Untreated Wastewater as a Reservoir of Carbapenem-Resistant Escherichia Coli and Klebsiella Pneumoniae Isolates

Chijioke A. Nsofor, James Efosa & Chibuike G. Uzoma

Federal University of Technology Owerri

ABSTRACT

Carbapenem-resistant Escherichia coli and Klebsiella pneumoniae are increasingly important pathogens with limited treatment options, and there is limited knowledge on the environmental factors contributing to their spread. We determined the occurrence of carbapenem- resistant E. coli and K. pneumoniae in hospital and slaughterhouse wastewater in Owerri, a city in southeast Nigeria. Samples of untreated hospital and slaughterhouse waste- water were collected monthly at the major tertiary hospital and slaughterhouse in Owerri between April and September 2023. E coli and K. pneumoniae strains were selectively isolated and identified using conventional microbiological technique, and antibiotic susceptibility testing performed using the Kirby Bauer disk diffusion assay. A total of 193 E. coli and K. pneumoniae were isolated from the 269 wastewater samples analyzed. Among the 193 isolates, 101 (52.3%) were identified as K. pneumoniae while 92 (45.7%) were identified as E. coli respectively.

Keywords: NA

Classification: NLM Code: WC 270

Language: English

Great Britain Journals Press

LJP Copyright ID: 392812

London Journal of Medical and Health Research

Volume 24 | Issue 2 | Compilation 1.0

Untreated Wastewater as a Reservoir of Carbapenem-Resistant *Escherichia Coli* and *Klebsiella Pneumoniae* Isolates

Chijioke A. Nsofor[α], James Efosa[σ] & Chibuike G. Uzoma[ρ]

ABSTRACT

Carbapenem-resistant Escherichia coli and Klebsiella pneumoniae are increasingly important pathogens with limited treatment options, and there is limited knowledge on the environmental factors contributing to their spread. We determined the occurrence of carbapenem- resistant E. coli and K. pneumoniae in hospital and slaughterhouse wastewater in Owerri, a city in southeast Nigeria. Samples of untreated hospital and slaughterhouse waste-water were collected monthly at the major tertiary hospital and slaughterhouse in Owerri between April and September 2023. E coli and K. pneumoniae strains were selectively isolated and identified using conventional microbiological technique, and antibiotic susceptibility testing performed using the Kirby Bauer disk diffusion assay. A total of 193 E. coli and K. pneumoniae were isolated from the 269 wastewater samples analyzed. Among the 193 isolates, 101 (52.3%) were identified as K. pneumoniae while 92 (45.7%) were identified as E. coli respectively.

The isolates had high resistance rates (≥48.1%) to 6 antibiotics tested, and resistance to carbapenems ranged from 22.8% to 30.6% with resistance to ertapenem, 30.6 %(59/193) being the highest carbapenem resistance observed. There was no statistical significant difference in carbapenem resistance rates between the hospital and slaughterhouse wastewater isolates (P > 0.05). This study has shown that the release of untreated wastewater into the environment may contribute to the increased spread of carbapenem-resistant E. coli and K. pneumoniae in Owerri, Nigeria. Therefore, there is pressing need to address wastewater as a crucial factor in curtailing the spread of carbapenem-resistant bacteria.

Author α σ ρ: Department of Biotechnology, Federal University of Technology Owerri Nigeria.
α: Department of Forensic Science, Federal University of Technology Owerri Nigeria.

I. INTRODUCTION

Untreated wastewaters from hospitals, agriculture, slaughterhouses, industry and, together with domestic wastewaters contribute to the contamination and degradation of aquatic environments (Oliveira et al., 2021). The release of untreated or poorly treated wastewater into the environment is being increasingly recognized as the major factor in spreading clinically relevant antimicrobial resistant bacteria and genes (Obayiuwana and Ibekwe, 2020). Antimicrobials in wastewater promote the emergence of antibiotic resistance, facilitated by selective pressure and transfer of resistant genes. The widespread use of antimicrobials in clinical practice to control infectious diseases, their application in veterinary medicine coupled with the discharge of non-treated pharmaceutical effluent into the environment, results in the selective pressure which is associated with the emergence and subsequent evolution of bacterial resistant to antibiotics (Tiwari et al ., 2022).

Contamination of water bodies is dependent on the amount, type and degradation potential of contaminants, and the self-purification ability of the recipient water body (Obasi et al 2017). Human and animal pathogens are considered to be important water contaminants; this is possible because, together with human and animal pathogens from all infected individuals and animals (symptomatic, asymptomatic, pre-symptomatic, and post-symptomatic), is excreted through feces, urine, nasal mucus, and sputum

London Journal of Medical & Health Research

from veterinary clinics, slaughterhouses, households, hospitals, and nursing homes, end up in the municipal sewage system. Within the concept of one health perspective, such discharges of clinically relevant antibiotic-resistant bacteria pose a significant health risk to both humans and animals. Therefore, determining the prevalence of clinically important antibiotic resistant bacteria in wastewater may help in controlling their circulation in the environment and saving human and environmental health.

Carbapenems are broad range β-lactam antibiotics used as a last resort in treating infections with multi-drug resistant *Enterobacteriaceae* (MDR-E). In the past few decades, the emergence of strains resistant to carbapenems has been a growing concern (Meletis, 2016). In February 2017, the World Health organization (WHO) published a list of the most dangerous pathogens for human health. At the top of that list are carbapenem-resistant *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacteriaceae* (WHO, 2017). There have been several reports of bacteria with acquired carbapenem resistance isolated from hospital wastewaters (Zhang et al., 2013; Chandran et al., 2014; Seruga-Music et al., 2017), as well as raw and secondary treated municipal wastewaters (Hrenovic et al., 2017). However, data on the prevalence of bacteria of clinical importance from wastewater sources in southeast Nigeria is scarce; most studies are centered on the clinical environment and there is no information about prevalence of carbapenem-resistant *E. coli* and *K. pneumoniae* in environmental sources in the region. This study was designed to bridge this knowledge gap and was therefore, aimed to investigate the prevalence of *E. coli* and *K. pneumoniae* isolated from hospital and slaughterhouse wastewater sources in Owerri, a city located in southeast Nigeria, during a regional surveillance program conducted in March to September 2023.

II. METHODOLOGY

2.1 Sample Collection and Analysis

Untreated wastewater samples were drawn from the Federal University Teaching Hospital Owerri wastewater discharge points and a discharge point of Owerri slaughterhouse. Samples were collected from the upper 5 cm portion of each of the two wastewater sources in triplicate and pooled to form a composite sample. The samples were taken aseptically in sterile plastic bottles under refrigerated conditions and processed in the laboratory within 4 h after collection. Wastewater samples were serially diluted with peptone water and a 100 μl volume of each dilution concentration (original, 1/10, 1/100, and 1/1000) was inoculated on MacConkey agar with a spread plate technique, following the manufacturer's instructions. The plates were incubated at 37°C for 18–24 h, after which colonies were counted and expressed as colony forming units (CFU) per milliliter (ml). Colonies resembling *E. coli* and *K. pneumoniae* were sub-cultured in nutrient agar and the isolates were identified using conventional microbiological techniques.

2.2 Antibiotics Susceptibility Testing

The antibiotic susceptibility pattern of the isolates was determined using the Kirby-Bauer Disk diffusion method on Mueller Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2012). Susceptibility was tested against ertapenem, imipenem, meropenem, amoxicillin/ clavulanic acid, ceftazidime, and cefuroxime (Oxoid, England). *E. coli* ATCC 25922 was included as a reference strain. Isolates found to have an ertapenem, meropenem or imipenem ≤22mm inhibition zone diameter were classified as carbapenem-resistant isolates.

III. RESULTS

3.1 Prevalence of Bacterial Isolates

In this study, 269 wastewater samples from Owerri Nigeria were processed for carbapenem-resistant *E. coli* and *K. pneumoniae* isolation. The samples included 135 hospital wastewater and 134 slaughterhouse wastewater respectively. In all, a total of 193 bacteria comprising of consecutive 92 (46.7%) *E. coli* and 101 (52.3%) *K. pneumoniae* was isolated. One bacteria isolate was selected from one wastewater sample and one sample;

either hospital or slaughterhouse wastewater was collected and not both. Sample collection was based on availability and we could not compare isolation rate from hospital or slaughterhouse wastewater The distribution of the 92 *E. coli* isolates according to sample sources showed that 59 (64.1%) came from hospital wastewater while 33 (35.9%) came from slaughterhouse wastewater Similarly, 61.4% (62/101) of the 101 *K. pneumoniae* isolates were from hospital wastewater while 38.6% (39/101) of the isolates came from slaughterhouse wastewater respectively Table 1.

Table 1: The Overall Growth and Distribution of *Escherichia Coli* and *Klebsiella Pneumoniae* Within the Different Wastewater Sampled

Sample Source	Total number of Samples	Escherichia Coli	Klebsiella Pneumoniae	Total
Hospital Wastewater	135	59 (64.1%)	33(35.9%)	92(46.7%)
Slaughterhouse Wastewater	134	62 (61.4%)	39 (38.6%)	101(52.3%)
Total	269	121(62.7%)	72(37.3%)	193(71.7%)

3.2 Antimicrobial Susceptibility Testing

As shown in Table 2, high antibiotic resistance rates were observed in the isolates, no isolate was susceptible to all the antibiotics tested. Among the carbapenems, moderate to high resistance rate was observed; ranging from 22.8% in imipenem to 30.6% in ertapenem. As expected, very high resistance rate was also observed in cephalosporins including 55.9% in cefuroxime and 58.0% in ceftazidime respectively. Amoxicillin clavulanic acid, an antibiotic used for phenotypic detection ESBL production was considerably inactive against the isolates with a 41.9% resistance rate.

There was no statistically significant difference in carbapenem resistance rates between the hospital and slaughterhouse wastewater isolates ($P > 0.05$).

Table 2: Antibiotics Susceptibility Patterns of *Escherichia Coli* and *Klebsiella Pneumoniae* Isolates against the 6 Antibiotics Tested

Antibiotics	Number of Resistance	Number of E-Coli	Number of K. Pneumoniae,
Imipenem	44/193(22.8%)	32/44 (72.7%)	12/44(27.3%)
Meropenem	56/193(29.1%)	27/44(48.2%)	29/44(51.8%)
Ertapenem	59/193(30.6%)	39/44(66.1%)	20/44(33.9%)
Amoxicillin clavulanic acid	81/193(41.9%)	37/44(45.7%)	44/44(54.3%)
Cefuroxime	108/193(55.9%)	51/44(47.2%)	57/44(52.8%)
Ceftazidime	112/193(58.0%)	58/44(51.8%)	54/44(48.2%)

IV. DISCUSSION

Antibiotics resistance profiling of pathogens helps to identify the emergence of rare or new resistance threats and prioritize possible actions to be taken against them. The analysis of wastewater can reveal the circulation of antibiotic resistant bacteria and antibiotics resistance genes among the catchment communities. In this study, we carried out untreated wastewater surveillance for carbapenem resistance traits among *E. coli* and *K. pneumoniae*. Our results indicate that carbapenem-resistant *E. coli* and *K. pneumoniae* are widely distributed among the wastewater sources. The isolates expressed highest resistance to ertapenem (30.6%) while the least resistance was observed in imipenem with 22.8% resistance rate. To our knowledge, these are the earliest carbapenem-resistant *E. coli* and *K. pneumoniae* detected in non-clinical samples in Owerri southeast Nigeria. In agreement with our result, a study conducted in 2020 also reported carbapenem-resistant traits as the most abundant resistant traits in hospital wastewater in Helsinki (Majlander *et al.,* 2021). However, their study included genotypic characterization while ours was based on only phenotypic characterization due to limited resources. Another study from the Netherlands, based on molecular characterization reported carbapenem-resistant genes from both hospital and municipal wastewater, but the abundance in hospital wastewater was higher than in the municipal wastewater (Buelow et al., 2018). As untreated wastewater is considered to provide a glimpse of the antibiotic resistant bacteria and antibiotic resistance genes circulating in a community, our findings indicate that ertapenem resistant *E. coli* and *K. pneumoniae* was the most prevalent carbapenem-resistant trait in Owerri during the study period.

The World Health Organization (WHO) has classified carbapenem-resistant *Enterobacterales* as a critical priority, driving the need to develop new antibiotics (Davies and Simeon, 2017). *E. coli* and *K. pneumoniae* isolates identified as carbapenem-resistant in this study have been described in the environment and reported to be potential clinical pathogens (Li et al., 2019).

Perhaps, some carbapenemase genes are related to aquatic environments and only occasionally cause infection. Many of these bacterial species could be asymptomatically carried by humans in their intestines, and thus be found in wastewater.

Clinical isolates can differ from wastewater-based isolates. The clinical isolates are pathogenic, but wastewater-based isolates better represent symbiotic and normal human bacteria. The presence of high concentrations of carbapenem resistant *E. coli* and *K. pneumoniae* isolates in both the hospital and slaughterhouse wastewater samples is not only a reflection of the increasing use of carbapenems in Nigeria, but also an additional evidence regarding the inefficiency of the conventionally applied wastewater treatments in the elimination of these microorganisms from the treated effluents, subsequently leading to their release into the environment (Oliveira et al., 2020).

V. CONCLUSION

The analysis of the antibiotic resistance phenotypes of the potentially pathogenic carbapenem resistant *E. coli* and *K. pneumoniae* isolated from hospital and slaughterhouse wastewater samples revealed that all were multidrug resistance, showing resistance phenotypes to more than three antibiotic classes. The findings of this study, together with the clinical reports from the previous studies indicate a steady increase in carbapenem resistant bacteria in Nigeria and support wastewater surveillance as a potential preparedness tool for antimicrobial resistance surveillance. Wastewater surveillance could supplement the clinical surveillance approach in monitoring the possible circulation of antimicrobial resistance in communities and shows potential as a public health tool. Future studies could cover larger geographical areas of Nigeria and include molecular tools to obtain a more representative national picture. The detection of carbapenem resistant *E. coli* and *K. pneumoniae* in the two wastewater samples underlines the importance of proper wastewater treatment to avoid the dissemination of antimicrobial resistant bacteria in the environment and reduce public health risks.

REFERENCES

1. Buelow, E., Bayjanov, J. R., Majoor, E., Willems, R. J. L., Bonten, M. J. M., Schmitt, H., et al. (2018). Limited influence of hospital wastewater on the microbiome and resistome of wastewater in a community sewerage system. FEMS Microbiol. Ecol. 94, 1–9. doi: 10.1093/femsec/fiy087.
2. Chandran SP, Diwan V, Tamhankar AJ, Joseph BV, Rosalesklintz S, Mundayor S, Lundborg CS and Macaden R (2014). Detection of carbapenem resistance genes and cephalosporin, and quinolone resistance genes along with oqxAB gene in Escherichia coli in hospital wastewater: a matter of concern. J. Appl. Microbiol. 117 (4) 984–995. https://doi.org/10.1111/jam.12591.
3. Clinical and Laboratory Standards Institute. (2012). Performance Standards for Anti-microbial Susceptibility Testing: Twenty-second Informational Supplement M100-S22. CLSI, Wayne, PA, USA.
4. Davies, O. L., and Simeon, B. (2017). WHO Publishes List of Bacteria for which New Antibiotics Are Urgently Needed, WHO. WHO publishes list of bacteria for which new antibiotics are urgently needed.
5. Hrenovic J, Ganjto M and Goicbarisic I (2017). Carbapenem-resistant bacteria in a

secondary wastewater treatment plant. Water SA 43 (2) 186–191. https://doi.org/10.4314/wsa.v43i2.02
6. Li, Y., Luo, L., Xiao, Z., Wang, G., Li, C., Zhang, Z., et al. (2019). Characterization of a Carbapenem-resistant Kluyvera Cryocrescens isolates carrying Blandm-1 from hospital sewage. Antibiotics 8:149. doi: 10.3390/antibiotics8030149.
7. Majlander, J., Anttila, V. J., Nurmi, W., Seppälä, A., Tiedje, J., and Muziasari, W. (2021). Routine wastewater-based monitoring of antibiotic resistance in two Finnish hospitals: focus on carbapenem resistance genes and genes associated with bacteria causing hospital-acquired infections. J. Hosp. Infect. 117, 157–164. Doi: 10.1016/j.jhin.2021.09.008.
8. Meletis G (2016) Carbapenem resistance: overview of the problem and future perspectives. Ther. Adv. Infect. Dis. 3 (1) 15–21. https://doi.org/10.1177/20499361156-21709.
9. Obasi A, Nwachukwu SC, Ugoji E, Kholer C, Gho¨ ler A, Balau V (2017). Extended-spectrum β-lactamase- producing *Klebsiella pneumoniae* from pharmaceutical waste waters in southwestern Nigeria. Microb Drug Resist. 23(8): 1013–1018. https://doi.org/10.1089/mdr.2016.0269 PMID: 28375698.
10. Obayiuwana A and Ibekwe AM. (2020). Antibiotic Resistance Genes Occurrence in Wastewaters from Selected Pharmaceutical Facilities in Nigeria. Water 2020, 12, 1897; doi: 10.3390/w12071897.
11. Oliveira, M.; Leonardo, I. C.; Nunes, M.; Silva, A. F.; Barreto Crespo, M. T. (2021). Environmental and Pathogenic Carbapenem Resistant Bacteria Isolated from a Wastewater Treatment Plant Harbour Distinct Antibiotic Resistance Mechanisms. Antibiotics 2021, 10, 1118. https://doi.org/10.3390/antibiotics10091118.
12. Oliveira, M.; Nunes, M.; Barreto Crespo, M. T.; Silva, A. F. (2020). The environmental contribution to the dissemination of carbapenem and (fluoro) quinolone resistance genes by discharged and reused wastewater effluents: The role of cellular and extracellular DNA. Water Res. 182, 116011.
13. Seruga Music M, Hrenovic J, Goibarisic I, Hunjak B, Skoric D and Ivanovic T (2017). Emission of extensively-drug-resistant Acinetobacter baumannii from hospital settings to the natural environment. J. Hosp. Infect. 96 (4) 323–327. https://doi.org/10.1016/j.jhin.2017.04.005.
14. Tiwari A, Paakkanen J, Österblad M, Kirveskari J, Hendriksen RS and Heikinheimo A (2022) Wastewater Surveillance Detected Carbapenemase Enzymes in Clinically Relevant Gram-Negative Bacteria in Helsinki, Finland; 2011–2012. Front. Microbiol.13: 887888. doi: 10.3389/fmicb.2022.887888.
15. WHO (World Health Organization) (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. URL: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1
16. Zhang C, Qiu S, Wang Y, Qi L, Hao R, Liu X, Shi Y, Hu X, An D, Li Z, Li P, Wang L, Cui J, Wang P, Huang L, Klena JD and Song H (2013). Higher isolation of NDM-1 producing Acinetobacter baumannii from the sewage of the hospitals in Beijing. PLoS ONE 8 (6) e64857. https://doi.org/10.1371/journal.pone.0064857.