selected schools. This approach was to ensure a good geographical coverage and to

make the study more practical and cost-effective.

Secondly, within the selected schools, stratified random sampling was used to stratify participants based on factors including age group (8–12 years and 12–18 years), educational level (primary vs secondary school) and gender. A simple random sampling was applied within each stratified group to select participants, which ensured that all relevant subgroups were adequately represented in the study.

Sample and data collection:

Clinical samples were randomly collected from a total of 441 pupils and students (234 males, and 207 females), with urine (n= 398), wound swab (n=15), armpit swab (n= 11), nasal swab (n=11), and groin swab (n= 6). The demographic data (age, sex) of the participants were collected using a designed proforma.

Clean-catch midstream freshly voided urine specimens were collected into sterile plastic specimen bottles. The samples were transported to the Department of Applied Microbiology Laboratory unit, Faculty of Science, Ebonyi State University, Abakaliki within two hours of collection for bacteriological analysis.

Isolation and identification of *Staphylococcus aureus* isolates:

Samples were aseptically inoculated on Mannitol Salt Agar (Oxoid, United Kingdom) and incubated aerobically at 37°C for 18–24 hours. Discrete colonies were sub-cultured on fresh MSA plates to obtain pure isolates. Presumptive *S. aureus* isolates were identified based on the appearance of yellow or golden colonies with mannitol fermentation on MSA. Further confirmation was carried out using standard microbiological procedures, including Gram staining, catalase test, coagulase test, haemolysis on blood agar, and assessment of colony morphology (30,31).

Isolation of *S. aureus* and MRSA from urine samples was considered clinically significant only when recovered as a pure culture at $\geq 10^5$ CFU/ml, or at $\geq 10^4$ CFU/mL with supportive evidence such as pyuria or repeat isolation, to minimize the inclusion of contaminants from skin flora.

Antibiotic susceptibility test of *Staphylococcus aureus* isolates:

The antibiotic susceptibility test of the identified isolates was performed by the disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines. Inoculum of the test isolate was prepared from pure colonies on the sub-culture plate and standardized to 0.5 McFarland standard equivalent, which was then inoculated on the surface of Mueller Hinton agar (Oxoid, UK) plates using sterile swab stick.

Antibiotic discs (Oxoid UK); ceftazidime (CAZ, 30µg), ciprofloxacin (CIP, 5µg), clindamycin (DA, 2µg), penicillin (P, 10µg), erythromycin (E, 15µg), nitrofurantoin (F_{200}, 300µg), gentamicin (CN, 5µg), sulphamethoxazole (RL, 25µg), and tetracycline (TE, 30µg) were placed on the surface of the inoculated agar plates 30mm apart using a sterile forcep. The antibiotics were allowed to diffuse for about 10 minutes and the plates incubated at 37°C for 18-24 hours. The diameters of inhibition zones were measured in millimeter with a meter rule, recorded and interpreted as sensitive or resistant according to the Clinical Laboratory Standard Institute (CLSI) guidelines (32).

Detection of methicillin resistant *S. aureus:*

This was done using the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Pure colonies of the isolated bacteria were inoculated into 5 ml nutrient broth. The turbidity of the broth was adjusted to 0.5 McFarland standards and inoculated on Mueller-Hinton agar plate with a sterile swab. Cefoxitin (FOX, 30µg) disc was placed on the inoculated agar plates to detect MRSA. The plates were incubated at 37°C for full 24 hours. Diameter of zone of inhibition was measured, recorded and interpreted as resistant if the inhibition zone diameter was ≤ 21 mm, according to the CLSI guidelines (32).

Determination of multiple antibiotic resistance index (MARI) of MRSA isolates:

Multiple antibiotic resistance index (MA RI) of each *S. aureus* isolate was calculated as the number of antibiotics to which the isolate was resistant to, divided by the total number of antibiotics that was tested on the isolate, as previously described by Ukpai et al., (31).

Statistical analysis of data:

The prevalence of *S. aureus* and MRSA colonization across the States, schools, and gender was compared using the Chi-square test of independence. Fisher's exact test was applied where expected cell counts were less than five. A p-value<0.05 was considered statistically significant. Additionally, binary logistic regression analysis was performed to identify factors associated with MRSA colonization.

Results

Prevalence of *Staphylococcus aureus* and MRSA colonization among the study participants:

Table 1 shows the distribution of *S. aureus* and MRSA isolates among the participants across the senatorial districts in both Enugu and Ebonyi States. The overall prevalence of *S. aureus* colonization is 46.7%, while the overall prevalence for MRSA is 31.7%. Ebonyi State showed a higher prevalence of both *S. aureus* (49.5%) and MRSA (32.9%) compared to Enugu, with 44.2% and 30.7%, respectively, indicating a marginally higher but insignificant colonization rate of pupils and students in Ebonyi State by *S. aureus* and MRSA (p=0.3017 and p=0.7073).

Although *S. aureus* colonization rate was similar between males and females in both States (p=0.9916 for Ebonyi, p=0.9849 for Enugu, and p=0.9704 for both States), MRSA prevalence was consistently but insignificantly higher among females in both States (p= 0.2849 for Ebonyi, p=0.4424 for Enugu, and p=0.1642 for both States), suggesting possible gender-related exposure and behavioral factors influencing transmission.

Comparative prevalence of *S. aureus* and MRSA colonization among the study participants:

The prevalence distribution of *S. aureus* and MRSA colonization among the participants across school types is shown in Table 2. In Enugu, secondary school students had significantly higher prevalence of *S. aureus* (57.8%) and MRSA (42.0%) colonization than primary school pupils (34.8% and 18.8%) (p=0.0083 and p=0.0002, respectively), whereas in Ebonyi, primary school pupils had higher but insignificant *S. aureus* (53.3%) and MRSA (38.1%) colonization compared to secondary school students (45.7% and 27.6%) (p=0.3340 and p= 0.1418, respectively).

Overall, secondary school students had higher but insignificant MRSA colonization rate (35.3%) than primary schools (28.1%) (p= 0.1305). These differences suggest that environmental factors, hygiene practices, or age-related behaviors may influence MRSA transmission differently in each State.

Table 1: Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* colonization among apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

States	**Total no examined**		**No +ve for *S. aureus* (%)**		**No +ve for MRSA (%)**	
	M	**F**	**M**	**F**	**M**	**F**
Enugu State						
SENE	26	14	9 (34.6)	6 (42.8)	2 (7.6)	4 (28.6)
PENE	18	24	8 (44.4)	2 (8.3)	4 (22.2)	1 (4.2)
SENN	28	12	11 (39.3)	8 (66.7)	11 (39.3)	8 (66.7)
PENN	15	12	5 (33.3)	1 (8.3)	5 (33.3)	1 (8.3)
SENS	13	26	11 (84.6)	18 (69.2)	8 (61.5)	17 (65.3)
PENS	21	22	10 (47.6)	13 (59.1)	4 (19.0)	6 (27.3)
Sub-Total	121	110	54 (44.6)	48 (43.6)	34 (28.1)	37 (33.6)
	231		102 (44.2)		71 (30.7)	
Ebonyi State						
SEBN	14	10	4 (28.6)	2 (20.0)	0	1 (10.0)
PEBN	22	18	7 (31.8)	6 (33.3)	5 (22.7)	5 (35.7)
SEBC	33	19	21 (63.6)	11 (57.9)	12 (36.4)	9 (47.4)
PEBC	17	13	10 (58.8)	7 (53.9)	5 (29.4)	3 (23.1)
SEBS	9	20	2 (22.2)	8 (40.0)	1 (11.1)	6 (30.0)
PEBS	18	17	12 (66.7)	14 (82.4)	10 (55.6)	12 (70.6)
Sub-Total	113	97	56 (49.6)	48 (49.5)	33 (29.2)	36 (37.1)
	210		104 (49.5)		69 (32.9)	
Total	**234**	**207**	**110 (47.0)**	**96 (46.4)**	**67 (28.6)**	**73 (35.3)**
	441		**206 (46.7)**		**140 (31.7)**	

SENE- Secondary school Enugu East Senatorial Zone PENE- Primary school Enugu East Senatorial Zone
SENN- Primary school Enugu North Senatorial Zone. PENN- Primary school Enugu North Senatorial Zone
SENS- Secondary school Enugu South Senatorial Zone. PENS-Primary school Enugu South Senatorial Zone
SEBN- Secondary school Ebonyi North Senatorial Zone. PEBN- Primary school Ebonyi North Senatorial Zone
SEBC- Secondary school Ebonyi North Senatorial Zone. PEBC- Primary school Ebonyi North Senatorial Zone
SEBS -Secondary school Ebonyi North Senatorial Zone. PEBS- Primary school Ebonyi North Senatorial Zone

Table 2: Comparative prevalence distribution of *Staphylococcus aureus* and MRSA colonization among apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

Location	Total no examined		No positive for *S. aureus* (%)		No positive for MRSA (%)	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
Enugu	112	119	39 (34.8)	63 (57.8)	21 (18.8)	50 (42.0)
Ebonyi	105	105	56 (53.3)	48 (45.7)	40 (38.1)	29 (27.6)
Total	**217**	**224**	**95 (43.8)**	**111 (49.6)**	**61 (28.1)**	**79 (35.3)**

Antibiotic resistance profile of MRSA isolates:

The antibiotic resistance profile (Table 3, Fig 2 and Fig 3), supported by the MARI distribution (Table 4), indicates widespread multi-drug resistance among the MRSA isolates. Very high resistance rates were observed for penicillin (97.9%), ceftazidime (93.0%), and sulph amethoxazole (90.7%), while moderate resistance was seen with erythromycin (76.8%), tetracycline (75.7%), and clindamycin (68.7%). In contrast, lower resistance rates were recorded for gentamicin (15.1%) and ciprofloxacin (23.3%).

The predominance of high MARI values (≥0.6) in both States, especially in Ebonyi indicates high antimicrobial consumption in the community.

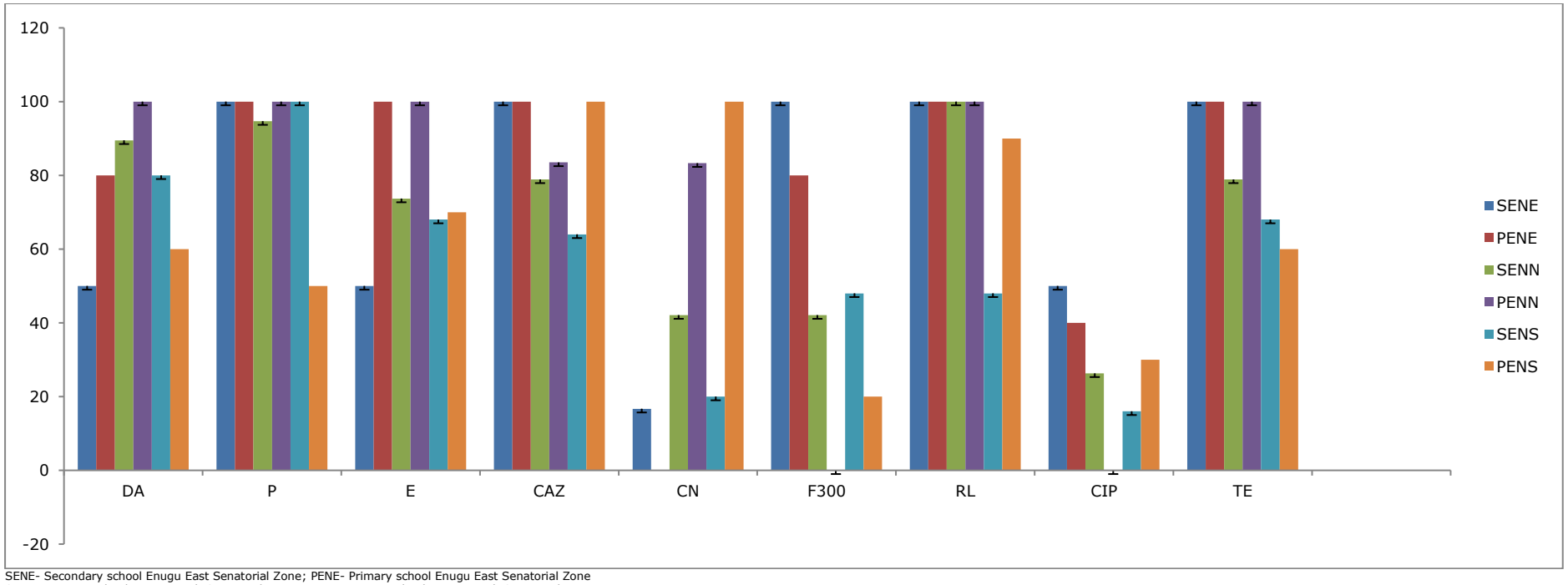
Table 4: Multiple Antibiotic Resistance Index (MARI) of MRSA isolates from apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

MARI value	Enugu	Ebonyi
	No of MRSA (%)	No of MRSA (%)
0.1	3 (4.2)	2 (2.9)
0.2	2 (2.8)	2 (2.9)
0.3	3 (4.2)	6 (8.5)
0.4	12 (16.9)	5 (7.3)
0.5	5 (7.0)	6 (8.5)
0.6	11 (15.5)	10 (14.5)
0.7	11 (15.5)	16 (23.2)
0.8	12 (16.9)	17 (24.6)
0.9	12 (16.9)	5 (7.3)
1.0	0	0
Total	**71 (100)**	**69 (100)**

No (%) = Number and percentage of MRSA with specified MARI value

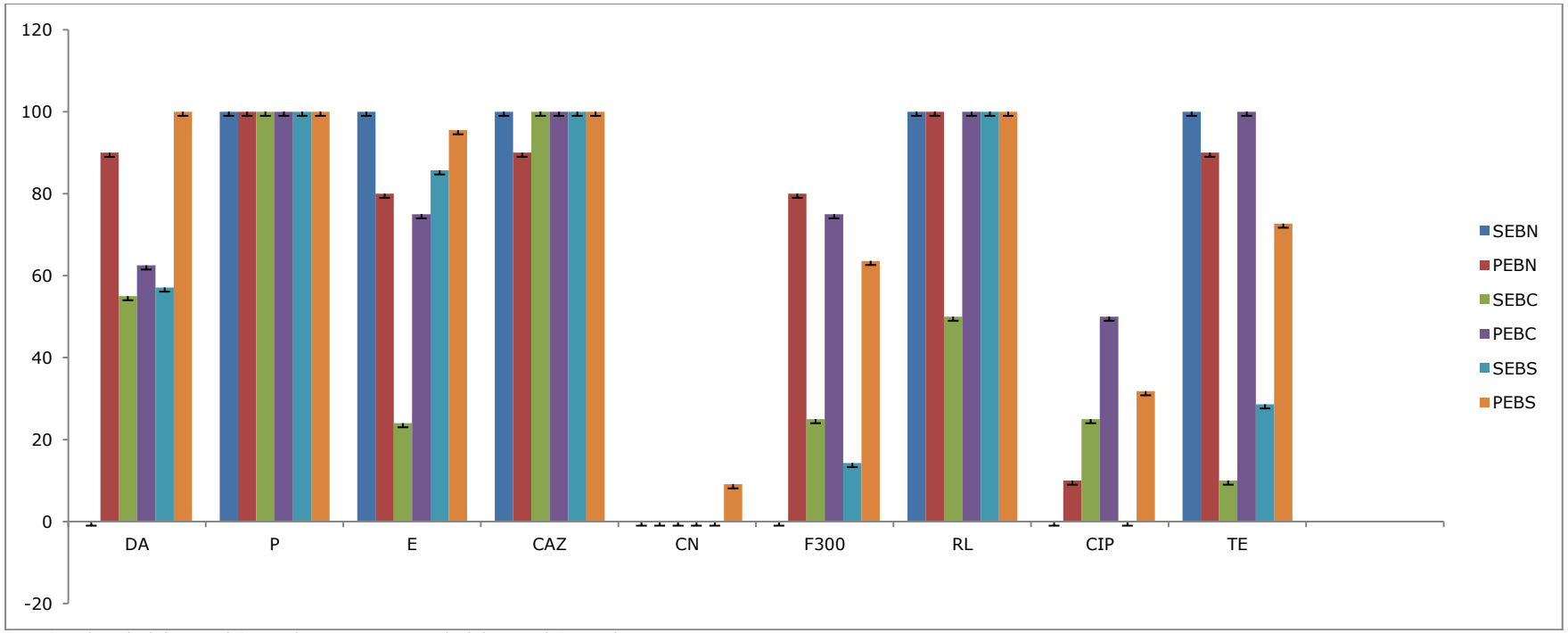
Table 3: Antibiotic resistance (%) of MRSA isolates from apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

Antibiotics	MRSA isolates		
	Enugu (%)	Ebonyi (%)	Average of both States (%)
Penicillin	95.6	100.0	97.9
Ceftazidime	87.7	98.3	93.0
Sulphamethoxazole	89.7	91.7	90.7
Erythromycin	76.8	76.7	76.8
Clindamycin	76.6	60.8	68.7
Nitrofurantoin	48.4	43.0	45.7
Ciprofloxacin	27.1	19.5	23.3
Gentamicin	28.7	1.5	15.1
Tetracycline	84.5	66.9	75.7



SENE- Secondary school Enugu East Senatorial Zone; PENE- Primary school Enugu East Senatorial Zone
SENN- Primary school Enugu North Senatorial Zone; PENN- Primary school Enugu North Senatorial Zone
SENS- Secondary school Enugu South Senatorial Zone; PENS-Primary school Enugu South Senatorial Zone
DA=Clindamycin; P=Penicillin; E= Erythromycin; CAZ= Ceftazidime; CN= Gentamicin; F_{200} = Nitrofurantoin;
RL=Sulphamethoxazole; CIP= Ciprofloxacin; TE= Tetracyline

Fig 2: Percentage antibiotic resistance pattern of MRSA isolated from schools (secondary and primary) in Enugu State, Nigeria



SEBN- Secondary school Ebonyi North Senatorial Zone. PEBN- Primary school Ebonyi North Senatorial Zone
SEBC- Secondary school Ebonyi North Senatorial Zone. PEBC- Primary school Ebonyi North Senatorial Zone
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RL=Sulphamethoxazole; CIP= Ciprofloxacin; TE= Tetracyline

Fig 3: Percentage antibiotic resistance pattern of MRSA isolated from schools (secondary and primary) in Ebonyi State, Nigeria

Discussion:

The burden of MRSA has been on the increase over the years despite several efforts. Although the overall prevalence of MRSA colonization is 31.7% in this study, the distribution varied significantly across senatorial zones in both Enugu and Ebonyi States. In Enugu State, MRSA prevalence was lowest in Enugu East (13.4%) and markedly higher in Enugu North (37.3%) and Enugu South (42.7%), and this difference was statistically significant (p<0.001). Similarly, in Ebonyi State, MRSA prevalence differed significantly across senatorial zones, with the lowest prevalence in Ebonyi North (17.2%), compared to Ebonyi Central (35.4%) and Ebonyi South (45.3%) (p=0.002). Overall, these findings indicate a significant geographical variation in MRSA colonization across senatorial zones, with consistently higher prevalence observed in southern zones of both States.

The results of this study demonstrate a high overall comparative prevalence of MRSA in both Enugu and Ebonyi States. However, the findings consistently reveal a greater burden in Ebonyi State compared to Enugu. Specifically, Ebonyi State recorded a prevalence of 32.9%, which exceeds the 30.7% reported in Enugu State. Ebonyi also had a higher *S. aureus* carriage rate (49.5%) compared with Enugu (44.2%), which expands the reservoir from which MRSA emerges. Similar *S. aureus* carriage rates have been reported by Abdullahi et al., (33) (31.5%) Rosal et al., (34) (33.0%); Ogefere et al., (35) (42.3%) and Akerele et al., (36) (49.5%).

The prevalence of MRSA reported in some previous studies by Okwu et al., (37) 6.0%; Rosal et al., (34) 4.4%; and Akerele et al., (36) 22.2% are lower than the rate reported in this study. In contrast, higher prevalence rates have been reported by Morufat et al., (18) 45.6%; Azzam et al., (38) 42.2%; Abdullahi et al., (33) 23.5% and Ogefere et al., (35) 37.2%. Furthermore, this pattern aligns with research suggesting that rural and peri-urban populations with limited hygiene infrastructure and reduced access to regulated healthcare practices often experience higher community MRSA transmission (39,40). Thus, the data indicate that Ebonyi State bears a heavier overall burden.

Gender-based analysis revealed notable differences in MRSA colonization, with females exhibiting higher carriage rates than males in both States. In Enugu State, MRSA prevalence was 33.6% among females compared to 28.1% among males, while in Ebonyi State, females had a prevalence of 37.1% versus 29.2% in males. These findings are consistent with previous reports indicating higher MRSA colonization among females, which have been attributed to behavioral factors such as sharing of personal items, close interpersonal contact and frequent grooming-related practices that enhance transmission (21,22,35,41).

However, some studies have reported male predominance in MRSA colonization in other settings, including those by Kodde et al., (42) in Iran, Rosal et al., (34) in Spain, and Akerele et al., (36) in Edo State, Nigeria. In this study, there was a statistically significant association of the prevalence of *S. aureus* and the female gender of the participants (p=0.02). However, there was no statistically significant association in the prevalence of MRSA between males and females in Enugu and Ebonyi States (p=0.051). The observed pattern in southeastern Nigeria may reflect the prevailing social and behavioral interactions among female pupils and adolescents within school environments.

Educational level comparisons further reveal important distinctions in MRSA colonization rate among the participants between the States. In Enugu, secondary school students had significantly higher MRSA colonization rate (42.0%) than primary pupils (18.8%) (p=0.0002), indicating that older students were the more affected educational group in that State. In contrast, a reverse pattern was seen in Ebonyi State, with primary school pupils showing higher but insignificant MRSA colonization rate of 38.1% compared to 27.6% in secondary school students (p=0.1418). These contrasting patterns suggest that younger children in Ebonyi State are the most vulnerable educational group, likely due to poorer personal hygiene practices, close-contact play behavior, overcrowding, and limited ability of school sanitation facilities factors documented to drive MRSA transmission in younger age groups (34). However, in line with the findings from Enugu State, a higher prevalence among age group 10-29 years where secondary school students fall within in another study has also been reported (21). The higher rates among older students in Enugu State may reflect greater mobility, wider social networks, and increased participation in peer-group activities.

Methicillin-resistant *S. aureus* isolates were highly resistant to several commonly used antibiotics in both Enugu and Ebonyi States. Markedly, elevated resistance levels of MRSA isolates were evident for against penicillin (97.9%), sulphamethoxazole (90.7%), erythromycin (76.8%), tetracycline (75.7%), and clindamycin (68.7%), reflecting broad decreased susceptibility to both β-lactam and various non-β-lactam antibiotics. The near universal resistance to penicillin observed in this study agrees with international findings that *S. aureus* exhibits widespread resistance to β-lactam antibiotics due to β-lactamase production and *mecA*-mediated alterations in penicillin-binding proteins. Comparable researches have also reported elevated MRSA resistance to erythromycin, clindamycin, and

tetracycline, indicating the persistent challenge of resistance to these commonly administered antibiotics across various regions, including Africa (14, 33,36,37,38). These trends clearly imply that misuse of antibiotics and unrestricted access to antimicrobial agents in community settings may promote the continued presence and proliferation of resistant MRSA strains.

Contrastingly in this study, MRSA isolates displayed relatively low resistance to gentamicin and ciprofloxacin suggesting these antibiotics may still be partially effective against MRSA isolates in the region. These results are consistent with previous records where *S. aureus* isolates exhibited lower resistance to gentamicin and ciprofloxacin compared to β-lactam antibiotics and tetracycline (35,36,43). Despite this, widespread multidrug-resistance reported in MRSA emphasize the urgent need for sustained antimicrobial surveillance and implementation of antibiotic stewardship programs. Infection control strategies need to be reinforced together with the adoption of evidence-based antibiotic policies is crucial to curb the spread of resistant strains and optimize treatment effectiveness. The MARI further reinforce that Ebonyi State is at higher risk for antimicrobial resistance emergence as more MRSA isolates clustered in the upper MARI categories (0.7–0.8), suggesting higher antibiotic pressure and more sustained transmission environments compared with Enugu.

Conclusion:

In conclusion, the findings from this study highlights the need for state-specific infection control strategies, with priority attention directed towards female pupils and primary school children in Ebonyi, as well as older students in Enugu. Ciprofloxacin and gentamicin may still be effective for the treatment of MRSA infections in this setting.

Contributions of authors:

UEG and OES contributed to the conception and design of the study; UEG coordinated the sample collection and performed the laboratory analysis. UEG and OES participated in the writing of the manuscript, critically reviewed and edited the manuscript. Both authors read and approved the final version of the manuscript.

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