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Antibiotic Resistance pattern of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Selected Pharmaceutical Company Effluents in Ogun State, Nigeria

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Abstract

Background: Pharmaceutical manufacturing effluents have emerged as significant environmental reservoirs of antibiotic-resistant bacteria, posing serious public health threats. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major healthcare-associated pathogen, and its presence in industrial wastewater represents a critical pathway for environmental dissemination of resistance determinants. This study investigated the antibiotic resistance patterns of MRSA isolated from wastewater effluents of selected pharmaceutical companies in Ogun State, Nigeria.

Objective: To determine the phenotypic antibiotic resistance profiles of MRSA isolates from pharmaceutical company effluents.

Methods: A total of 164 *Staphylococcus aureus* isolates were recovered from pretreated wastewater samples collected from three pharmaceutical companies (A, B, and C) in Ogun State, Nigeria. MRSA was phenotypically identified using cefoxitin disk diffusion (30 µg) according to CLSI guidelines. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method against 10 antibiotics from multiple classes, including β-lactams, glycopeptides, aminoglycosides, fluoroquinolones, and lincosamides.

Results: The overall MRSA prevalence among *S. aureus* isolates was 35 (49.3%) in Pharma A, 29 (48.3%) in Pharma B, and 15 (45.5%) in Pharma C. Alarmingly, all MRSA isolates (100%) exhibited resistance to aztreonam, cefoxitin, clindamycin, ceftriaxone, imipenem, trimethoprim-sulfamethoxazole, and vancomycin. High resistance rates were also observed for gentamicin (55.2-71.4%), ofloxacin (72.4-80.0%), and levofloxacin (85.7-86.7%).

Conclusion: This study reveals an alarmingly high prevalence of multidrug-resistant MRSA in pharmaceutical wastewater, including complete resistance to vancomycin, a last-resort antibiotic. These findings highlight pharmaceutical effluents as critical hotspots for the environmental dissemination of highly resistant MRSA strains, necessitating urgent regulatory interventions and enhanced wastewater treatment protocols.

Keywords: MRSA; Antibiotic resistance; Pharmaceutical wastewater; Environmental contamination.

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1. Introduction

Antimicrobial resistance (AMR) constitutes one of the most significant global public health challenges of the 21st century, threatening the effective treatment of a wide range of infections [1,2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a particularly critical concern, being a major cause of both healthcare-associated and community-acquired infections worldwide [3-5]. The emergence and spread of MRSA have been driven by the extensive use of β-lactam antibiotics, with resistance primarily mediated by the *mecA* gene encoding an altered penicillin-binding protein (PBP2a) that confers resistance to all β-lactam antibiotics [6].

While clinical settings have traditionally been considered the primary reservoirs for MRSA, increasing evidence indicates that environmental sources, particularly wastewater from pharmaceutical manufacturing facilities, play a crucial role in the dissemination of antibiotic-resistant bacteria and resistance genes [7,8]. Pharmaceutical effluents contain high concentrations of antibiotic residues, creating selective pressure that promotes the development and persistence of resistant bacterial populations [9]. Furthermore, wastewater treatment plants often fail to completely remove antibiotic-resistant bacteria and resistance genes, leading to their release into receiving water bodies and subsequent environmental dissemination [10,11].

Nigeria, as Africa's most populous nation, has a significant pharmaceutical manufacturing sector with over 120 registered companies [12]. However, regulatory oversight of pharmaceutical waste management practices remains limited, particularly regarding wastewater treatment [13]. This regulatory gap potentiates the release of antibiotic compounds and resistant bacteria through insufficiently treated wastewater into the environment [14]. Studies conducted in Nigeria have reported the presence of multidrug-resistant bacteria in pharmaceutical effluents [15,16], yet comprehensive data on MRSA prevalence and resistance patterns in these environmental reservoirs remain scarce.

The presence of MRSA in pharmaceutical wastewater carries serious public health implications, as these effluents may contaminate water sources used for drinking, irrigation, and recreation, creating a direct pathway for human exposure [17]. Moreover, the horizontal gene transfer potential in wastewater environments can facilitate the spread of resistance determinants among diverse bacterial species [18]. The One Health approach, which recognizes the interconnection between human, animal, and environmental health, underscores the importance of monitoring environmental reservoirs of antibiotic resistance as part of comprehensive AMR containment strategies [19].

This study was designed to investigate the antibiotic resistance patterns of MRSA isolated from wastewater effluents of selected pharmaceutical companies in Ogun State, Nigeria. Specifically, the study aimed to: (1) determine the prevalence of MRSA among *S. aureus* isolates from pharmaceutical effluents, and (2) characterize the phenotypic antibiotic resistance profiles of MRSA isolates against clinically relevant antibiotics.

2. Materials and Methods

2.1. Study Area and Sampling Sites

The study was conducted in Ogun State, Nigeria, which hosts approximately 65% of the nation's pharmaceutical manufacturing capacity. Three pharmaceutical companies (designated Pharma A, B, and C) located within the Sango-Ota and Agbara Industrial Estates were selected for sampling. These facilities produce various classes of antibiotics, including β-lactams, fluoroquinolones, macrolides, and tetracyclines.

2.2. Ethical Considerations

Ethical approval was obtained from the Ebonyi State University Ethics Committee (Approval Number: EBSU/DRIC/UREC/026/034). Informed consent was obtained from each pharmaceutical company after comprehensive explanation of the study's objectives and methods.

2.3. Sample Collection

A total of 300 wastewater samples (100 from each pharmaceutical company) were collected from pre-treated discharge points. Samples were collected in sterile polypropylene bottles, transported to the laboratory on ice, and processed within 6 hours of collection.

2.4. Bacterial Isolation and Identification

Serial ten-fold dilutions of wastewater samples were prepared, and 0.5 mL aliquots were plated on Mannitol Salt Agar (MSA) (bioMérieux, France) using the pour plate method. Plates were incubated at 37°C for 24 hours. Golden-yellow colonies suggestive of *S. aureus* were sub-cultured onto Nutrient Agar (bioMérieux, France) for purification. Identification was based on Gram staining, catalase test, coagulase test, DNase agar, and mannitol fermentation [20]. Further confirmation was performed using the VITEK® 2 COMPACT Automated system (bioMérieux, France) according to manufacturer instruction.

2.5. Phenotypic Detection of MRSA

MRSA was identified using oxacillin and cefoxitin disk diffusion (1 µg) and (30 µg) respectively according to Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. Oxacillin and Cefoxitin disk diffusion has been widely utilized for phenotypic detection of MRSA in both clinical and environmental settings [3]. Zones of inhibition ≤ 10 mm and ≤ 21 mm respectively were interpreted as MRSA.

2.6. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to CLSI guidelines [21]. The following antibiotics were tested with their respective disk potencies: aztreonam (30 µg), cefoxitin (30 µg), clindamycin (15 µg), ceftriaxone (30 µg), imipenem (10 µg), gentamicin (10 µg), ofloxacin (5 µg), levofloxacin (5 µg), trimethoprim-sulfamethoxazole (25 µg), and vancomycin (10 µg) (Oxoid, Uk). Results were interpreted using CLSI zone diameter breakpoints, and isolates were categorized as susceptible, and resistant [21].

2.7. Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26.0. Descriptive statistics were used to calculate frequencies and percentages of antibiotic resistance. Results are presented as percentages and frequencies.

3. Results

3.1. Prevalence of MRSA

A total of 164 *S. aureus* isolates were recovered from the three pharmaceutical companies: 71 (43.3%) from Pharma A, 60 (36.6%) from Pharma B, and 33 (20.1%) from Pharma C. Phenotypic screening using cefoxitin disk diffusion revealed MRSA prevalence rates of 35 (49.3%) in Pharma A, 29 (48.3%) in Pharma B, and 15 (45.5%) in Pharma C, with an overall prevalence of 79 (48.2%) across all companies (Table 1).

Table 1 Prevalence of MRSA in Pharmaceutical Company Effluents

Pharmaceutical Company	Total *S. aureus* Isolates	MRSA-Positive (%)	MRSA Prevalence (%)
Pharma A	71	35	49.3%
Pharma B	60	29	48.3%
Pharma C	33	15	45.5%
Total	**164**	**79**	**48.2%**

The distribution of MRSA varied across different effluent sources within the companies. In Pharma A, the highest MRSA rate was observed in the pre-treated effluent from sample point 2 (68.4%), while the lowest was in sample point 4 (23.8%), indicating significant variation between different production or treatment stages (Table 2).

Table 2 Distribution of MRSA by Effluent Source in Pharma A

Sampling Site	Total *S. aureus*	MRSA Positive (%)	Non-MRSA (%)
Pre-treated (Site 1)	21	11 (52.4)	10 (47.6)
Pre-treated (Site 2)	19	13 (68.4)	6 (31.6)

Pre-treated (Site 3)	10	6 (60.0)	4 (40.0)
Pre-treated (Site 4)	21	5 (23.8)	16 (76.2)
Total	**71**	**35 (49.3)**	**36 (50.7)**

Table 2B Distribution of MRSA by Effluent Source in Pharma B

Sampling Site	**Total *S. aureus***	**MRSA Positive (%)**	**Non-MRSA (%)**
Pre-treated (Site 1)	15	7 (46.7%)	8 (53.3%)
Pre-treated (Site 2)	15	8 (53.3%)	7 (46.7%)
Pre-treated (Site 3)	15	7 (46.7%)	8 (53.3%)
Pre-treated (Site 4)	15	7 (46.7%)	8 (53.3%)
Total	60	29 (48.3%)	31 (51.7%)

Table 2C Distribution of MRSA by Effluent Source in Pharma C

Sampling Site	**Total *S. aureus***	**MRSA Positive (%)**	**Non-MRSA (%)**
Pre-treated (Site 1)	8	4 (50.0%)	4 (50.0%)
Pre-treated (Site 2)	9	4 (44.4%)	5 (55.6%)
Pre-treated (Site 3)	8	3 (37.5%)	5 (62.5%)
Pre-treated (Site 4)	8	4 (50.0%)	4 (50.0%)
Total	33	15 (45.5%)	18 (54.5%)

3.2. Antibiotic Resistance Patterns of MRSA

The antibiotic susceptibility profiles of MRSA isolates are presented in Table 3. Alarmingly, all MRSA isolates from all three pharmaceutical companies exhibited 100% resistance to multiple antibiotics, including aztreonam, cefoxitin, clindamycin, ceftriaxone, imipenem, trimethoprim-sulfamethoxazole, and vancomycin. Complete resistance to vancomycin, a last-resort antibiotic for MRSA infections, was observed in all isolates.

Resistance to other antibiotics was also notably high: gentamicin resistance ranged from 55.2% to 71.4%, ofloxacin resistance from 72.4% to 80.0%, and levofloxacin resistance from 85.7% to 86.7%. No significant differences in resistance patterns were observed between the three pharmaceutical companies.

Table 3 Antibiotic Resistance Profile of MRSA Isolates from Pharmaceutical Company Effluents

Antibiotic Class	**Antibiotic (Disk Potency)**	**Pharma A MRSA (n=35)**		**Pharma B MRSA (n=29)**		**Pharma C MRSA (n=15)**	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Monobactam	Aztreonam (30 µg)	35 (100)	0 (0.0)	29 (100)	0 (0.0)	15 (100)	0 (0.0)
β-Lactam/Cephem	Cefoxitin (30 µg)	35 (100)	0 (0.0)	29 (100)	0 (0.0)	15 (100)	0 (0.0)

Lincosamide	Clindamycin (15 µg)	35 (100)	0 (0.0)	29 (100)	0 (0.0)	15 (100)	0 (0.0)
β-Lactam/Cephem	Ceftriaxone (30 µg)	35 (100)	0 (0.0)	29 (100)	0 (0.0)	15 (100)	0 (0.0)
β-Lactam/Carbapenem	Imipenem (10 µg)	35 (100)	0 (0.0)	29 (100)	0 (0.0)	15 (100)	0 (0.0)
Aminoglycoside	Gentamicin (10 µg)	25 (71.4)	10 (28.6)	16 (55.2)	13 (44.8)	9 (60.0)	6 (40.0)
Fluoroquinolone	Ofloxacin (5 µg)	28 (80.0)	7 (20.0)	21 (72.4)	8 (27.6)	11 (73.3)	5 (26.7)
Fluoroquinolone	Levofloxacin (5 µg)	30 (85.7)	5 (14.3)	25 (86.2)	4 (13.8)	13 (86.7)	2 (13.3)
Sulfonamide	Trimethoprim-Sulfamethoxazole (25 µg)	35 (100)	0 (0.0)	29 (100)	0 (0.0)	15 (100)	0 (0.0)
Glycopeptide	Vancomycin (10 µg)	35 (100)	0 (0.0)	29 (100)	0 (0.0)	15 (100)	0 (0.0)

Key: R = Resistant; S = Susceptible; n = Number of MRSA isolates tested

3.3. Multi-Drug Resistance Patterns

All MRSA isolates (100%) exhibited multi-drug resistance (MDR), defined as resistance to three or more classes of antibiotics. The isolates showed resistance to 7-9 different antibiotic classes, with all isolates being resistant to β-lactams (cefoxitin, ceftriaxone, imipenem), glycopeptides (vancomycin), lincosamides (clindamycin), monobactams (aztreonam), and sulfonamides (trimethoprim-sulfamethoxazole). This pattern indicates extensive drug resistance with limited therapeutic options. All MRSA isolates (100%) exhibited resistance to 10 antibiotics across multiple classes. All MRSA isolates across all three pharmaceutical companies exhibited multidrug resistance (resistance to ≥3 antibiotic classes).

Table 4 Multidrug Resistance Profile of MRSA Isolates by Company

Pharmaceutical Company	MRSA Isolates (n)	Antibiotic Codes Resisted	Number of Codes	MDR Status
Pharma A	35	ATM, FOX, CD, CRO, IPM, CN, OFX, LEV, SXT, VA	10	100% MDR
Pharma B	29	ATM, FOX, CD, CRO, IPM, CN, OFX, LEV, SXT, VA	10	100% MDR
Pharma C	15	ATM, FOX, CD, CRO, IPM, CN, OFX, LEV, SXT, VA	10	100% MDR

Key: R = Resistant; S = Susceptible; n = Number of MRSA isolates tested; ATM = Aztreonam; FOX = Cefoxitin; CD = Clindamycin; CRO = Ceftriaxone; IPM = Imipenem; CN = Gentamicin; OFX = Ofloxacin; LEV = Levofloxacin; SXT = Trimethoprim-Sulfamethoxazole; VA = Vancomycin.

4. Discussion

The overall MRSA prevalence of 79 (48.2%) observed in this study is alarmingly high and exceeds rates reported in many other environmental and clinical settings. This prevalence is substantially higher than the 27.5% reported in municipal wastewater in Finland [22] and the 21.6% reported from clinical and environmental sources in Jakarta [23]. However, it is comparable to the 55% prevalence reported in clinical settings by Thakar et al. [24]. The high MRSA rates in pharmaceutical effluents likely reflect the intense selective pressure exerted by antibiotic residues present in these wastewaters, which promote the survival and proliferation of resistant strains [25].

Notably, the distribution of MRSA varied significantly across different effluent sources within the same pharmaceutical company, ranging from 23.8% to 68.4% in Pharma A. This variation suggests that certain production or treatment stages may be more prone to harboring MRSA, possibly due to differences in antibiotic concentrations, nutrient availability, or treatment efficiency. Similar variability has been reported in other studies, with higher MRSA prevalence in influent compared to effluent samples in wastewater treatment plants [26,27].

The most concerning finding of this study is the complete (100%) resistance to vancomycin observed in all MRSA isolates. Vancomycin has traditionally been the first-line antibiotic for treating severe MRSA infections, including those that are multidrug-resistant [28]. The emergence of vancomycin-resistant MRSA (VRSA) in pharmaceutical effluents is alarming, as it significantly limits treatment options for MRSA infections.

This finding aligns with recent reports of increasing vancomycin resistance in environmental and clinical settings globally [29,30]. In Nigeria, studies have previously reported vancomycin-intermediate S. aureus (VISA) and VRSA in clinical settings [31,32], but the detection of VRSA in pharmaceutical wastewater suggests environmental dissemination of these resistance traits.

The mechanism of vancomycin resistance in *S. aureus* typically involves acquisition of the vanA or vanB gene clusters from enterococci via horizontal gene transfer [33]. While we did not specifically screen for van genes in this study, the phenotypic resistance observed warrants further investigation to characterize the underlying genetic mechanisms. The presence of VRSA in pharmaceutical effluents is particularly concerning given the potential for these resistant bacteria to be released into the environment and subsequently transferred to humans through contaminated water sources or food chains [34].

The MRSA isolates in this study also exhibited complete resistance to aztreonam, cefoxitin, clindamycin, ceftriaxone, imipenem, and trimethoprim-sulfamethoxazole. Resistance to imipenem, a carbapenem antibiotic, is particularly concerning as carbapenems are considered last-resort antibiotics for treating infections caused by multidrug-resistant Gram-negative bacteria [35]. While carbapenem resistance is more commonly associated with Gram-negative organisms, its detection in MRSA from environmental sources suggests potential cross-resistance or co-selection mechanisms.

The high resistance to clindamycin (100%) is also significant, as clindamycin remains an important treatment option for skin and soft tissue infections caused by MRSA [36]. The resistance observed in all isolates suggests that this antibiotic would be ineffective in treating infections potentially originating from these environmental sources.

Moderate but substantial resistance was observed for gentamicin (55.2-71.4%), ofloxacin (72.4-80.0%), and levofloxacin (85.7-86.7%). These resistance rates are consistent with findings from other Nigerian studies that have reported high resistance to aminoglycosides and fluoroquinolones among MRSA isolates [31,32,37]. The slightly lower resistance to gentamicin compared to other antibiotics may indicate that this antibiotic retains some activity against certain MRSA strains; however, the high resistance rates still limit its therapeutic utility.

All MRSA isolates in this study exhibited multidrug resistance (MDR), with resistance to at least seven different antibiotic classes. This high prevalence of MDR is consistent with findings from other studies in Nigeria [31,37] and other African countries [38,39]. MDR MRSA is a major public health concern as it limits treatment options, increases morbidity and mortality, and drives the need for more expensive and potentially more toxic alternative therapies [40].

The presence of MDR MRSA in pharmaceutical effluents is particularly concerning given the environmental context. These effluents serve as potential reservoirs and dissemination routes for MDR bacteria, which can contaminate water sources, soil, and agricultural products, creating a pathway for human exposure and potential infection [18]. Moreover, the high prevalence of MDR phenotypes suggests that pharmaceutical wastewater may serve as an environment where horizontal gene transfer and the accumulation of multiple resistance determinants are favored [18].

The MRSA prevalence and resistance patterns observed in this study are generally higher than those reported in many other environmental studies. For example, studies in Sweden, Spain, and the USA reported MRSA prevalence of 50-55% in wastewater treatment plants, with prevalence decreasing through treatment stages [26,42,43]. In contrast, the prevalence in our study (48.2%) remained consistently high across different stages.

The complete vancomycin resistance observed in this study is higher than that reported in most other studies. Shariati et al. (2020) reported a global prevalence of 67% vancomycin-intermediate S. aureus (VISA) in Asia [44], while studies

in Africa have reported variable vancomycin resistance rates [31,32]. The higher rates observed in our study may reflect the intense selective pressure in pharmaceutical effluents, where high antibiotic concentrations may promote the emergence and persistence of highly resistant strains.

The high prevalence of MRSA in pharmaceutical effluents can be attributed to several interconnected factors. First, the presence of antibiotic residues in wastewater exerts continuous selective pressure, favoring the survival and proliferation of resistant strains [9]. Second, pharmaceutical wastewaters often contain high organic loads and nutrients that support bacterial growth, potentially facilitating the out-competition of susceptible strains by resistant ones [45]. Third, the manufacturing process itself may introduce S. aureus through human carriers during handling, processing, and purification stages, as suggested by Lateef (2003) [46] and Oguntunnbi et al. (2024) [47].

The persistence of MRSA in pre-treated effluents indicates that current wastewater treatment processes are insufficient to eliminate these resistant bacteria. This is consistent with findings from other studies that have reported the survival of antibiotic-resistant bacteria through conventional treatment processes [10,11,48]. The failure to remove MRSA effectively from wastewater creates an ongoing risk of environmental dissemination and subsequent human exposure.

The findings of this study have significant public health implications. Pharmaceutical effluents containing high concentrations of MDR MRSA, including VRSA, pose a direct threat to environmental and human health. These effluents, if discharged without adequate treatment, can contaminate water bodies used for drinking, irrigation, and recreation, creating multiple pathways for human exposure [17].

The presence of VRSA in pharmaceutical effluents is particularly concerning given the limited therapeutic options for infections caused by these strains. Vancomycin has been a cornerstone for treating severe MRSA infections, and the emergence of resistance significantly complicates clinical management [28]. The potential for these resistant strains to spread from the environment to healthcare settings or communities underscores the need for integrated surveillance and control measures [19].

Furthermore, the high prevalence of MDR MRSA in environmental sources raises concerns about community-acquired infections. The presence of these strains in the environment suggests that they may be circulating in the community, posing a risk of infection to individuals who come into contact with contaminated water or soil.

Several limitations should be acknowledged in interpreting these findings. First, the study included only three pharmaceutical companies in Ogun State, and the results may not be generalizable to all pharmaceutical facilities in Nigeria or other countries. Second, the study relied on culture-based methods for bacterial isolation, which may underestimate the true prevalence of MRSA and other antibiotic-resistant bacteria [49]. Third, the study did not investigate the presence of van genes or other resistance mechanisms, which would have provided additional insight into vancomycin resistance. Finally, the lack of comprehensive antibiotic history and discharge data from the pharmaceutical companies limits our ability to correlate specific resistance patterns with manufacturing practices.

5. Conclusion

This study reveals an alarmingly high prevalence of multidrug-resistant MRSA, including complete vancomycin resistance, in wastewater from pharmaceutical companies in Ogun State, Nigeria. The overall MRSA prevalence of 48.2% among *S. aureus* isolates, coupled with resistance to multiple antibiotic classes, identifies pharmaceutical effluents as critical environmental reservoirs for highly resistant MRSA strains.

The complete resistance to vancomycin observed in all MRSA isolates is particularly concerning, as it significantly limits therapeutic options for infections that may originate from environmental sources. These findings demonstrate that current wastewater treatment practices are insufficient to eliminate MRSA and may even contribute to the selection and dissemination of highly resistant strains.

The implications of this study extend beyond Nigeria to the broader global context of AMR containment. Pharmaceutical manufacturing facilities represent hotspots for the environmental dissemination of antibiotic resistance, and their contribution to the global AMR burden requires urgent attention. There is a critical need for enhanced wastewater treatment technologies capable of removing antibiotic-resistant bacteria and resistance genes, stricter regulatory oversight of pharmaceutical effluent discharge, and integrated surveillance systems that monitor environmental reservoirs of resistance.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

- Regulatory Enforcement: The National Agency for Food and Drug Administration and Control (NAFDAC) and environmental protection agencies should establish and enforce mandatory minimum standards for pharmaceutical wastewater treatment, including specific requirements for the removal of antibiotic-resistant bacteria.
- Advanced Treatment Technologies: Pharmaceutical companies should be required to implement advanced treatment systems, such as membrane bioreactors, advanced oxidation processes, or constructed wetlands, specifically designed to remove resistant bacteria and resistance genes from effluents.
- Comprehensive Surveillance: A national surveillance program should be established to routinely monitor antibiotic-resistant bacteria in pharmaceutical effluents and receiving water bodies, using standardized culture-based methods.
- Antibiotic Stewardship: Pharmaceutical companies should implement antibiotic stewardship programs to minimize antibiotic release into wastewater, including process optimization, waste minimization, and alternative waste treatment strategies.
- Research Priority: Further research is needed to investigate the prevalence of vancomycin resistance genes (vanA, vanB) in environmental MRSA isolates, understand the mechanisms of vancomycin resistance, and track the dissemination pathways of highly resistant MRSA strains from pharmaceutical effluents to clinical settings.
- One Health Approach: The findings of this study should be incorporated into Nigeria's National Action Plan on Antimicrobial Resistance, with specific objectives and allocated funding addressing the environmental dimension, particularly pollution from pharmaceutical manufacturing.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

This study was approved by the Ebonyi State University Ethics Committee (Approval Number: EBSU/DRIC/UREC/026/034).

Statement of informed consent

Written informed consent was obtained from all participating pharmaceutical companies after comprehensive explanation of the study objectives and methods.

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