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METHICILLIN-RESISTANT *Staphylococcus aureus* AND MULTIDRUG-RESISTANT *Escherichia coli* IN BENIN CITY, NIGERIA: A CROSS – SECTION STUDY

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Escherichia coli* (MDRE) continuously pose a threat to global health, particularly in low- and middle-income countries, such as Nigeria. Therefore, given the dearth of locally-generated evidence, this study aimed to assess the prevalence of MRSA and MDRE with a view to informing local public health research, practice and policy. This was a cross-sectional study of outpatients presenting to three purposively selected healthcare facilities in Benin City, Edo State, Nigeria. Standard microbiological procedures were performed using nasal swabs and urine specimens. The outcome variables were the identification of MRSA and MDRE, defined as the proportion of persons diagnosed as carrying *Staphylococcus aureus* and *E. coli*, respectively, with these resistant bacterial strains. Descriptive analysis using frequencies and percentages by participant's characteristics was presented. Two hundred and thirty-three persons participated in this study between January 2021 and July 2021, majority of whom were females (67%) and aged 18-24 (45%). Growth of *S. aureus* was detected in 55.1% (91) of 165 participants who provided nasal swabs. Of these 91 participants, 91.2% (83/91) were confirmed as carrying MRSA. Additionally, 51.7% (i.e., 89) of the 172 participants who provided urine for culture were positive for *E. coli* growth, of which 92.1% (82/89) were identified as carrying MDRE. This study recorded a high prevalence of both MRSA and MDRE in the study setting, underlining the need for an urgent preventive public health measure, such as awareness and antimicrobial stewardship promotion.

Keywords: Benin City, Multidrug-resistant *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus*, Nigeria, Prevalence

INTRODUCTION

There has been considerable attention on reducing the burden of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Multi-Drug Resistant (MDR) *Escherichia coli* (*E. coli*) in Nigeria, given their re-emergence and attendant public health impacts in the last few years. MDR strains of *E. coli* can occur both in the community and healthcare settings and are a significant concern as they limit treatment options, making infections more challenging to manage, and exhibiting non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012). Consequently, the treatment of infections by such resistant strains of *E. coli* is less efficient and more costly. A systematic review found a substantial increase in the prevalence of MRSA to commonly prescribed and inexpensive antibiotics (cotrimoxazole and tetracycline) in Nigeria between 2007 and 2017, with a significant increase from 18.3% in 2009 to 42.3% in 2013, albeit with varying prevalence across regions in the country (Abubakar and Sulaiman, 2018). Furthermore, there is evidence to support widespread antimicrobial resistance (AMR) in enteric *E. coli* in Nigeria, particularly to penicillins, aminoglycosides, cephalosporins, chloramphenicol, tetracyclines and cotrimoxazole (Nigeria Centre for Disease Control, 2017). For example, a cross-sectional study of 122 randomly selected healthy poultry workers in the Municipal and Kuje area of the Federal Capital Territory in Nigeria from December, 2018 to April, 2019 found the prevalence of *E. coli* to be 39.7% (n=48), of which 79.2% (38/48) were MDR to commonly used antibiotics (Aworh *et al*., 2019).

Generally, despite the concerted efforts to promote antimicrobial stewardship, AMR remains a serious public health threat globally, particularly in low- and middle-income countries (LMICs). In Nigeria, the usefulness of most antimicrobial drugs for managing infectious diseases—the major determinant of morbidity and mortality in the country—is threatened by AMR (Nigeria Centre for Disease Control, 2017). The widespread antibiotic use and consequent increase in bacterial resistance are diverse (Bell *et al*., 2014), ranging from increased healthcare costs, increased morbidity and mortality (Levy, 2001) to complications of advanced treatments (e.g., treatment of malignancies and transplantations) that rely on effective infection control (Montassier *et al*., 2013). Therefore, the prompt identification and treatment of patients carrying resistant bacteria is crucial to preventing in-hospital transmission of resistant strains of pathogens (Mogensen *et al*., 2018), especially in a setting with a suboptimal healthcare system, such as Nigeria.

Nonetheless, there is limited evidence on the epidemiology of MRSA and MDR E. coli, particularly concerning prevalence at various levels of healthcare and, equally important, context-specific risk factors in Nigeria. Understanding the epidemiology of MRSA and MDR *E. coli* becomes even more critical for Nigeria where antimicrobial drugs are readily available over-the-counter, with attendant high potential for indiscriminate use by poultry farmers and the populace. A situation analysis of AMR in Nigeria by the Nigeria Centre for Disease Control in conjunction with its partners further underlines the importance of providing additional data on the epidemiology of AMR in the country, especially in the south-south region (Nigeria Centre for Disease Control, 2017). Thus, a study seeking to fill this research gap will contribute to the limited evidence available to researchers and public health policymakers in making an informed decision regarding AMR control in the country. Therefore, this project aims to provide a comprehensive outlook of the epidemiology of MRSA and MDR *E. coli* carriage in patients presenting at contrasting health facilities in Benin City, Edo State of Nigeria. Specifically, the project sought to describe the prevalence of MRSA and MDR *E. coli* in patients and to identify contextual risk factors for MRSA and MDR *E. coli* in Benin City.

MATERIALS AND METHOD

Study design

This study utilised a cross-sectional design, with data on both the outcome and exposure variables collected concurrently.

Study setting

This study was conducted in Benin City, the capital of Edo State, which is one of the 36 states in Nigeria. Administratively, Benin City comprises three Local Government Areas (LGAs), including Oredo, Egor and Ikpoba-Okha. In terms of the healthcare system, the city populace and those from neighbouring towns and states are serviced by primary healthcare centres (PHCs), two government-owned secondary hospitals (Stella Obasanjo Specialist Hospital and Central Hospital), and a tertiary hospital (University of Benin Teaching Hospital). For this study, however, we restricted participant recruitment to those presenting at two consenting PHCs (Evbuotubu and Evbuogida in Egor LGA) and one secondary hospital (Central Hospital). The choice of these health facilities was informed by their expressed willingness to participate in the study and formal approval for data collection. All the study participants were non-emergency and outpatients.

Eligibility analysis

To be eligible for the study, participants of any age who presented to the study healthcare facilities on the day of data collection needed to be willing to provide informed consent (informed ascent in the case of children aged 5-17 years). However, participants who had been admitted for more than 16 hours before enrolment were ineligible for the study, as were participants who could not be swabbed in the nose or throat due to anatomic or surgical reasons.

Sample size

The formula below is used for the sample size estimation.

$$n = \frac{3.84\pi(1-\pi)}{w^2}$$

Where:

n=required minimum sample size; π=proportion of interest; and w=precision of estimate.

The prevalence of MRSA (42%) and MDR *E. coli* (70%) in Benin City appeared to have been overestimated due to available studies being conducted in an Otorhinolaryngology Department at the University of Benin Teaching Hospital (Akerele *et al.,* 2014) and underpowered (e.g., 70% of 20 *E. coli* isolates was detected from urine samples in one LGA) (Aibuedefe Osagie & Joy Imuetiyan, 2018). Thus, given the diversity of health facilities for this study, we worked with an assumption (per standard practice) that the prevalence of both MRSA and MDR *E. coli* carriage to be between 10% and 15% (π=0.1 to 0.15), and wanted to estimate the sample size to within 4% (w=0.04) with 95% certainty. We will err on caution and use π=0.15 for our estimation.

Applying the formula with these values gives:

$$n = \frac{3.84 \times 0.15\ (1-0.15)}{0.04^2}$$

Thus, we would need to recruit 306 participants to be 95% confident that the prevalence of MRSA and MDR *E. coli* carriage was estimated to be within 4% of the true prevalence. However, after adjusting for 10% non-response[1], the final sample size was increased to 340 participants.

Outcome variables

The primary outcome variables for this study were the presence of MRSA and MDR *E. coli* carriage in persons who presented at the selected health facilities, defined as the proportion of persons having these bacteria among all the study participants. The secondary outcome variables were the risk factors for carriage of antibiotic resistance, defined as the proportion and odds ratios between patients with and without MRSA and MDR *E. coli*.

Potential risk factors for MRSA and MDR *E. coli* carriage

We also collected information from each study participant on the following variables: gender, age, ethnicity, occupation, average income per month (for those working), religion, tattoos/piercings, contact with farm produce (poultry, live pigs, or cattle), hospitalization in the past six months, invasive surgery performed in the last six months, ulcers and abscesses within the previous six months, antibiotic treatment within the last six months and history of diarrhoea in previous three months.

Enrolment of study participants

Under the supervision of KE and ADO, all patients presenting at the selected health facilities were informed about the study (including the requirement for ethics) by trained research assistants using both verbal and written approaches. Where applicable, a potential study participant was allowed to have a family member, friend or acquaintance as a lay representative present at the information collection point. Privacy was secured during the information collection, and no treatment was delayed because of enrolment into the study. All participants were offered at least 1 hour to consider their study participation before providing informed written consent. The consent information included the voluntary permission of the study participant to obtain a swab from the nose and urine sample, as well as information on the microbiological analysis and strategies for disseminating findings. The participant also received information about who they could contact afterwards for additional questions as needed.

Data collection methods

Information on sociodemographic and potential risk factors for carrier status was obtained using an interviewer-administered questionnaire (previously pre-tested for ambiguity and clarity before data collection). To collect specimens for microbiological analyses, a study participant was swabbed in the nose using a standard swab stick. A nasal sample was obtained by inserting a swab into the anterior nares and rotating it along the mucous membrane by an experienced nurse. The swab sample container was labelled with information on study health facility and the participant's unique identification number. Additionally, the urine sample was collected using a sterile, wide-mouthed container with screw cap tops per conventional procedures. Again, the urine sample was labelled as before. All samples were aseptically transported and analysed at the Department of Medical Laboratory Services, University of Benin Teaching Hospital, to minimise bias from variations in microbiological techniques.

Microbiological analysis

An experienced microbiologist (NI) examined the collected samples for adequacy and quality. Nose swabs were examined for the presence of MRSA. For this, an enhancement broth [Tryptic soy broth supplemented with 2.5%

[1] Adjustment factor for 10% non-response =100/ (100-10)

NaCl, 3,5 mg/L Cefoxitin and 20 mg/L Aztreonam] was inoculated with 100 µL from the swab media and incubated at 35–37 °C for 16–24 hours. Subsequently, a selective chromogenic medium [ChromAgar MRSA II agar] was inoculated with 100 µL of the enhancement broth and incubated for an additional 42–48 hours in atmospheric conditions. Isolates of *Staphylococcus aureus* were identified using standard microbiological techniques, as previously reported (Moraes *et al*., 2021). MRSA was identified using Oxacillin disc/Cefoxitin disc as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (European Committee on Antimicrobial Susceptibility Testing, 2022). For the isolation of *E. coli* from urine samples, bacterial growth was identified based on cultural characteristics, gram stain and conventional biochemical tests, then confirmed by the API 20E identification system as previously documented (Maina *et al*., 2014). When confirmed as *E. coli*, antimicrobial susceptibility testing was performed on the Muller-Hinton agar plate by the Kirby-Bauer disk diffusion method as per the Clinical and Laboratory Standards Institute (Clinical & Laboratory Standards Institute, 2023). *E. coli* ATCC 25922 was used as the control strain and tested each time susceptibility testing was performed. Test results were only validated in cases where inhibition zone diameters of the control strains were within performance ranges per microbiological guidelines (Clinical & Laboratory Standards Institute, 2023). MDR *E. coli* was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Resistant and intermediate results were considered non-susceptible.

Data management and analysis

All information, including sociodemographic and microbiological assessment outcomes, was entered directly into an electronic database (ODK) on smartphones designed solely for the study (ODK, 2022). Data were extracted from the ODK storage system as MS Excel after the end of the study. The missing indicator approach was used to handle missing data. After data cleaning and validation, the final dataset was anonymized before data analysis. The prevalence of the resistant bacteria was calculated using the combined data, and described 'participants' socioeconomic and clinical characteristics (potential risk factors). However, contrary to our initial plan of identifying MRSA and MDRE risk factors, we could not perform univariable and multivariable logistic regression analyses given the consistent non-significance of association (see details in the discussion chapter). That is, based on our predefined statistical significance at a $p< 0.05$. All data management and analyses were performed in STATA 16. This study was reported per STROBE (Strengthening the Reporting of Observational Studies in Epidemiology).

RESULTS AND DISCUSSION

Baseline characteristics of the study participants

Overall, 233 participants were recruited for this study between January 2021 and July 2021 (Table 1). There was a predominance of female over male participants (67% vs 33%). Participants aged 18-24 years accounted for the highest proportion of the participants at 45%, seconded by those aged 25-34 years at 37%. Regarding the participants' occupation, there was a predominance of persons who identified as students (20%) and businessmen or women (17%); those classified as being engaged in '"other' occupation accounted for the highest proportion at 43%. Over half (53%) of the participants were recruited at the secondary level of care compared to those recruited at the primary level (47%).

Prevalence of multidrug-resistant *S. aureus* and *E. coli*

The prevalence of *S. aureus* and *E. coli* and their respective multidrug-resistant strains are outlined in Table 2. Of the 165 participants who provided nasal swabs for culture, we detected a growth of *S. aureus* in 55.1% (91/165). Of the 91 specimens with *S. aureus* growth, we detected MRSA in 91.2% (83/91). In a similar trend, 51.7% of the 172 participants who provided urine for culture had *E. coli* growth. Of the specimens with *E. coli* growth, 92.1% (82/89) were MDRE.

Table 1: Baseline characteristics of the study participants (N=233)

Characteristic	Frequency (%)
Sex	
Female	157 (67.4)
Male	76 (32.6)
Age group, year	
6-17	8 (3.4)
18-24	104 (44.6)
25-34	85 (36.5)
35-49	25 (10.7)
≥50	11 (4.7)
Mean (SD) age, year	27.4 (9.4)
Occupation	
Student	47 (20.2)
Business	39 (16.7)
Housewife	14 (6.0)
Teacher	8 (3.4)
Driver	8 (3.4)
Caterer	7 (3.0)
Factory worker	5 (2.2)
Farmer	3 (1.3)
Others (e.g., barber, mechanic)	100 (42.9)
Unemployed	2 (0.9)
Religion	
Christianity	230 (98.7)
Islam	3 (1.3)
Reported income per month, Naira	
≤10,000	64 (27.5)
11,000-99,900	92 (39.5)
≥100,000	7 (3.0)
Missing	70 (30.0)
Health facility type	
Primary healthcare centre	109 (46.8)
Secondary hospital	124 (53.2)

SD: Standard Deviation

Table 2: Prevalence of multidrug-resistant *S. aureus* and *E. coli*

Variable	Frequency (%)
	n=165
S. aureus	
No	74 (44.9)
Yes	91 (55.1)
	n=91
Multi-drug Resistant *S. aureus*	
No	8 (8.8)
Yes	83 (91.2)
	n=172
E. coli	
No	83 (48.3)
Yes	89 (51.7)
	n=89
Multi-drug Resistant *E. coli*	
No	7 (7.9)
Yes	82 (92.1)

Association between 'participants' sociodemographic and clinical characteristics with multidrug-resistant *S. aureus* and *E. coli*

The association between 'participants' sociodemographic and clinical characteristics with multidrug-resistant *S. aureus* and *E. coli* is outlined in Table 3. Overall, none of the 'participants' characteristics were statistically significant in relation to MRSA and MDRE. Briefly, female participants recorded a higher proportion of MRSA (69% of 83) and MDRE (77% of 82) than their male counterparts. Participants aged 18-24 recorded the highest proportion of MRSA (57% of 83), while those aged 25-34 had the highest MDRE (45% of 82). The mean age of participants who tested positive for MRSA (27 years) was slightly higher than that of those who tested negative (25 years); a similar trend was recorded for MDRE in relation to 'participants' mean age. Participants earning 11,000-99,999 monthly accounted for the highest proportion of recorded MRSA (45%) and MDRE (42%). A higher proportion of MRSA and MDRE was recorded among participants who presented to secondary and primary healthcare, respectively. Regarding known risk factors for both MRSA and MDRE, there were lower proportions of MRSA and MDRE among those who had tattoo/piecing (6% vs 6%), had contact with farm produce (29% vs 27%), had recently been hospitalized (7% vs 7%), had invasive surgery (1% vs 1%), have ulcer (21% vs 18%), and have abscess (7% vs 5%).

Summary of principal findings

This study found a high prevalence of MRSA and MDRE among 233 participants presenting to secondary hospitals and primary healthcare centres in Benin City. Overall, *S. aureus* was 55.1% among 165 participants who provided nasal swabs; MRSA prevalence was 91.2% (83/91). Similarly, the prevalence of *E. coli* among the 172 participants who provided urine specimens was 51.7%; MDRE prevalence was 92.1% (82/92.1).

Interpretation of principal findings in the context of existing evidence

MRSA and MDRE are well-recognized public health problems that contribute to substantial nosocomial infections worldwide (Ferri *et al*., 2017). The high prevalence of both MRSA and MDRE found in the present study underlines the potential threat pose by these pathogens to the healthcare sector in the study setting. However, the prevalence in this study is far higher than that of 11% in Ile-Ife (Shittu *et al*., 2011), 28.6% in Kano (Nwankwo *et al.*, 2009), 34.7% in Ilorin (Taiwo *et al*., 2004) and 11% in Benin City (Obasuyi and Akerele, 2015). These observed differences may be due to different population studied, the antibiotic consumption pattern and the periods when the studies were

conducted. Notably, our study population is lower than those in the aforementioned studies, which could potentially explain the very high prevalence.

We found a higher prevalence of MRSA and MDRE in females than in males, with the former at 68.7% and 76.8%, respectively, and the latter at 31.3% and 23.2%, respectively. Evidence suggests that biological differences and behavioural patterns contribute to the difference in pathogenic resistance between the sexes (Garoy *et al*., 2019). Some of these disparities also can be attributed to hormonal differences, variations in immune responses and skin structure, which tend to favour female over the male gender (Ferri *et al*., 2017). Primarily, MRSA and MDRE transmissions occur through direct contact with contaminated skin or surfaces. A study conducted in Nigeria observed that females tend to have higher rates of healthcare exposure due to factors such as pregnancy, gynecological procedures and extended hospital stays (Ogbolu *et al*., 2015). Therefore, these might have contributed to the increased susceptibility to MRSA and MDRE among the female participants compared to their male counterparts.

Oxacillin antibiotic, used as a marker of Methicillin resistance, was used in this study to screen for MRSA, and a 91.2% resistance was recorded. This was higher than the 46.3% and 52.4% reported by Ibadin *et al*. (2017) and Garoy *et al.* (2019) respectively. This could potentially be explained by variations in prior antibiotic exposure, prescription pattern and policies of health facilities in the various locations (Olorunfemi *et al.,* 2020).

Strengths and limitations

A strength of this study is adding to the limited literature on MRSA and MDRE prevalence in the study setting (Benin City). In addition, our systematic sampling approach minimised selection bias. However, this study has substantial limitations worth outlining. First, we initially estimated a sample size of 340 participants but could only recruit 233 participants within the study timeline. Therefore, this could have affected the estimated prevalence, most likely overestimating the reported prevalence. Secondly, identifying the socioeconomic and clinical characteristics associated with the outcome variables was one of our *a priori* objectives. However, the collected data did not favour the performance of multivariable logistic regression. Understanding the reason for this unexpected finding would benefit from a follow-up study.

CONCLUSIONS

A high percentage of apparently healthy participants in this study were carriers of methicillin-resistant *Staphylococcus aureus and multi-drug-resistant Escherichia coli,* placing them at risk of severe infections. Therefore, we recommend an urgent community-driven risk communication on the public health threats of these microorganisms and antimicrobial stewardship in the study setting.

Table 3: Factors associated with MRSA and MDRE

Variable	MRSA (N=91)		MDRE (N=89)	
	Negative (n=8)	Positive (n=83)	Negative (n=7)	Positive (n=82)
Sex				
Female	3 (37.5)	**57 (68.7)**	6 (85.7)	**63 (76.8)**
Male	5 (62.5)	26 (31.3) NS	1 (14.3)	19 (23.2) NS
Age group, year				
6-17	0 (0.0)	2 (2.41)	1 (14.3)	2 (2.4)
18-24	5 (62.5)	**47 (56.6)**	4 (57.1)	35 (42.7)
25-34	2 (25.0)	22 (26.5)	2 (28.6)	**37 (45.1)**
35-49	1 (12.5)	7 (8.4)	0 (0.0)	6 (7.3)
≥50	0 (0.0)	5 (6.0) NS	0 (0.0)	2 (2.4) NS
Mean (SD) age, year	25.1 (5.1)	27.0 (10.1)	23.3 (4.3)	26.8 (8.7)
Reported income per month, Naira				
≤10,000	2 (25.0)	20 (24.1)	4 (57.1)	23 (28.1)
11,000-99,999	4 (50.0)	**37 (44.6)**	2 (28.6)	**34 (41.5)**
≥100,000	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.1)
Missing	2 (25.0)	25 (30.1) NS	1 (14.3)	24 (29.3) NS
Health facility type				
Primary healthcare centre	2 (25.0)	35 (42.2)	3 (42.9)	**45 (54.9)**
Secondary hospital	6 (75.0)	**48 (57.8)** NS	4 (57.1)	37 (45.1) NS
Tattoo/piercing				
No	8 (100.0)	78 (94.0)	6 (85.7)	77 (93.9)
Yes	0 (0.0)	5 (6.0) NS	1 (14.3)	5 (6.1) NS
Contact with farm produce				
No	7 (87.5)	59 (71.1)	7 (100.0)	60 (73.2)
Yes	1 (12.5)	24 (28.9) NS	0 (0.0)	22 (26.8) NS
Recent hospitalization				
No	8 (100.0)	77 (92.8)	6 (85.7)	76 (92.7)
Yes	0 (0.0)	6 (7.2) NS	1 (14.3)	6 (7.3) NS
Invasive surgery				
No	8 (100.0)	81 (97.6)	7 (100.0)	81 (98.8)
Yes	0 (0.0)	2 (2.4) NS	0 (0.0)	1 (1.2) NS
Ulcer				
No	5 (62.5)	66 (79.5)	4 (57.1)	67 (81.7)
Yes	3 (37.5)	17 (20.5) NS	3 (42.9)	15 (18.3) NS
Abscess				
No	7 (87.5)	77 (92.8)	7 (100.0)	78 (95.1)
Yes	1 (12.5)	6 (7.2) NS	0 (0.0)	4 (4.9) NS

NS=not statistically significant (i.e., p-value ≥0.05)

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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