International Journal of Advanced Health Science and Technology
Multidisciplinary : Rapid Review : Open Access Journal
eISSN: 2808-6422: p-ISSN:3037

RESEARCH ARTICLE
OPEN ACCESS

Manuscript received February 18, 2025; accepted April 20, 2025; date of publication April 27, 2025
Digital Object Identifier (**DOI**): https://doi.org/10.35882/ijahst.v5i2.442

How to cite: Daniel Tunde Abbey, Adamu Hafsa Abdullahi, "Persistence, Prevalence and Antibiotic Susceptibility of Pseudomonas aeruginosa Isolated from Hospital Wastewater", International Journal of Advanced Health Science and Technology, vol. 5, no. 2, pp. 33-39, April 2025

Persistence, Prevalence and Antibiotic Susceptibility of *Pseudomonas aeruginosa* Isolated from Hospital Wastewater

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Acknowledgement: Our profound gratitude goes to our supervisor Dr. Peter G. Shiaka and also extend our sincere thanks to the Head of Department, and the entire staff of the Microbiology Department at Federal University Dutse. Their dedication and assistance have been instrumental in the completion of this research. Special thanks to Mr. Hanson Etukudor and the entire technical staff, for their technical support and assistance in the laboratory. Their expertise and patience were crucial in conducting the experiments and analyses.

ABSTRACT The increasing prevalence of antibiotic-resistant bacteria in hospital wastewater poses significant public health risks. This study investigates the persistence, prevalence, and antibiotic susceptibility of *Pseudomonas aeruginosa* in hospital wastewater collected from three healthcare facilities in Dutse, Jigawa State, Nigeria: Rasheed Shekoni Teaching Hospital, Dr. Sambo Hospital, and Dutse General Hospital. Wastewater samples were analyzed for bacterial isolation, characterization, and antibiotic susceptibility. Wastewater samples were analyzed for bacterial isolation, characterization, and antibiotic susceptibility. Results showed that *P. aeruginosa* was just present everywhere in samples collected from Dr. Sambo Hospital and Dutse General Hospital. But at Rasheed Shekoni Teaching Hospital prevalence was a bit lower, at 60%. Physiochemical analysis showed that P. aeruginosa exhibited optimal growth at 37°C with pH variations influencing its persistence. The bacterium sticks around for up to 72 hours floating around in wastewater and hospital water runoffs. Yes, this shows just how tough this microbe can be. Antibiotic susceptibility testing indicated high resistance to multiple antibiotics, with no zones of inhibition observed for several drugs, including ampicillin, streptomycin, and nalidixic acid. However, some isolates showed susceptibility to ciprofloxacin and gentamycin. The findings underscore the urgent need for improved hospital wastewater treatment to mitigate the spread of antibiotic-resistant bacteria in the environment. Future scientific research should dive into the mechanisms at the molecular level that drive resistance from microorganisms found in wastewater from hospitals.

Keywords: *Pseudomonas aeruginosa,* hospital wastewater, antibiotic resistance, prevalence, persistence, public health.

1. INTRODUCTION

Hospital wastewater is a mixture of toxic liquid waste produced from the medical and clinical area which usually carry biological, chemical and pharmaceutical pollutants [1,2]. Hospital wastewater is another major concern with respect to presence of antibiotic-resistant bacteria, potentially creating a substantial public health risk. Because hospitals are important centers for antibiotic use, bacteria in hospital wastewater often are chronically exposed to these antibiotics and thereby have a high rate of acquiring and disseminating antibiotic resistance genes to other pathogens [3,4,5]. After the release into the environment, these pathogens can be found in water sources, and humans can be exposed to resistant infections if wastewaters are not sufficiently treated [6,7]. Among bacterial pathogens in hospital wastewater, *P. aeruginosa* is of special concern. *P. aeruginosa* is a Gram-negative, rod (rod-shaped) bacterium that is capable of surviving in wet areas and drug-resistance to a variety of antibiotics ranging from broad-spectrum to narrow-spectrum types [8,9,10] The adaptive mechanisms, e.g., biofilm formation and efflux pump activity, play a role to resist its eradication in hospital wastewater. *P. aeruginosa* causes severe infections, especially in people with weak immune systems or long-term illnesses. [11,12,13]. This bacterium can be found practically anywhere—soil, water and plants with hospitals being no exception too, where it can lead to many infections[14]. As a developing country, Nigeria has to contend with many challenges in the treatment of hospital wastewater owing to the lack of treatment infrastructure. Many hospitals, especially in rural and underserved areas like Jigawa State, lack proper wastewater treatment systems [15] Due to this fact, highly transmissible, untreated effluents are frequently discharged directly into the environment, both raising the chance of waterborne disease transmission and the transmission of antimicrobial resistance. [16,17,18]. The lack of infrastructure for wastewater treatment further exacerbates this issue, allowing resistant bacteria like P. aeruginosa to thrive and spread within communities [19,20] Persistence and prevalence of *P. aeruginosa* in wastewater from hospital environments is one of the main issues in infection control [21,22]. The bacterium's resistance to treatment of effluents allows it to act as a reservoir of antibiotic resistance, which may result in increasingly resistant bacterial populations that compromise hospital acquired infection treatment [23] Although there has been an increasing worldwide interest in antimicrobial resistance, few studies in developing countries have been concerned with the microbial contamination of hospital wastewater. [24]

This study investigates the prevalence and persistence of *P. aeruginosa* in hospital wastewater from three healthcare facilities in Jigawa State, Nigeria: Rasheed Shekoni Teaching Hospital, General Hospital, Dutse, Dr. Sambo Hospital. Through the analysis of *P. aeruginosa* in hospital wastewater, this work seeks to illustrate the contribution of hospital effluent to distribution of resistant isolates. These results will deliver empirical evidence to inform public health policies and programmes, enhance areas of infection control and enhance wastewater management practices in healthcare settings.

2. MATERIALS AND METHODS

A. STUDY AREA AND HOSPITAL SELECTION

The research was conducted in the Microbiology Laboratory of Federal University Dutse. The study area is Dutse, the capital of Jigawa State, in the North-Western region of Nigeria. Dutse is home to several healthcare facilities, notably Dutse General Hospital, Rasheed Shekoni Teaching Hospital, and Dr. Sambo Hospital. Hospitals were selected based on their size, patient turnover, and the volume of wastewater generated. The chosen hospitals for sample collection were Rasheed Shekoni Teaching Hospital (RS), Dutse General Hospital (DG), and Dr. Sambo Hospital (DS), all located within Dutse metropolis, Jigawa State, Nigeria.

B. *SAMPLE COLLECTION*

Prior to sample collection, necessary permissions and ethical approval were obtained from the appropriate authorities and hospital management. Wastewater discharge points in each hospital were mapped to identify optimal sampling locations. Wastewater samples were collected in triplicates from designated points: hospital effluent discharge points, internal sewage lines, and outflow points where hospital wastewater enters the public sewage system. Sampling was conducted in the morning (7:00–9:00 AM), and evening (5:00–7:00 PM). Each sample consisted of 25 ml of wastewater collected using sterile glass bottles. To maintain microbial integrity, samples were placed in sterile containers, labeled with specific information (location, date, and time of collection), and stored in a cool box containing ice blocks. The collected samples were transported to the laboratory within 2–4 hours following standard microbiological transport protocols to minimize microbial alterations.

C. MEDIA PREPARATION

Cetrimide agar, Mueller-Hinton agar, and peptone water broth were prepared according to the manufacturer's instructions. The compositions of the media included: Cetrimide Agar (selective for *P. aeruginosa*) which is composed of peptone, magnesium chloride, potassium sulfate, and cetrimide, which inhibit the growth of competing organisms. Mueller-Hinton Agar (used for antibiotic susceptibility testing) is composed of beef infusion, casein hydrolysate, and starch, providing a non-selective and differential medium. Peptone Water Broth (used for bacterial enrichment) is composed of peptone and sodium chloride to support bacterial growth. All media were sterilized using autoclaving at 121°C and 15 psi for 15 minutes. Quality control was ensured by checking for sterility through negative controls (un-inoculated plates) and performance testing using reference bacterial strains.

D. ISOLATION AND IDENTIFICATION OF PSEUDOMONAS AERUGINOSA

Upon arrival at the laboratory, wastewater samples were homogenized by shaking to ensure even bacterial distribution. Samples were inoculated using the pour plate technique, where 1 ml of wastewater was transferred onto sterile Petri dishes, followed by the addition of molten cetrimide agar. Plates were allowed to solidify, inverted, and incubated at 37°C for 24–48 hours.

After incubation, plates were examined for colonies exhibiting characteristic *P. aeruginosa* features, such as green pigmentation due to pyoverdine production and a distinct grape-like odor. Suspected colonies were subjected to Gram staining (confirming Gram-negative rods) and biochemical tests, including oxidase and catalase tests, for further confirmation.

E. PERSISTENCE OF PSEUDOMONAS AERUGINOSA

The persistence of *P. aeruginosa* was evaluated by inoculating isolates into peptone water broth under different environmental conditions. Bacterial cultures were incubated at 25°C, 37°C, and 40°C to simulate various environmental conditions. Growth was monitored by measuring bacterial counts at intervals of 0, 24, 48, and 72 hours. To assess bacterial survival, samples were plated on cetrimide agar at each time point, and colony-forming units (CFU/ml) were recorded. Additional controlled parameters included aeration (shaking at 120 rpm) and nutrient concentration (standardized peptone broth composition) to reflect real-world persistence conditions.

F. *ANTIBIOTIC SUSCEPTIBILITY TESTING*

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. A 0.5 McFarland standard turbidity solution was prepared, corresponding to a bacterial concentration of 1.5×10^8 CFU/ml. Standardized inoculum from 16–18-hour-old *P. aeruginosa* cultures were swabbed onto Mueller-Hinton agar following Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic disks tested included ciprofloxacin, septrin, streptomycin, ampicillin, ceporex, tarvid, nalidixic, reflacine, gentamycin, augmentin. These antibiotics were selected based on their clinical relevance in treating *P. aeruginosa* infections and resistance patterns observed in previous studies [26]. Using sterile forceps, antibiotic disks were placed on inoculated plates, which were then inverted and incubated at 37°C for 18–24 hours. Zones of inhibition were measured in millimeters and categorized as susceptible, intermediate, or resistant based on CLSI breakpoints.

3. RESULT

The results from this study provide comprehensive data on the identification, prevalence, persistence, and antibiotic susceptibility of Pseudomonas aeruginosa isolated from hospital wastewater samples across three locations: Rasheed Shekoni Teaching Hospital (RS), Dr. Sambo Hospital (DS), and Dutse General Hospital (DG). In Table 1, all isolates across the three hospitals displayed characteristic features of P. aeruginosa, with colonies described as smooth, shiny or opaque, and greenish-yellow in color some with a distinctive grape-like odor. All isolates were Gram-negative rods, catalase-positive, and oxidase-positive, confirming their identity as P. aeruginosa. Notably, sample IDs such as RS3, DS1, DS2, DG1, and others consistently met these criteria, reinforcing the bacterium's commonality in hospital effluents (TABLE 1). The prevalence data presented in Table 2 showed that P. aeruginosa was detected in 60% of samples from RS, while DS and DG had a 100% prevalence rate, indicating that these two hospitals discharge wastewater highly contaminated with this pathogen, posing potential public health and environmental threats. In examining the persistence of P. aeruginosa in different temperature conditions (Table 3 and 3b), it was evident that the bacterium exhibited notable resilience, particularly at higher temperatures (37°C and 40°C), where growth was generally more sustained and intense. For instance, in RS3 and DS1 samples, moderate to heavy bacterial growth was observed at 37°C and 40°C after 48 hours, while growth at 25°C was often minimal or absent after the same duration. This thermotolerance suggests that P. aeruginosa can survive and

TABLE 1
Identification of *Pseudomonas aeruginosa*

Sample ID	Morphology	Gram reaction	Catalase	Oxidase	Inference
RS3	Smooth, shiny, greenish-yellow	-	+	+	*P. aeruginosa*
DS1	Smooth, opaque, greenish-yellow	-	+	+	"
DS2	Smooth, opaque, greenish-yellow	-	+	+	"
DS3	Smooth, shiny, opaque, greenish-yellow	-	+	+	"
DG1	Smooth, shiny, greenish-yellow	-	+	+	"
DG2	Smooth, opaque, greenish-yellow	-	+	+	"
DG3	Smooth, shiny, greenish-yellow	-	+	+	"
RS1	Opaque, shiny, greenish-yellow	-	+	+	"
RS2	Opaque, grape-like odor, greenish-yellow	-	+	+	"
DS1	Smooth, opaque, greenish-yellow	-	+	+	"
DS2	Smooth, shiny, greenish-yellow	-	+	+	"
DG1	Smooth, opaque, greenish-yellow	-	+	+	"
DG2	Opaque, grape-like odor, greenish-yellow	-	+	+	"

Keys: RS=Rasheed Shekoni Hospital, DS= Dr Sambo Hospital, DG= Dutse General Hospital, P= *Pseudomonas*, Negative= -, Positive=

TABLE 2
Prevalence of *P. aeruginosa* in each hospital wastewater

Hospital	Total Samples Collected	Positive Samples	Prevalence (%)
RS	5	3	60
DS	5	5	100
DG	5	5	100

Keys: RS= Rasheed Shekoni Teaching Hospital, DS= Dr. Sambo, DG= Dutse General Hospital

TABLE 3
Persistence of *Pseudomonas aeruginosa* in Sample 1

Sample ID	Time Interval (hours)	25°C	37°C	40°C
RS3	0	−	−	−
	24	+	++	++
	48	−	+++	+++
	72	−	−	++
DS1	0	−	−	−
	24	+	+	++
	48	+	++	+++
	72	−	−	++
DS2	0	−	−	−
	24	++	+	+++
	48	+++	++	++
	72	+	+	+
DS3	0	−	−	−
	24	++	++	+
	48	+	+	+
	72	−	−	−
DG1	0	−	−	−
	24	++	++	++
	48	+	+++	+++
	72	+	−	+
DG2	0	−	−	−
	24	++	++	+++
	48	+	+	++
	72	+	−	+
DG3	0	−	−	−
	24	++	++	++
	48	+++	+	+++
	72hours	+	−	−

Keys: No growth =(−), Scanty growth =(+), Moderate growth = (++), Heavy growth =(+++)

proliferate in warmer conditions typically found in tropical climates, further complicating efforts to control its spread. Samples like DG1 and DS2 remained viable for up to 72 hours at elevated temperatures, indicating strong persistence capabilities. Meanwhile, Table 4 details the antibiotic susceptibility profile of the isolates, revealing significant resistance patterns. Many isolates showed no zones of inhibition against multiple antibiotics, including commonly used ones like streptomycin (S), ampicillin (PN), and nalidixic acid (NA). Ciprofloxacin (CPX), gentamycin (CN), and augmentin (AU) displayed moderate effectiveness in some samples, such as RS3 and DG2, suggesting partial susceptibility. DS1, DS2, and DS3 particularly highlighted strong resistance, with DS1 showing inhibition only to ampicillin and augmentin, and DS2 presenting very limited susceptibility across all tested antibiotics (TABLE 2). Notably, several samples recorded zero response to nearly all antibiotics tested, raising alarms about multidrug resistance among these environmental isolates. The variation in susceptibility, with some isolates like RS3 and DG2 reacting to a broader range of antibiotics, may suggest different levels of exposure or antibiotic pressure in the respective hospitals (TABLE 3 and TABLE 4). Overall, the study demonstrates that P. aeruginosa is not only prevalent in hospital wastewater but also exhibits remarkable persistence in various environmental conditions and a worrying resistance to multiple antibiotics. These findings underscore the urgent need for effective wastewater treatment strategies and antimicrobial stewardship in healthcare facilities to curb the environmental dissemination of this opportunistic pathogen and to prevent potential outbreaks caused by resistant strains in community settings.

4. DISCUSSION

This study highlights the significant presence, persistence, and antibiotic resistance of *Pseudomonas aeruginosa* in hospital

TABLE 4
Persistence of *Pseudomonas aeruginosa* in Sample 2

Sample ID	Time Interval (hours)	25°C	37°C	40°C
RS1	0	−	−	−
	24	++	++	++
	48	+	+	+
	72	−	−	−
RS2	0	−	−	−
	24	++	+	+
	48	+	++	++
	72	−	−	−
DS1	0	−	−	−
	24	++	++	+
	48	+	+	++
	72	−	−	−
DS2	0	−	−	−
	24	++	++	++
	48	+	+	+
	72	−	−	−
DG1	0	−	−	−
	24	+	++	+
	48	+++	+++	+
	72	++	++	−
DG2	0	−	−	−
	24	++	++	++
	48	+++	+	+
	72	−	−	−

Keys: No growth =(−), Scanty growth =(+), Moderate growth = (++), Heavy growth =(+++)

wastewater within Dutse metropolis Nigeria. The findings emphasize the potential risks associated with untreated hospital effluents, particularly in resource-limited settings, where wastewater treatment infrastructure is inadequate. The results revealed that *P. aeruginosa* was present in all three hospitals sampled, with a 100% prevalence in Dr. Sambo Hospital (DS) and Dutse General Hospital (DG), while Rasheed Shekoni Teaching Hospital (RS) had a slightly lower prevalence of 60%. The widespread presence of *P. aeruginosa* aligns with previous studies conducted in Nigeria, such as [25], who reported a similarly high prevalence of this pathogen in hospital wastewater, underscoring its ability to thrive in such environments. Additionally, [26] identified *P. aeruginosa* as one of the dominant multidrug-resistant pathogens in wastewater treatment plants, reinforcing its persistence in untreated hospital effluents. In contrast, [27] found a comparatively lower prevalence of *P. aeruginosa* in hospital wastewater in China. This disparity may be attributed to more advanced wastewater treatment systems in developed regions, which effectively reduce bacterial loads before effluent discharge. The results highlight the need for improved wastewater management practices in Nigerian hospitals to minimize environmental contamination and prevent the potential spread of antibiotic-resistant bacteria into community water sources. The ability of *P. aeruginosa* to persist in wastewater environments for up to 72 hours, with optimal growth observed at 37°C and notable survival at 25°C and 40°C, further underscores its resilience. These findings are consistent with the study by [28], who demonstrated that *P. aeruginosa* can survive under diverse environmental conditions, making it a formidable opportunistic pathogen in hospital settings. The presence of organic nutrients, antibiotic residues, and disinfectants in hospital wastewater may further contribute to its prolonged survival and adaptability. The environmental resilience of *P. aeruginosa* suggests that hospital wastewater could serve as a continuous source of infection, potentially contributing to nosocomial outbreaks and community-acquired infections. The findings highlight the need for targeted interventions, including the development of effective disinfection protocols to limit bacterial persistence in hospital effluents before their release into the environment (TABLE 5).

Antibiotic susceptibility testing revealed high resistance of *P. aeruginosa* isolates to multiple antibiotics, with no zones of inhibition recorded for commonly used drugs such as streptomycin, ampicillin, and nalidixic acid. However, some

TABLE 5
Antibiotic susceptibility of *P. aeruginosa* in mm

Sample ID	CPX	SXT	S	PN	CEP	OFX	NA	PEF	CN	AU
RS3	16	18	-	-	17	16	18	17	17	15
DS1	-	-	-	14	-	-	-	-	-	13
DS2	-	-	-	-	11	12	-	13	-	-
DS3	-	-	18	-	-	-	-	13	-	-
DG1	15	-	-	-	16	-	9	-	-	16
DG2	-	15	-	12	-	-	-	-	-	-
DG3	-	-	7	-	11	-	9	-	15	-
RS1	16	-	-	-	-	-	-	-	-	-
RS2	-	14	-	-	13	-	12	11	8	10
DS1	-	-	-	-	-	-	-	-	-	-
DS2	14	-	12	8	9	-	-	-	-	14
DG1	-	14	-	-	-	-	18	-	13	-
DG2	13	14	11	11	-	14	-	-	15	13

Keys: (CPX)=Ciprofloxacin, (SXT)=Septrin, (S)=Streptomycin, (PN)=Ampicillin, (CEP)=Ceporex, (OFX)=Tarvid, (NA)=Nalidixic, (PEF)=Reflacine, (CN)=Gentamycin, (AU)=Augmentin, (-)= No Zone of Inhibition

susceptibility was observed for ciprofloxacin, gentamicin, and cephalosporins. These resistance patterns are in agreement with findings from [26], who reported high levels of multidrug resistance in *P. aeruginosa* isolates from wastewater treatment plants. Similarly, [30] observed that *P. aeruginosa* strains from hospital effluents exhibited significant resistance to beta-lactam and aminoglycoside antibiotics, raising concerns about the effectiveness of these treatment options in clinical settings. The high resistance levels observed in this study highlight the growing threat of antimicrobial resistance (AMR), which is exacerbated by the discharge of untreated hospital wastewater into the environment. Hospital effluents containing antibiotic residues create selective pressure that favors the survival of resistant bacterial strains, increasing the risk of AMR dissemination. [13] emphasized that hospital wastewater can act as a reservoir for resistant pathogens, facilitating their transmission to natural water bodies and posing a significant public health risk. The presence of multidrug-resistant *P. aeruginosa* in hospital wastewater has serious public health implications. If left untreated, these effluents can contaminate water sources used for domestic and agricultural purposes, leading to the spread of resistant pathogens in the community. Previous studies, such as [25], have emphasized the need for urgent interventions to address the growing problem of antibiotic resistance linked to hospital wastewater discharge.

5. CONCLUSION

The findings of this study along with previous research reinforce the critical need for improved wastewater treatment infrastructure in Nigerian hospitals to curb the spread of *P. aeruginosa* and other antibiotic-resistant pathogens. The high prevalence, persistence, and resistance of *P. aeruginosa* in hospital wastewater pose significant challenges that require immediate attention from public health authorities, researchers, and policymakers. Future research should focus on exploring novel treatment approaches for hospital effluents and evaluating the effectiveness of existing disinfection strategies in reducing bacterial contamination. Without urgent intervention, the continued release of resistant pathogens into the environment could contribute to an escalating AMR crisis, threatening global health security.

REFERENCES

[1] Mena, K. D., and Gerba, C. P. Risk assessment of *Pseudomonas aeruginosa* in water. *Rev. Environ. Contam. Toxicol.* 201: 71-115. 2009 DOI: 10.1007/978-0-387-96259-6_3
[2] Chonova T., Keck F., Labanowski J., Montuelle B., Rimet F., Bouchez A. Separate treatment of hospital and urban wastewaters: a real scale comparison of effluents and their effect on microbial communities. *Sci. Total Environ.*; 542:965–975. 2016 DOI: 10.1016/j.scitotenv.2015.10.150
[3] Hu, Y., Zhu, Y., and Niu, Z. *Pseudomonas aeruginosa* in hospital wastewater: A review on survival, prevalence, and resistance. *Environ.*

Sci. Pollut. Res. 27(6): 5565–5578. 2020 DOI: 10.1007/s11356-019-07104-5

[4] Perez, R. E., Garcia, M. C., and Villanueva, R. R. Antibiotic resistance in *Pseudomonas aeruginosa* from hospital wastewater. *J. Water Health* 21(3):201-215. 2023 DOI: 10.2166/wh.2023.010

[5] Obasi A.I., Amaeze N.H., Osoko D.D. Microbiological and toxicological assessment of pharmaceutical wastewater from the Lagos Megacity, Nigeria. *Chinese J. Biol.*; 20-24. 2014

[6] Cydzik-Kwiatkowska A., Zielińska M. Bacterial communities in full-scale wastewater treatment systems. *World J. Microbiol. Biotechnol.*; 32(4):66. 2016 DOI: 10.1007/s11274-016-2006-5

[7] Bäumlisberger M., Youssar L., Schilhabel M.B., Jonas D. Influence of a non-hospital medical care facility on antimicrobial resistance in wastewater. *PLoS One.* 10(3): e0122635. 2015 DOI: 10.1371/journal.pone.0122635

[8] Korzeniewska E., Harnisz M. Beta-lactamase-producing *Enterobacteriaceae* in hospital effluents. *J. Environ. Manage.*; 123:1–7. 2013 DOI: 10.1016/j.jenvman.2013.03.025

[9] Tiwari B., Sellamuthu B., Ouarda Y., Drogui P., Tyagi R.D., Buelna G. Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. *Bioresour. Technol.* 224:1–12. 2017 DOI: 10.1016/j.biortech.2016.11.020

[10] Asfaw T., Negash L., Kahsay A., Weldu Y. Antibiotic-resistant bacteria from treated and untreated hospital wastewater at Ayder Referral Hospital, Mekelle, North Ethiopia. *Adv. Microbiol*; 7(12):871. 2017 DOI: 10.4236/aim.2017.712064

[11] Resende A.C.B., Soares R., de Bastos A., dos Santos D., Montalvão E.R., do Carmo Filho J. Detection of antimicrobial-resistant Gram-negative bacteria in hospital effluents and in the sewage treatment station of Goiânia, Brazil. *O Mundo da Saúde* 33(4):385–391. 2009

[12] Huang, J. J., Hu, H. Y., Tang F., Li, Y., Lu, S. Q., and Lu, Y. Monitoring and evaluation of antibiotic-resistant bacteria and their genes in wastewater treatment systems: a review. *Sci. Total Environ.* 731, 138939. 2019 DOI: 10.1016/j.scitotenv.2020.138939

[13] Brown-Jaque M., Calero-Cáceres W., Muniesa M. Transfer of antibiotic-resistance genes via phage-related mobile elements. *Plasmid*; 79:1–7. 2015 DOI: 10.1016/j.plasmid.2015.01.001

[14] Buelow E., Bayjanov J.R., Majoor E., Willems R.J., Bonten M.J., Schmitt H. Limited influence of hospital wastewater on the microbiome and resistome of wastewater in a community sewerage system. *FEMS Microbiol. Ecol.* 94(7): fiy087. 2018 DOI: 10.1093/femsec/fiy087

[15] Akin, B.S. Contaminant properties of hospital clinical laboratory wastewater: a physiochemical and microbiological assessment. *Journal of Environmental Protection*, 7(05), 635. 2016 . DOI: 10.4236/jep.2016.75057

[16] Buelow, E., Bayjanov, J.R., Willems, R.J., Bonten, M.J., Schmitt, H., & Van Schaik, W. The microbiome and resistome of hospital sewage during passage through the community sewer system. *bioRxiv*, 216242. 2017.

[17] Chagas, T., Seki, L., Cury, J., Oliveira, J., Dávila, A., & Silva, D. Multiresistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro, Brazil. *Journal of Applied Microbiology*, 111(3), 572–581. 2011.

[18] Ibrahim, C., Hassen, A., Pothier, P., Mejri, S., & Hammami, S. Molecular detection and genotypic characterization of enteric adenoviruses in a hospital wastewater. *Environmental Science and Pollution Research International*, 25(11). 2018.

[19] Kizny Gordon, A.E., Mathers, A.J., Cheong, E.Y., Gottlieb, T., Kotay, S., & Walker, A.S. The hospital water environment as a reservoir for carbapenem-resistant organisms causing hospital-acquired infections—a systematic review of the literature. *Clinical Infectious Diseases*, 64(10), 1435–1444. 2014.

[20] Lamba, M., Graham, D.W., & Ahammad, S.Z. Hospital wastewater releases of carbapenem-resistance pathogens and genes in urban India. *Environmental Science & Technology*, 51(23). 2017.

[21] Li, D., Qi, R., Yang, M., Zhang, Y., & Yu, T. Bacterial community characteristics under long-term antibiotic selection pressures. *Water Research*, 45(18). 2011.

[22] Nunez, L., & Moretton, J. Disinfectant-resistant bacteria in Buenos Aires city hospital wastewater. *Brazilian Journal of Microbiology*, 38(4), 644–648. 2007.

[23] Szekeres, E., Baricz, A., Chiriac, C.M., Farkas, A., Opris, O., & Soran, M.-L. Abundance of antibiotics, antibiotic resistance genes and bacterial community composition in wastewater effluents from different Romanian hospitals. *Environmental Pollution*, 225, 304–315. 2017.

[24] Wang, H.-P., Zhang, H.-J., Liu, J., Dong, Q., Duan, S., & Ge, J.-Q. Antimicrobial resistance of 3 types of gram-negative bacteria isolated from hospital surfaces and the hands of health care workers. *American Journal of Infection Control*, 45(11), e143–e147. 2017.

[25] Ayandele, A.A., & Akinyanju, J.A. Prevalence and antibiogram profile of *Pseudomonas aeruginosa* from hospital wastewater in Nigeria. *African Journal of Clinical and Experimental Microbiology*, 21(3), 195-202. 2020.

[26] Varela, A.R., André, S., & Nunes, O.C. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* in wastewater treatment plants. *Antibiotics*, 10(6), 720. 2021.

[27] Zhao, X., Wang, L., & Wang, J. Environmental adaptability of *Pseudomonas aeruginosa* in healthcare and environmental settings. *Journal of Applied Microbiology*, 134(2), 528-539. 2023.

[28] Rahman, M. A., Islam, M. T., and Haque, A. Survival of multidrug-resistant *Pseudomonas aeruginosa* in wastewater environments. *Int. J. Environ. Res. Public Health.* 17(5):1845. 2020 DOI: 10.3390/ijerph17051845

[30] Chen J., Liu C., and Zhang Z. Antibiotic resistance patterns of *Pseudomonas aeruginosa* isolated from hospital effluents: A global perspective. *J. Environ. Sci. Health.* 56(9):1-12. 2021 DOI: 10.1080/03601234.2021.1932176