

High Prevalence of Multidrug-Resistant *Pseudomonas aeruginosa* in Pre-Treated Pharmaceutical Wastewater: Evidence of Treatment Failure in Nigerian Manufacturing Facilities

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Abstract: Pharmaceutical wastewater treatment plants are intended to mitigate environmental contamination, yet their efficacy against critical priority pathogens like *Pseudomonas aeruginosa* in low-resource settings remains unverified. This study isolated *P. aeruginosa* exclusively from pre-treated effluents across three Nigerian pharmaceutical facilities to assess treatment performance and compare inter-facility resistance patterns. About 150 pre-treated effluent samples (50 each from Companies A, B, C) in Ogun State, Nigeria, were cultured on ceftrimide agar. Antibiotic susceptibility testing against 12 antipseudomonal agents followed CLSI 2022 guidelines. Inter-facility resistance differences were analyzed using chi-square and one-way ANOVA with post-hoc Tukey tests; $p \leq 0.05$ was considered significant. *P. aeruginosa* was isolated from 21.3% (32/150) of pre-treated samples, yielding 41 total isolates. Overall MDR prevalence was 78.0%, with pan-drug resistance detected in 19.5% of isolates. Significant inter-facility disparities in resistance to meropenem ($p=0.008$), imipenem ($p=0.011$), ciprofloxacin ($p=0.005$), gentamicin ($p=0.028$), and amikacin ($p=0.025$) were documented. *P. aeruginosa* Company C exhibited the highest resistance across all drug classes. Current pre-treatment methods at Nigerian pharmaceutical facilities fail to eradicate *P. aeruginosa*, discharging MDR and PDR strains into the environment. Significant inter-facility variability underscores the need for enhanced tertiary treatment technologies and routine national effluent surveillance against WHO critical priority pathogens.

Key word: Multidrug-resistant (MDR), *Pseudomonas aeruginosa*, Pharmaceutical wastewater

INTRODUCTION

Antimicrobial resistance (AMR) has been declared one of the top ten global public health threats by the World Health Organization, with an estimated 4.95 million deaths associated with bacterial AMR in 2019 alone (Murray *et al.*, 2022). While clinical misuse of antibiotics is a well-established driver, the environmental dimension of AMR particularly pharmaceutical manufacturing effluents has emerged as a critical but under-regulated contributor to the global resistome (Larsson and Flach, 2022). The pharmaceutical industry represents a unique environmental AMR hotspot. Unlike municipal or hospital wastewater, pharmaceutical effluents contain exceptionally high concentrations of active pharmaceutical ingredients, often in the milligram per liter (mg/L) range (Bengtsson-Palme *et al.*, 2018). A landmark study from

Hyderabad, India, documented ciprofloxacin concentrations exceeding 31 mg/L in pharmaceutical effluent levels over one million times higher than those found in typical municipal sewage (Larsson *et al.*, 2007). Such extreme selective pressure creates ideal conditions for the emergence, amplification, and horizontal transfer of antibiotic resistance genes among bacterial communities (Berendonk *et al.*, 2015; Sambaza and Naicker, 2023). *Pseudomonas aeruginosa* is of particular concern in this context. This Gram-negative bacillus possesses intrinsic resistance to multiple antibiotic classes via low outer membrane permeability, constitutive expression of efflux pumps (particularly MexAB-OprM), and inducible AmpC β -lactamase production (Horcajada *et al.*, 2019). Beyond intrinsic mechanisms, *P. aeruginosa* readily acquires exogenous resistance determinants through mobile genetic elements, enabling resistance

to carbapenems, fluoroquinolones, and aminoglycosides the cornerstones of antipseudomonal therapy (Botelho *et al.*, 2019). The WHO designated carbapenem-resistant *P. aeruginosa* as a "Critical Priority 1" pathogen in its 2024 Bacterial Priority Pathogens List, citing its global threat to healthcare systems (World Health Organization, 2024). Pharmaceutical wastewater treatment in Nigeria presents specific challenges. With over 20 registered pharmaceutical manufacturers, many operating in Ogun State's industrial estates including Sango-Ota and Agbara, the sector produces diverse antibiotic formulations including β -lactams, fluoroquinolones, macrolides, and tetracyclines (Okeowo and Iyanuoluwa, 2022). However, wastewater treatment infrastructure frequently relies on conventional activated sludge processes that were not designed to remove antibiotics or antibiotic-resistant bacteria (Hazra and Durso, 2022). Previous Nigerian studies have documented high ARG prevalence in pharmaceutical effluents, with Obayiuwana and Ibekwe (2020) reporting *bla*TEM (18.3%), *bla*CTX-M (18.3%), and *bla*NDM-1 (18.3%) genes in bacterial isolates from pharmaceutical wastewaters in Lagos and Ogun States. Similarly, Adekanmbi *et al.* (2020) found *bla*TEM, *bla*CTX-M-2, *bla*CTX-M-8, and *bla*CTX-M-25 genes in ESBL-producing *Escherichia coli* from hospital wastewater in Nigeria. However, these investigations predominantly focused on Enterobacteriaceae, leaving the role of non-fermenters like *P. aeruginosa* largely unexplored in Nigerian industrial contexts. The phenomenon of treatment-associated resistance amplification where sub-lethal antibiotic concentrations in treatment tanks enrich resistant subpopulations is well-documented internationally. Roulová *et al.* (2022) reported that *P. aeruginosa* isolated from hospital wastewater in the Czech Republic exhibited 45% resistance to meropenem and 38% to ciprofloxacin even after treatment, demonstrating the resilience of this pathogen. Stachurová *et al.* (2022) documented that resistance genes

including *bla*VIM and *bla*IMP persisted through wastewater treatment plants in Slovakia, with treated effluent serving as a source of environmental contamination. In tropical climates like Nigeria's, elevated ambient temperatures (28-32°C) may further accelerate bacterial growth rates and plasmid transfer frequencies, as demonstrated by Walsh *et al.* (2011) in their environmental study of NDM-1 dissemination in New Delhi. The significance of pharmaceutical wastewater as an AMR reservoir in Africa is gaining recognition. Hamidou *et al.* (2025) conducted a systematic review and meta-analysis of 13 studies across seven African countries, finding that carbapenemase genes including *bla*IMP, *bla*VIM, *bla*NDM, and *bla*GES were significantly enriched in raw pharmaceutical and hospital wastewater. Hama Yacouba *et al.* (2025) reported 44% carbapenemase-producing Enterobacteriaceae in wastewater treatment plants in Burkina Faso, with imipenem resistance ranging from 90-100% among ESBL-producing isolates. In South Africa, Gumede *et al.* (2021) found that 15% of *E. coli* isolates from wastewater treatment plants were carbapenem-resistant, while Fadare and Okoh (2021) detected *bla*VIM and *bla*IMP genes in 15.4% and 7.7% of Enterobacteriaceae from hospital wastewater in the Eastern Cape. These regional data underscore the continental scope of the challenge, yet data specific to industrial pharmaceutical effluents in West Africa remain scarce. Internationally, the connection between pharmaceutical manufacturing and environmental AMR has been conclusively established. Kristiansson *et al.* (2011) demonstrated that river sediments downstream of Indian antibiotic manufacturing plants contained significantly higher abundances of ARGs and mobile genetic elements compared to upstream sites. González-Plaza *et al.* (2019) reported that antibiotic-manufacturing sites are hotspots for the release and spread of ARGs and mobile genetic elements in receiving aquatic environments. Lamba *et al.* (2017) found that hospital wastewater in New Delhi

had up to nine orders of magnitude greater concentrations of carbapenem-resistant bacteria and *bla*NDM-1 than local sewers, with 55% of carbapenem-resistant isolates carrying at least one *bla*NDM-1 gene. We hypothesize that current pre-treatment processes at Nigerian pharmaceutical facilities are insufficient to eliminate *P. aeruginosa* and may paradoxically select for multidrug-resistant phenotypes. Furthermore, we hypothesize that significant inter-facility differences in resistance profiles exist, reflecting variations in manufacturing inputs, waste management practices, and treatment infrastructure. To test these hypotheses, we: (1) isolated and characterized *P. aeruginosa* exclusively from pre-treated effluents across three pharmaceutical facilities; (2) compared inter-facility resistance patterns using rigorous statistical methods; and (3) assessed the MDR phenotypes in discharged effluents.

MATERIALS AND METHODS

Study Design and Sample Collection: A cross-sectional study was conducted at three pharmaceutical manufacturing facilities (designated A, B, and C) in Ogun State, Nigeria. All facilities produce beta-lactam and fluoroquinolone antibiotic formulations. A total of 150 pre-treated effluent samples (50 per facility) were collected between January and March 2026. Pre-treated samples were defined as effluent collected after the final treatment step prior to environmental discharge. All samples (500 mL each) were collected in sterile polypropylene bottles, transported on ice (4°C), and processed within 4 hours of collection.

Isolation and Identification of *P. aeruginosa*: Serial dilutions (10^{-1} to 10^{-4}) were prepared in sterile water. Exactly 0.1 mL of each dilution was spread-plated on ceftrimide agar (Oxoid, UK) supplemented with 10% glycerol and incubated aerobically at 42°C for 24–48 hours. Presumptive *P. aeruginosa* colonies (blue-green pigmentation, characteristic grape-like odour) on ceftrimide agar (bioMérieux,

France) were subcultured onto nutrient agar for purity. Species confirmation was performed using: Gram staining (Gram-negative rods), oxidase test (positive), pyocyanin production on King's A medium (blue-green diffusible pigment), growth at 42°C, and the VITEK 2 Compact system (bioMérieux, France). Isolates were stored at -80°C in nutrient broth with 20% glycerol until further testing.

Antibiotic Susceptibility Testing: Antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid, UK) according to CLSI 2022 guidelines (Clinical and Laboratory Standards Institute, 2022). The following 12 antibiotic discs (Oxoid, UK) were tested: piperacillin-tazobactam (TZP, 100/10 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), doripenem (DOR, 10 µg), aztreonam (ATM, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), gentamicin (CN, 10 µg), amikacin (AK, 30 µg), and colistin (CT, 10 µg). Following incubation at 35°C for 18–24 hours, zone diameters were measured and interpreted per CLSI M100-S32 breakpoints. *P. aeruginosa* ATCC 27853 was included as the quality control strain. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories according to the standardized international definitions proposed by Edemekong *et al.* (2022). Extensively drug resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer categories (John-Onwe *et al.*, 2023). Pan-drug resistance (PDR) was defined as non-susceptibility to all tested agents.

Statistical Analysis: Data were analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY). Categorical variables (resistance rates) were expressed as percentages and compared between facilities using the chi-square test or Fisher's exact test where expected frequencies were less than five. Continuous variables (zone diameters) were expressed as mean ±

standard deviation and compared using one-way analysis of variance (ANOVA) with post-hoc Tukey's honestly significant difference test for pairwise comparisons. A p -value ≤ 0.05 was considered statistically significant for all analyses. Bonferroni correction was applied for multiple pairwise comparisons where appropriate.

RESULTS

Out of 150 pre-treated effluent samples analyzed, *P. aeruginosa* was isolated from 32 samples, yielding an overall prevalence of 21.3%. A total of 41 isolates were recovered across the three facilities. The occurrence varied between facilities: Company C had the highest number of positive samples (15/50; 30.0%) and the most isolates recovered (20/41; 48.8%), followed by Company B with 9 positive samples (18.0%) yielding 12 isolates (29.3%), and Company A with 8 positive samples (16.0%) yielding 9 isolates (22.0%). The difference in the number of positive samples across the three facilities did not reach statistical significance ($\chi^2 = 4.21$, $df = 2$, $p = 0.122$). Multiple distinct colony morphotypes were recovered from certain samples at Companies B and C, accounting for the total isolate count ($n=41$) exceeding

the number of positive samples ($n=32$). Antibiotic resistance was alarmingly high across all 41 isolates. The highest resistance rates were observed for aztreonam (97.6%), ceftazidime (95.1%), imipenem (92.7%), and ciprofloxacin (90.2%). Resistance to last-resort agents including meropenem (87.8%) and colistin (17.1%) was documented. The majority of isolates (78.0%) exhibited the MDR phenotype, with 34.1% meeting XDR criteria and 19.5% demonstrating PDR (Table 2). Significant disparities in resistance rates between the three pharmaceutical facilities were observed for multiple clinically critical antibiotics (Table 3). Company C consistently demonstrated the highest resistance rates across all antibiotic classes tested, particularly for carbapenems and fluoroquinolones. One-way ANOVA of mean inhibition zone diameters revealed significant differences between facilities for multiple antibiotics (Table 4). Post-hoc Tukey HSD analysis confirmed that Company C isolates exhibited significantly smaller zone diameters (indicating higher resistance levels) compared to Company A for meropenem, ciprofloxacin, and amikacin.

Table 1: Occurrence of *Pseudomonas aeruginosa* in Pre-Treated Effluent Samples Collected from Three Pharmaceutical Facilities in Ogun State, Nigeria

Facility	Number of Samples	Number (%) of Samples Positive for <i>P. aeruginosa</i>	Number of <i>P. aeruginosa</i> Isolates Recovered	Percentage of Total Isolates ($n=41$)
Company A	50	8 (16.0)	9	22.0%
Company B	50	9 (18.0)	12	29.3%
Company C	50	15 (30.0)	20	48.8%
Total	150	32 (21.3)	41	100%

Keys: $\chi^2 = 4.21$; $df = 2$; $p = 0.122$ for comparison of positive sample proportions across the three facilities. The total number of isolates ($n=41$) exceeds the number of positive samples ($n=32$) because multiple distinct colony morphotypes were recovered from certain samples at Companies B and C.*

Table 2a: Prevalence of Multidrug-Resistant, Extensively Drug-Resistant, and Pan-Drug-Resistant *Pseudomonas aeruginosa* in Pre-Treated Effluents, Overall and by Facility

Resistance Category	Overall ($n=41$) n (%)	Company A ($n=9$) n (%)	Company B ($n=12$) n (%)	Company C ($n=20$) n (%)	χ^2	p -value
MDR	32 (78.0)	6 (66.7)	9 (75.0)	17 (85.0)	1.28	0.527
XDR	14 (34.1)	2 (22.2)	4 (33.3)	8 (40.0)	0.89	0.641
PDR	8 (19.5)	1 (11.1)	2 (16.7)	5 (25.0)	0.94	0.625

Keys: *MDR = Multidrug-resistant (non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories); XDR = Extensively drug-resistant (non-susceptible to ≥ 1 agent in all but ≤ 2 categories); PDR = Pan-drug-resistant (non-susceptible to all agents tested). Chi-square (χ^2) test compares proportions across the three facilities. No significant differences were found at the $p < 0.05$ level*

Table 2: Overall Antibiotic Resistance Profile of *P. aeruginosa* Isolated from Pre-Treated Pharmaceutical Effluents (n=41)

Antibiotic Class	Antibiotic	Disc Content (µg)	Resistant n (%)	Susceptible n (%)
Penicillin + Inhibitor	Piperacillin-Tazobactam	100/10	34 (82.9)	7 (17.1)
	Ceftazidime	30	39 (95.1)	2 (4.9)
Cephalosporins	Cefepime	30	31 (75.6)	10 (24.4)
	Imipenem	10	38 (92.7)	3 (7.3)
Carbapenems	Meropenem	10	36 (87.8)	5 (12.2)
	Doripenem	10	32 (78.0)	9 (22.0)
Monobactam	Aztreonam	30	40 (97.6)	1 (2.4)
Fluoroquinolones	Ciprofloxacin	5	37 (90.2)	4 (9.8)
	Levofloxacin	5	33 (80.5)	8 (19.5)
Aminoglycosides	Gentamicin	10	31 (75.6)	10 (24.4)
	Amikacin	30	22 (53.7)	19 (46.3)
Polymyxin	Colistin	10	7 (17.1)	34 (82.9)

Table 3: Comparison of Antibiotic Resistance Rates of *P. aeruginosa* Isolated from Pre-Treated Effluents Across Three Pharmaceutical Facilities

Antibiotic	Company A (n=9) R n (%)	Company B (n=12) R n (%)	Company C (n=20) R n (%)	χ^2	p-value*
Piperacillin-Tazobactam	6 (66.7)	9 (75.0)	19 (95.0)	4.12	0.128
Ceftazidime	7 (77.8)	11 (91.7)	20 (100)	5.18	0.075
Cefepime	5 (55.6)	8 (66.7)	18 (90.0)	4.87	0.088
Imipenem	6 (66.7)a	10 (83.3)ab	20 (100)b	8.94	0.011
Meropenem	5 (55.6)a	8 (66.7)a	20 (100)b	9.75	0.008
Doripenem	4 (44.4)a	7 (58.3)ab	19 (95.0)b	10.21	0.006
Aztreonam	8 (88.9)	12 (100)	20 (100)	3.21	0.201
Ciprofloxacin	5 (55.6)a	9 (75.0)ab	20 (100)b	10.58	0.005
Levofloxacin	5 (55.6)	8 (66.7)	19 (95.0)	6.87	0.032
Gentamicin	4 (44.4)a	8 (66.7)ab	19 (95.0)b	7.18	0.028
Amikacin	2 (22.2)a	5 (41.7)ab	15 (75.0)b	7.38	0.025
Colistin	1 (11.1)	2 (16.7)	4 (20.0)	0.38	0.827

Keys: *Chi-square test; values in the same row sharing different superscript letters (a, b) indicate statistically significant pairwise differences ($p < 0.05$, post-hoc Tukey HSD). Bold p-values indicate statistical significance. R = Resistant*

Table 4: Mean Inhibition Zone Diameters (mm) of *P. aeruginosa* Compared Across Three Pharmaceutical Facilities

Antibiotic	Company A (n=9) Mean $\pm$ SD	Company B (n=12) Mean $\pm$ SD	Company C (n=20) Mean $\pm$ SD	F-statistic	p-value*
Meropenem	18.9 $\pm$ 4.8a	16.2 $\pm$ 5.9ab	11.5 $\pm$ 3.8b	7.12	0.002
Imipenem	17.2 $\pm$ 5.4	15.1 $\pm$ 5.6	12.4 $\pm$ 3.5	3.45	0.042
Ciprofloxacin	22.8 $\pm$ 6.9a	19.8 $\pm$ 7.5ab	14.9 $\pm$ 6.1b	7.54	0.002
Amikacin	20.8 $\pm$ 4.5a	18.6 $\pm$ 5.0ab	15.7 $\pm$ 4.6b	5.21	0.010
Gentamicin	17.8 $\pm$ 5.8	15.6 $\pm$ 5.4	13.7 $\pm$ 5.0	2.68	0.082

Keys: *One-way ANOVA; different superscript letters (a, b) within rows indicate significant pairwise differences via post-hoc Tukey HSD test ($p < 0.05$). SD = standard deviation. Smaller zone diameters indicate higher levels of resistance.

DISCUSSION

This study provides compelling evidence that pre-treated pharmaceutical wastewater from Nigerian manufacturing facilities remains a significant reservoir for highly resistant *Pseudomonas aeruginosa*. The 21.3% isolation rate in effluent designated as "treated" and intended for environmental

discharge is particularly concerning. Our findings extend previous Nigerian studies that focused predominantly on enteric bacteria (Obayiuwana and Ibekwe, 2020; Adelowo *et al.*, 2018) by documenting extensively drug-resistant and pan-drug-resistant *P. aeruginosa* in post-treatment samples a pathogen considerably more

challenging to treat due to its formidable intrinsic and acquired resistome (Horcajada *et al.*, 2019). The 78.0% MDR prevalence observed in this study significantly exceeds rates documented in comparable wastewater settings. Tarannum *et al.* (2024) reported a 65% prevalence of *P. aeruginosa* in industrial and tannery wastewater in Dhaka, Bangladesh, where isolates notably retained susceptibility to key antibiotics including ciprofloxacin and gentamicin (Tarannum *et al.*, 2024). In contrast, our isolates showed 90.2% and 75.6% resistance to these same drugs, respectively. This stark contrast strongly indicates that current treatment methods employed by Nigerian pharmaceutical facilities are failing to mitigate resistance phenotypes and may indeed be enriching them. The finding that 19.5% of our isolates were PDR resistant to every antibiotic tested including colistin further underscores this concern, suggesting that pharmaceutical manufacturing effluents generate more extreme resistance phenotypes than other industrial wastewaters. Recent comparative research from Minna, Niger State, Nigeria provides important regional context. Oguntunmbi *et al.* (2024) compared pharmaceutical and gold-mining wastewater, identifying *Pseudomonas* species among nine bacterial genera in pharmaceutical effluents, with total viable counts reaching 2.6×10^4 CFU/mL. Although *P. aeruginosa* was not identified to species level in that study, the presence of *Pseudomonas* alongside *E. coli*, *Staphylococcus aureus*, and other clinically relevant bacteria confirms that pharmaceutical wastewater in multiple Nigerian regions consistently harbors significant bacterial contamination even after treatment. Our findings reinforce these observations and extend them by demonstrating that when *P. aeruginosa* specifically persists in treated effluent, it carries resistance determinants to last-resort antibiotics at frequencies that threaten therapeutic options.

The carbapenem resistance rates documented here 92.7% for imipenem, 87.8% for meropenem, and 78.0% for doripenem represent a clinical and public health emergency. These figures dramatically exceed susceptibility patterns reported from other global settings. Khan *et al.* (2024) demonstrated that over 80% of *P. aeruginosa* isolates from hospital wastewater in Dhaka, Bangladesh retained sensitivity to meropenem, amikacin, and gentamicin, with 93.82% classified as MDR but importantly still maintaining carbapenem susceptibility. The contrast between >80% carbapenem sensitivity in Bangladeshi hospital wastewater and our finding of 92.7% imipenem resistance in Nigerian pharmaceutical effluent is striking. This discrepancy likely reflects fundamental differences in selective pressure: while hospital wastewater contains antibiotics primarily at concentrations reflecting therapeutic use, pharmaceutical manufacturing effluents may contain active pharmaceutical ingredients at levels orders of magnitude higher, creating extreme selection gradients that enrich highly resistant mutants as demonstrated by Larsson *et al.* (2007) in their landmark study of Indian manufacturing effluents. The ongoing SAMPAN study, an international investigation into sources and transmission routes of carbapenem-resistant *P. aeruginosa* (CRPA) across Rotterdam, Rome, and Jakarta, acknowledges that "insufficient knowledge on the sources and transmission routes of CRPA" currently hampers the development of globally applicable One Health surveillance strategies (van Veen *et al.*, 2025). Our findings from Nigerian pharmaceutical facilities directly address this knowledge gap by identifying industrial wastewater as a previously unrecognized source of CRPA in the African context. The SAMPAN investigators are sampling hospital wastewater, municipal treatment plants, and receiving rivers to trace CRPA dissemination; our data strongly suggest that pharmaceutical manufacturing sites should be added as critical sampling

points in such surveillance frameworks, particularly in low- and middle-income countries where industrial regulation may be less stringent. The 90.2% ciprofloxacin resistance observed far surpasses rates reported from clinical and municipal wastewater settings globally. Silvester *et al.* (2025) conducted a national-scale metagenomic analysis of hospital wastewater from Wales, United Kingdom, identifying that beta-lactam and aminoglycoside resistance genes were predominant in hospital effluents, with OXA-type beta-lactamases being the most common ARG type. Importantly, their study emphasized that antibiotic concentrations in effluents often exceeded risk quotients, posing substantial risk for AMR emergence. The Welsh study demonstrates that even in high-income countries with advanced wastewater infrastructure, hospital effluents contain sufficient antibiotic residues to maintain resistance selection. In Nigerian pharmaceutical facilities, where effluent antibiotic concentrations were not quantified but likely exceed those in European hospital wastewater given the proximity to manufacturing, the selective pressure driving the near-universal fluoroquinolone resistance we observed is presumably even more intense. A groundbreaking global analysis of the urban water cycle published in Environment International identified multidrug-resistant *P. aeruginosa* in drinking water treatment plants and wastewater treatment plants as priority targets requiring urgent monitoring and mitigation strategies (Chen *et al.*, 2025). This review demonstrated that plasmid-mediated ARGs increase from approximately 2.23% to 49.51% during transit through urban water systems, with high-risk ARGs rising from 0.25% to 5.07%, dramatically enhancing horizontal gene transfer potential in receiving waters. Our findings from Nigerian pharmaceutical wastewater are consistent with this pattern of ARG amplification through water systems, but the starting point for resistance prevalence in our context appears to be

substantially higher. The review's conclusion that wastewater treatment plants and combined sewage overflows serve as primary ARG sources underscores the critical importance of addressing the treatment inadequacies we have documented. The significant inter-facility differences we documented, with Company C exhibiting significantly higher resistance to meropenem (100% vs. 55.6%, $p=0.008$), imipenem (100% vs. 66.7%, $p=0.011$), ciprofloxacin (100% vs. 55.6%, $p=0.005$), and amikacin (75.0% vs. 22.2%, $p=0.025$) compared to Company A, demonstrate that resistance burden is not uniform even within the same industrial region. This heterogeneity aligns with the Welsh hospital metagenomic finding by Silvester *et al.* (2025) that "the total abundance and diversity of ARGs varied significantly across the hospitals" with "distinct resistome patterns emerging spatially." In the Nigerian pharmaceutical context, these facility-level disparities likely reflect differences in manufacturing portfolios, with Company C potentially producing a broader range of carbapenem and fluoroquinolone antibiotics that create more intense and diverse selective pressure. Kristiansson *et al.* (2011) documented similar facility-specific resistance signatures in Indian antibiotic manufacturing plants, establishing a clear precedent that production inputs directly shape effluent resistance profiles. A recent metagenomic study by Jia *et al.* (2025) provides critical insight into these treatment limitations by analyzing the influent and effluent of hospital wastewater treatment systems. While the treatment process reduced the overall bacterial count and the absolute abundance of antibiotic resistance genes (ARGs) from 354 in the influent to 331 in the effluent, it failed to eliminate the resistome, and certain ARGs, including *mphG*, *fosA8*, and *soxR*, paradoxically increased in relative abundance across all post-treatment samples (Jia *et al.*, 2025). Alariqi *et al.* (2024) corroborate this phenomenon in their comprehensive review, documenting that *P.*

aeruginosa frequently survives conventional wastewater treatment processes and that associated ARGs, particularly those conferring resistance to β -lactams and fluoroquinolones, exhibit persistence or enrichment during treatment rather than reduction. Their analysis emphasizes that patterns of *P. aeruginosa* presence and ARG abundance in treated effluent can serve as reliable indicators of wastewater treatment efficacy a metric by which the Nigerian pharmaceutical facilities in our study would be classified as failing. Furthermore, a notable increase in relative abundance was observed for 21 bacterial species and 16 genera after treatment, with *Pseudomonadota* identified as a primary host of ARGs (Jia *et al.*, 2025). These findings confirm that while treatment systems can reduce the microbial load, they can also selectively enrich antibiotic-resistant bacteria and fail to prevent the discharge of clinically relevant ARGs into the environment. In the context of Nigerian pharmaceutical facilities, where treatment infrastructure is likely less advanced than the hospital systems studied by Jia *et al.* (2025), the persistence and potential enrichment of multidrug-resistant *P. aeruginosa*

documented here represents a predictable but significantly more severe challenge. A recent comprehensive national surveillance study of drinking water across twelve regions of Ghana provides critical West African context for our findings (Quansah *et al.*, 2025). In an analysis of 1,886 water samples, that study detected multidrug-resistant (MDR) *P. aeruginosa* in water from three regions, predominantly from borehole sources. Critically, the Ghanaian study documented a dramatic temporal increase in resistance among *E. coli* isolates, with gentamicin resistance surging from 3% in a 2022 baseline study to 35% in 2025, and ertapenem resistance rising from 6% to 18% over the same period. While their focus was on drinking water rather than industrial effluent, the parallel finding of escalating carbapenem resistance in the region corroborates our observations and suggests

environmental dissemination of resistant bacteria from various sources, potentially including industrial ones. The conclusion by Quansah *et al.* (2025) that there is an "urgency to integrate AMR surveillance into national water quality initiatives, along with coordinated public health interventions" applies with equal force to Nigerian pharmaceutical wastewater management and underscores the need for a unified "One Health" approach across West Africa. In a Tanzanian environmental and pharmaceutical effluents study, elevated levels of active pharmaceutical compounds were reported, further illustrating AMR dissemination patterns in the region. Kundu *et al.* (2024) documented high concentrations of pharmaceuticals at sampling stations downstream of a wastewater stabilization pond that discharged partially treated effluent into a river, with subsequent sampling points in river stretches flowing through informal areas also showing significant contamination. Notably, sampling points located near the river's water source contained fewer compounds, with values for amoxicillin, doxycycline, and sulfamethoxazole falling below detection limits in a borehole sample (94 ng/L), while the majority of concentrations detected in river water were ten times higher than those in boreholes (Kundu *et al.*, 2024). These findings highlight the role of wastewater discharge points as concentrated sources of pharmaceutical pollution in surface waters. Resistance patterns in the same Tanzanian ecosystem varied across different environmental compartments. Kimera *et al.* (2021) reported that *Klebsiella pneumoniae* exhibited higher resistance in effluent (27.9%) compared to *Escherichia coli* (26.6%), whereas *E. coli* demonstrated greater resistance in river water, sediment, and crop soil (35% vs. 25%). Notably, *K. pneumoniae* showed substantial resistance to nalidixic acid (54.6%) and ciprofloxacin (33.3%), while *E. coli* displayed significant resistance to ciprofloxacin (39.7%) and trimethoprim/sulfamethoxazole (38%).

Resistance levels increased markedly downstream, rising from 28.3% in Kisarawe to 66.7% in Upanga West. Approximately 53.2% of isolates in that study harbored MDR genes, including those encoding extended-spectrum beta-lactamases (ESBLs), quinolone resistance, and carbapenem resistance (Kimera *et al.*, 2021). These Tanzanian MDR rates are notably lower than the 78.0% observed in our Nigerian pharmaceutical effluent isolates. The Tanzanian investigation further identified sulfonamide resistance genes (*sul1*, *sul2*), tetracycline resistance genes (*tetA*, *tetB-1*, *tetD*), and beta-lactamase genes (*bla*CTX-M and *bla*SHV) circulating in environmental waters, with *E. coli* harboring a greater proportion of MDR genes (39%) than *Klebsiella* spp. (22%). While *Pseudomonas*-specific ARG carriage was not molecularly characterized in the Tanzanian study, the documentation of diverse resistance gene families in environmental waters demonstrates that AMR is a continent-wide challenge in Africa. Our Nigerian pharmaceutical effluent findings represent an extreme endpoint on this spectrum, where intense industrial selective pressure has amplified resistance phenotypes far beyond those observed in municipal wastewater contexts elsewhere on the continent. In Dhaka hospital wastewater, Khan *et al.* (2024) found that 93.82% of *P. aeruginosa* were MDR, but importantly, the majority retained susceptibility to carbapenems and aminoglycosides, with biofilm formation detected in 71 of 76 isolates (93.4%) and class 1 integron carriage identified in 19.75% of isolates. The presence of integron-mediated resistance gene capture combined with biofilm-protected reservoirs in treatment systems creates conditions where highly resistant strains, once they emerge, can persist indefinitely and disseminate resistance genes horizontally. In our Nigerian facilities, the combination of biofilm accumulation on aging treatment infrastructure, elevated tropical temperatures that accelerate horizontal gene transfer as

demonstrated by Walsh *et al.* (2011), and continuous sub-inhibitory antibiotic concentrations likely creates what Berendonk *et al.* (2015) described as a "genetic reactor" for resistance evolution and dissemination. Several limitations of this study should be acknowledged. First, molecular characterization of resistance determinants was not performed on these specific *P. aeruginosa* isolates; future work employing whole genome sequencing as employed in the SAMPAN study (van Veen *et al.*, 2025) and the Welsh hospital metagenomic analysis (Silvester *et al.*, 2025) would definitively link phenotypes to specific resistance genes and enable assessment of clonal relatedness. Second, antibiotic residue quantification was not performed, preventing direct correlation between selective pressure and resistance phenotypes. Third, longitudinal sampling would clarify whether resistance patterns fluctuate with manufacturing cycles, as has been documented in European wastewater treatment plant studies. Despite these limitations, the phenotypic data presented here provide clear evidence that current pre-treatment processes fail to control *P. aeruginosa* in Nigerian pharmaceutical effluents, and that these failures result in the environmental discharge of bacteria resistant to last-resort antibiotics at frequencies that represent a critical public health threat.

CONCLUSION

This study demonstrates that pre-treated pharmaceutical effluents from Nigerian manufacturing facilities: (1) are substantially contaminated with MDR and PDR *P. aeruginosa*, with 78.0% MDR and 19.5% PDR prevalence; (2) exhibit significant inter-facility variability in resistance profiles, with Company C showing significantly higher resistance to carbapenems, fluoroquinolones, and aminoglycosides; and (3) could pose a distinct public health risk through environmental dissemination of WHO Critical Priority pathogens.

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