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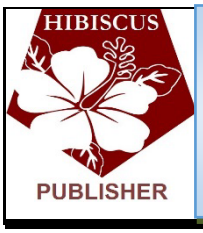

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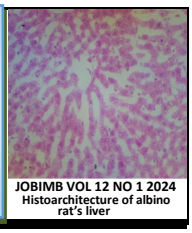

Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) And Extended-Spectrum β-Lactamases (ESBLs) Producing *Enterobacteriaceae* Among Nosocomial Bacteria in Kaduna, Nigeria

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ABSTRACT

Despite continuous control efforts, antimicrobial resistance remains a global public health problem that undermines the treatment of infectious diseases. Methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended-spectrum β-lactamases (ESBLs) producing bacteria represent two important groups of multi-drug resistant pathogens that are associated with a high rate of treatment failure, prolonged morbidity, and increased cost of treatment. Therefore, keeping such superbugs under surveillance, particularly in healthcare facilities, is required for informed action, designing of control interventions for further antimicrobial emergence, and determining strategies for infection control. Thus, this study was designed to determine the prevalence of MRSA and ESBLs production *Enterobacteriaceae* isolated from four hospitals. Using CLSI guidelines, a total of 96 *S. aureus* were screened for methicillin resistance, and 137 isolates of *Enterobacteriaceae* for ESBLs production. The study observed a prevalence rate of 35.42 % for MRSA and 26.3 % for ESBLs production among *Enterobacteriaceae.* Furthermore, species-wise analysis of the data indicated the individual prevalence rate of ESBLs production among *E. coli*, *K. pneumoniae,* and *P. aeruginosa* to be 25.6 %, 28.8 % and 23.0, respectively. This indicates the occurrence of antimicrobial resistance strains among bacteria isolated from hospital settings. The results obtained here could be important in informing public health policies/strategies for the control of antimicrobial resistance and infection.

INTRODUCTION

The acquisition and development of antimicrobial resistance by bacteria to a number of antibiotics is constantly increasing, constituting a constant threat to global public health [1]. Antimicrobial resistance (AMR) is developed when microbes change over time due to selective pressure incited by inadequate concentration of antibiotics or other stresses, thus rendering the antibiotics inactive against such microbes. Alternatively, some microbes can acquire resistance genes from the environment via horizontal gene transfer processes such as transformation, conjugation, or even transduction [2]. This greatly influences chemotherapeutic-based treatment outcomes and morbidity period, which invariably increases the cost of treating infectious diseases [1]. Strikingly, any inappropriate use of antibiotics in all biological systems, including human, animal and plant, can induce antimicrobial resistance [3].

Staphylococcus aureus has been recognised as an important nosocomial pathogen that causes a wide range of clinical infections, from minor skin and chronic osteoarticular infections to life-threatening septicaemia and endocarditis [4]. Over the years, *S. aureus* has evolved and acquired resistant to virtually all antibiotics used for its treatment. At first, the penicillin-resistant strain of S. aureus emerged only after one year of antibiotic introduction [5]. Later, resistance to other antibiotics, such as tetracyclines, erythromycin and streptomycin were reported [6]. Methicillin was then introduced to combat penicillin and other β-

lactam antibiotics-resistant *S. aureus*. This treatment option lasted for only 2 years, after which the bacterium acquired resistance to methicillin. In the recent years, vancomycin has been and remains the drug of last resort for the treatment of methicillin-resistant *S. aureus* (MRSA) infections. However, reports of reduced susceptibility to vancomycin in clinical isolates of *S. aureus* from Japan in 1997 raised significant medical concern regarding the evolution of vancomycin-resistant *S. aureus* (VRSA) [7]. Unfortunately, the nightmare of medical and healthcare practitioners came true when the VRSA was first identified in 2002 in the United States [8]. Presently, the global burden of the resistant strains of *S. aureus* is considerably high and the molecular basis of the resistance mechanisms are not fully delineated. For example, an estimated 80,461 cases of invasive MRSA infections occurred in the United States in 2011 [9].

Extended-spectrum beta-lactamases (ESBLs) producing bacteria is another multi-drug resistant bacteria that could inhabit surfaces in hospital environments. ESBLs are commonly produced by Gram-negative bacteria, especially in *Enterobacteriaceae* and *Pseudomonas aeruginosa*, which afford them the ability to confer resistance against penicillins, first, second and third generation cephalosporins and aztreonam (but not cephamycins or carbapenems). However, ESBLs are inhibited by inhibitors called "β-lactamase inhibitors," such as clavulanic acid [10,11]. The broader coverage (extended-spectrum) of ESBLs susceptibility to β-lactam antibiotics was demonstrated to be due to the expansion of active site as a result of mutations in the parent enzymes [12]. Resistance to cephalosporins has become widespread throughout the world, and many types of ESBLs have been identified in various bacteria [13]. More than 300 different types of extended-spectrum beta-lactamases (ESBLs) have been described around the world so far; they were frequently identified in *Enterobacteriaceae* family with *Klebsiella pneumoniae and Escherichia coli* being the most common [14].

There are several reports on the rising occurrence of MRSA and ESBLs producing bacteria in hospital settings from Nigeria and, specifically Kaduna Sate, which can lead to nosocomial and other community-acquired infections. [15–18]. This is alarming and call for the need to address the challenge pose by these antibiotic resistance strains of pathogens (i.e. MRSA and ESBLs producing bacteria). MRSA and ESBLs occurrence, particularly in hospital settings, will pose serious health concerns, especially as the treatment of infections due to such pathogens could be difficult due to antibiotic resistance and the high cost of other treatment options. Therefore, against this background, the current research assesses the susceptibility of S. aureus isolated from hospital fomites to methicillin and screens all Gram-negative bacteria isolated from the same source for ESBLs production.

MATERIALS AND METHODS

Bacterial Isolates

A total of 270 bacteria constituting *Staphylococcus aureus* (110), *Escherichia coli* (82), *Klebsiella pneumonia* (52), and *pseudomonas aeruginosa* (26) isolated from hospital fomites, including bedding, patient side tables and files; wash hand basin, nurse station tables, floors and doorknobs of four hospitals were obtained from our previous work (unpublished). Bacteria were isolated from the swabs of fomites from Sabo General hospital, Gwamna Awan hospital, Horeb hospital and Harmony hospital all situated within Kaduna metropolis between June-December 2019. Both cultural and biochemical test reconfirmed the identities of the isolates according to the Bergey's Manual of determinative bacteriology [19].

Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Using cefoxitin, Kirby-Bauer's' disc diffusion antibiotic susceptibility testing technique as described by Clinical and Laboratory Standards Institute (CLSI) (20) identified MRSA. The bacterial suspensions were adjusted to 0.5 McFarland standard turbidity and inoculated on Mueller-Hinton agar by spread plate method. Cefoxitin disk (30 µg) (Oxoid, Basingstoke, UK) was appropriately placed on the center of plate and was incubated at 33-35 ºC. Zones of inhibition around cefoxitin disc were observed after 16-18 hours. Zone of inhibition around the cefoxitin disc ≤ 21 mm in diameter was recorded positive for MRSA.

Screening and Confirmation for Extended Spectrum β-Lactamases (ESBLs) Production

All the Gram-negative isolates were subjected to initial screening test using disc diffusion method based on CLSI guidelines [20]. Sensitivity discs of third generation cephalosporins constituting, ceftazidime (30 µg), cefpodoxime (10 µg), and cefixime (30 µg) were used; and any isolate susceptible to one or more of the cephalosporins was recoded as a potential ESBL producer. The antibiotic susceptibility endpoints used were cefpodoxime ≤17mm, cefixime ≤22mm and ceftazidime ≤24mm zone of inhibition diameter.

Isolates that were positive for the screening were further subjected to ESBLs production confirmatory test using the Double Disc Synergy Test (DDST) [21]. Prior to the antibiotic susceptibility testing, the isolates were cultured overnight in peptone water to obtain confluent growth. The turbidity was adjusted to 0.5 McFarland standard and lawn culture was made on Mueller-Hinton agar using a sterile swab. A disc of amoxicillin + clavulanic acid (20 + 10 µg) was placed in center of the plate already inoculated with the test organism while cefpodoxime (10 µg), ceftazidime (30 µg) and cefixime (30 µg) discs were placed at a distance of 30 mm from the amoxicillin + clavulanic acid disc on the same plate. Zones of inhibition around the third-generation cephalosporin discs and the amoxicillin + clavulanic acid were observed after 24 h of incubation at 37°C. Isolates were labelled ESBL positive if the zone of inhibition around one or more cephalosporin discs was extended on the side nearest to the amoxicillin + clavulanic acid, oftentimes resulting in a characteristic shape-zone referred to as 'champagne-cork' or 'keyhole.'

RESULTS AND DISCUSSION

Methicillin-Resistant *S. aureus*

A total of 270 bacterial isolates were used in this study, but only 233 were culturable; thus, their identities were reconfirmed and shown in **Table 1**. The antibiotic susceptibility testing of 96 isolates of *S. aureus* revealed 34 of the isolates to be methicillin-resistant, amounting to 35.42 % prevalence rate of MRSA among *S. aureus* in the hospital environment (**Fig. 1**). Considering the robust and versatile nature of *S. aureus*, it is expected to record high burden in healthcare facilities as reported by many studies. However, the high prevalence rate of the multi-drug resistance strain of S. aureus, MRSA reported here is striking and should be of serious health concern. It has been established that MRSA is responsible for most of the global bacteremia due to *S. aureus,* leading to increased financial burden and poorer prognosis as compared to methicillin-sensitive strain of *S. aureus* [22]. A

prevalence rate of 78 %, 54 %, 34 %, 28 % and 18 % were reported from the same area previously by other researchers [15–18] while the global prevalence of MRSA varies among countries, ranging from 13 % to over 78 % [23,24]. Correspondingly, this high prevalence rate of MRSA is linked to it high burden in healthcare-associated infections, accounting for approximately 44 % of the total healthcare-associated infections [23].

A systemic review, which analysed 12 studies to evaluate the trend in prevalence of MRSA infection in Nigeria showed a rising pattern from 18.3% in 2009 16.5% to 42.3% in 2013 [25]. More specifically, the study reported the 8 % prevalence of MRSA infection as of 2012 in northern Nigeria, the region of the current studies. Thus, indicates an almost four-fold increase in the MRSA prevalence in the region, compared to the figure (35.42 %) observed in the current study. This calls for urgent medical intervention to limit or at least reduce the adverse effects of MRSA infection or the acquisition/development of the resistance by the susceptible strain.

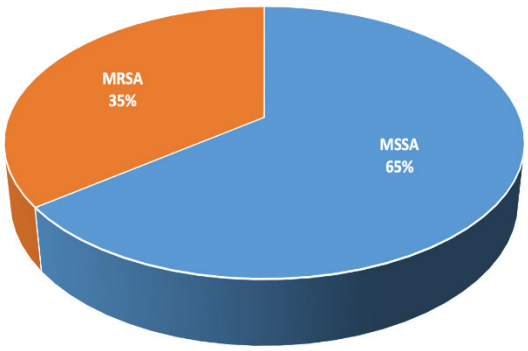

Fig. 1. Plot for occurrence of Methicillin-resistant *S. aureus*. MRSA represents Methicillin-resistant *S. aureus* and MSSA represent Methicillin-suceptible *S. aureus.*

Development and/or acquisition of antibiotic resistance by bacteria is multifaceted and could be due to various factors that are largely categorised into four groups [26]. The first one is associated with the pathogen, and it constitutes the incorrect diagnosis and the pathogen molecular characteristics such as survival fitness, transmissibility, and virulence. The second group is linked to physicians by way of inappropriate prescription of antimicrobial drugs. These two groups are the major causes of antibiotic overuse and misuse, eventually driving selective pressure and increasing resistance rate. The third group is related to the patient's population, and it is largely characterised by their attitudes towards antibiotic therapy and activities such as travel. Finally, the fourth group is connected to the hospital environment and the regulatory institutions. The factors include inconsistent implementation of healthcare regulations and infection control guidelines as well as inappropriate application of technological development and new drugs.

Apparently, no single intervention is ideal for effective control of antimicrobial resistance. However, multidimensional intervention is perceived to be the most efficient approach [27]. Again, there is no universal definition for antimicrobial resistance control program as it can vary at different local and national levels, depending on the peculiarities of the area and the country. Regardless, the program should comprise some basic common activities. These among others include surveillance [28], universal application of infection prevention and control measures by CDC and WHO or national/regional healthcare regulatory authorities [29], antimicrobial stewardship in human and animal health as well as and communication, education and training population on proper antibiotic usage [30].

A good antibiotic resistance control program should contain robust surveillance system that ensures close monitoring and real time update on antimaterial resistance and antibiotic usage. This invariably will inform actions and design of the of the intervention program. It is recommended that for every nation, a functional surveillance system should be available at both local and national levels in order to allow comparison of data at international level for identification of novel antibiotics resistance and determination of antibiotic usage policies and strategies for infection control.

Extended-Spectrum Beta-Lactamases (ESBLs) Producing Bacteria

Out of a total of 160 isolates screened for ESBLs production, 42 were confirmed to produce ESBLs giving an overall prevalence of 26.3% among Gram negative bacteria isolated from healthcare facilities. The specific prevalence of the individual bacterial species is presented in **Table 2.** Generally, the prevalence rate of ESBLs production among *Enterobacteriacea* in Nigeria is between 10- 27 % representing the least prevalent country, after Senegal (10 %) in the West African region with Ghana, Mali and Niger Republic recording about 49 %, 63-96 % and 40 %, respectively [31].

It is important to note that the prevalence rate of ESBLs production among bacteria largely depends on the source from which the bacteria are isolated. For example, as is evident in the current study and other previous studies (32–34), ESBLs producing bacteria occur at a higher rate in healthcare facilities than in communities or in food. This is probably due to the possibility of bacterial encounters with antibiotics during chemotherapy to in the hospital. This assertion is further corroborated by the high prevalence rate of 34.1 % reported in *E. coli* isolated from poultry farm in Jalingo, Nigeria [32]. Although poultry farm is not a healthcare facility, it is strongly associated with antibiotic administration, thus the source of antibiotic pressure that might drive the antibiotic resistance. Consistently, food drinks that are not known to be associated with antibiotics were reported to harbour bacteria with low ESBLs occurrence rate of about 18.75 % [33].

Table 1. Morphology and biochemical characterization of isolates.

Bacteria	Colony Morphology	Gram Reaction	Cell Morphology	Biochemical Testes									Frequency
				CA	CO	IN	MR	VP	OX	CI	MO	UR	
S. aureus	Yellow	Positive	Cocci	+	+	+	+	+	–	+	–	+	96
E.coli	Green Metalic Sheen	Negative	Rod Shaped	+	–	+	+	–	–	–	+	–	69
k. pneumoniea	Mucoid Pink	Negative	Rod Shaped	+	–	–	+	–	–	+	–	+	48
P .aeruginosa	Green	Negative	Rod Shaped	+	–	–	–	–	+	+	+	+	20
Total													233

Table 2. Prevalence of ESBLs Production Among *Enterobacteriacea.*

Organism	No. tested	Presumptive ESBLs	Confirmed ESBLs	Prevalence (%)
Escherichia coli	*82*	56	21	25.6
Klebsiella pneumoniae	*52*	38	15	28.8
Pseudomonas aeruginosa	*26*	13	6	23.0
Total	160	107	42	26.3

Among the *Enterobacteriaceae*, the present study observed *Klebsiella pneumoniae* to have the highest ESBLs producing occurrence rate of 28.8 % followed by *E. coli* with 25. 6 %. Similarly, many studies reported findings of similar pattern. One such work is the one that investigated the prevalence of ESBLs producing *Enterobacteriaceae* among food handlers at the University of Gondar, Ethiopia [34]. The study reported that *E. coli* and *Klebsiella pneumoniae* alone constitute 20.7 % out of the total 21.7 % ESBLs prevalence of *Enterobacteriaceae.* The other species that constitute the remaining 1 % were *Proteus mirabilis* and *Citrobacter freundii.*

CONCLUSION

This study highlights a significant public health concern with the high prevalence of multidrug-resistant Staphylococcus aureus (MRSA) in a hospital environment. Among the 96 *S. aureus* isolates tested, 35.42% were found to be methicillin-resistant, underscoring the robust nature of this pathogen and its capacity for resistance. The prevalence rate observed is notably higher than previous reports from northern Nigeria, indicating an alarming rise in MRSA cases. This increase necessitates urgent medical intervention to mitigate the adverse effects of MRSA infections. The development of antibiotic resistance is complex, driven by pathogen characteristics, physician prescribing practices, patient behaviors, and hospital regulations. Effective control of antimicrobial resistance requires a multifaceted approach, including robust surveillance systems, stringent infection control measures, and comprehensive education on antibiotic use. Additionally, the study reveals a 26.3% prevalence of ESBL-producing Gram-negative bacteria, positioning Nigeria as a relatively low-prevalence country in West Africa. These findings emphasize the need for continuous monitoring and adaptive strategies to combat antibiotic resistance in healthcare settings.

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