



Antimicrobial Resistance Profile of Multi-Drug Resistant Gram-Negative Bacteria Associated with Hospital Wastewater in Niger State, Nigeria

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ABSTRACT

Antimicrobial resistance (AMR) is an increasingly urgent global issue, with multidrug-resistant (MDR) bacteria posing a significant threat to public health. This study evaluated the MDR patterns of the isolates from hospital wastewater. Samples were collected from three hospitals in Minna, and the samples were identified using the spread plate method. The isolates were then identified using Gram staining and other biochemical characteristics. Antibiotic sensitivity testing was conducted using the Kirby-Bauer disc diffusion method. The study identified a total of 24 bacteria of four different types of Gram-negative isolates, including *Proteus mirabilis*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The result revealed that sample GH (2.15×10^7) had the highest microbial count while sample SH (2.5×10^6) had the least. All the isolates were found to be multi-drug resistant, with high Multiple Antibiotic Resistance (MAR) index for both isolated bacteria (0.61 to 0.70) and sampling sites (0.62 to 0.63). All bacterial isolates showed 100% resistance to Augmentin, Ampicillin and Chloramphenicol. The study concluded that hospital sewage serves as a hotspot for antibiotic-resistant bacteria, which are usually associated with infections difficult to control in the environment. Therefore, there is need for appropriate treatment of hospital wastewater to prevent the spread of MDR bacteria.



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1.0 INTRODUCTION

Antimicrobials are natural or synthetic chemicals that kill or inhibit the growth of susceptible microorganisms. They have been used to treat and prevent microbial infections in human and veterinary medicine for decades (Álvarez-Martínez et al., 2020). The discovery of antibiotics penicillin by Alexander Fleming in 1928, followed by the introduction of these life-saving medications into clinical use was undoubtedly the greatest medical breakthrough of the 20th century (Hutchings et al., 2019). In addition to treating infectious diseases, antibiotics made many modern medical procedures possible, including cancer treatment, organ transplants and open-heart surgery (Gautam et al., 2022) in most hospitals. However, in most hospitals where antibiotics are frequently used, such antibiotics are said to occur in the environments, particularly the hospital wastewaters. Hospital wastewaters are the primary hotspots for the selection of resistant microbes where several types of antimicrobials are flushed frequently inducing high selection pressure in the bacterial community (Tesfaye et al., 2019). In addition, the misuse and overuse of antimicrobial agents in food animal production are also posing serious risk of selection for resistant bacterial pathogens and commensal organisms. According to the World Health Organization, it has been presumed that 700,000 people worldwide die each year from infections that are resistant to drugs and if no measures are taken, this number could rise to 10 million deaths annually by 2050 (Uddin et al., 2021). The continuous contamination of multi-drug resistant (MDR) Gram-negative bacteria is the leading cause of high MDR in the

environment (Zainab et al., 2020). These bacteria pose a significant threat to public health, as they are often resistant to multiple antibiotics, making them difficult to treat and control (Zainab et al., 2020).

The presence of multi-drug resistant (MDR) Gram-negative bacteria in hospital wastewater is a great menace to the public health and the environment at large. Most of these multidrug resistant bacteria are often resistant to multiple antibiotics, thus making their treatment and control difficult (Uddin et al., 2021), which has led to delay in hospital stay, high morbidities and mortalities in most developing countries such as Nigeria. It is therefore necessary to determine the multidrug resistant Gram-negative bacteria associated with most wastewater, especially the hospital wastewater and this is the basis of the study which aims to determine degree of resistance associated with multidrug resistant Gram-negative bacteria isolated from hospital wastewater in Minna, Niger State.

2.0 MATERIALS AND METHODS

2.1 Study area

The research was conducted in Minna Metropolis of Niger State. Located within longitude 633'E and latitude 93'N with a land mass of 88km². The vegetation of the area reflect that of Savannah zone dominated by grass with scattered trees species. The climate present two different seasons, a rainy season between April and October and a dry season between November- march (completely devoid of rain).

2.2 Sample collection and processing

A total of three (3) wastewater samples were collected from three (3) different hospitals namely; General Hospital, Standard Hospital and the University Health Services in Federal University of Technology Minna, North Central, Nigeria. The samples were collected in sterile conical flask and were transported aseptically to the Microbiology laboratory in Federal University of Technology, Minna for further analysis. The collected wastewater samples were processed to concentrate the bacteria. This involved centrifugation at low speed 300 rpm for 15 min to separate the bacterial cells from the liquid portion. The resulting pellets were resuspended in 15ml of sterile saline solution.

2.3 Isolation of bacteria

The resuspended bacterial cells were serially diluted in nine (9) test tubes and 1mL of bacterial suspension from the fifth test tube was inoculated on Nutrient agar, followed by Differential and Selective media, namely MacConkey agar, Eosin Methyl Blue and *Salmonella-Shigella* Agar (SSA) respectively via spread plate method. The inoculated plates were then incubated at an appropriate temperature (37°C) for 24-48 h to allow the growth of colonies. After incubation, the MacConkey agar, SSA, and EMB agar plates were observed for the presence of bacterial colonies. The colonies were examined for their size, shape, color, texture, and other physical characteristics.

2.4 Identification of bacteria

The isolated bacteria were identified via Gram staining and only Gram-negative bacteria were subjected to other conventional biochemical tests.

2.5 Antibiotic susceptibility testing

The identified Gram-negative bacteria were tested for their susceptibility to various antibiotics using the Kirby-Bauer disk diffusion method. A pure culture of the test organism was grown in a nutrient agar. The inoculum was standardized accordingly to MacFarland Standard and was spread onto a Mueller-Hinton agar plate. Antibiotic discs such as Ofloxacin (5 µg), Streptomycin (10 µg), Sparfloxacin (5 µg), Chloramphenicol (30 µg), Septrin (30 µg), Pefloxacin (5 µg), ciprofloxacin (5 µg), augmentin (30 µg), gentamicin (10 µg), ampicillin (10 µg) were placed onto the agar plate, and the plates were incubated at 35-37°C for 16-18 h. After incubation, the diameter of the zone of inhibition around each antibiotic disc was measured and compared to a standardized chart to determine the susceptibility of the bacteria to each antibiotic. The results were interpreted as susceptible, intermediate, or resistant, based on the size of the zone of inhibition.

2.6 Multiple antibiotic resistance index (MARI) determination

The MARI of each isolate was determined using the formula first described by Krumperman (1983). The MAR index formula is defined as a/b , where 'a' = number of antibiotics to which the isolate is resistant, and 'b' = total number of antibiotics tested.

3.0 RESULTS

Out of all the wastewater samples analyzed, sample GH (2.15×10^7 cfu/mL) had the highest microbial count while sample SH had the least microbial count (2.5×10^6 cfu/mL) as seen in Table 1. The biochemical tests revealed that a total of 24 bacteria were isolated from the three hospitals. *Klebsiella pneumoniae* had the highest frequency of occurrence 37.5%, followed by *Pseudomonas aeruginosa* 25%, *Salmonella enterica typhi* 20.8%, and *Proteus mirabilis* had the least frequency of occurrence 16.7% as shown in Table 2 below. Standard hospital had the highest bacterial isolates (10 bacterial isolates) while General hospital had the least bacterial isolates (6 bacterial isolates) as seen in Figure 1. The antibiogram

revealed that 100% of most isolated Gram-negative bacteria were resistant to multiple classes of antibiotics. Most of the isolates exhibited total resistance to amoxicillin and augmentin as seen in Table 5

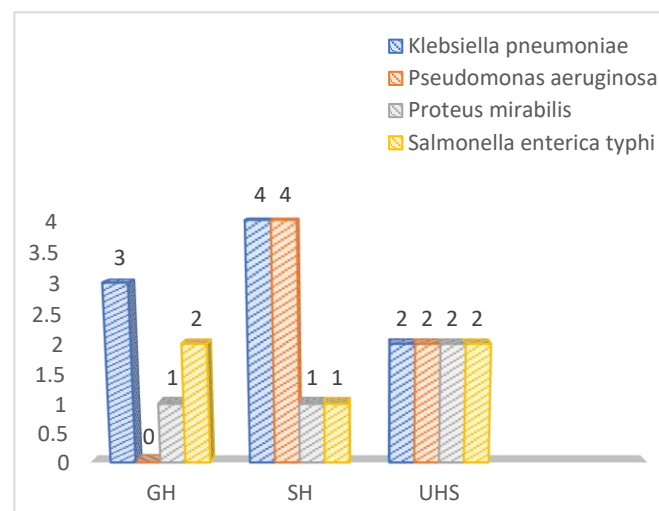


Figure 1. Graphical representation of the number of bacterial isolates associated with Various hospital location

Table 1. Microbial count on nutrient agar for the various samples

Sample	CFU/ML	Shape	Elevation	Colour	Edge
SH	2.5×10^6	Round	Convex raised	Milky	Regular
UHS	1.35×10^7	Round	Raised	Milky	Regular smooth
GH	2.15×10^7	Circular	Flat	Colourless	Regular

Table 2. Microbial morphology on MacConkey agar for the various samples

Sample	Shape	Elevation	Colour	Edge
SH	Round	Convex	Pinkish	Regular
UHS	Round	Flat	Colourless	Entire smooth
GH	Circular	Flat	Colourless	Regular

Table 3. Microbial morphology on *Salmonella Shigella* agar for the various samples

Sample	Shape	Elevation	Colour	Edge
SH	Round	Raised	Pinkish-red	Smooth
UHS	Round	Flat	blackish	Entire smooth
GH	Regular	Rough	Pinkish	Smooth

SH= Standard Hospital; UHS= University Health Service; GH=General Hospital

Table 4. Frequency of occurrence of bacterial isolates associated with hospital wastewater

Isolates	Frequency of Occurrence	Percentage of Occurrence (%)
<i>Klebsiella pneumoniae</i>	9	37.5
<i>Pseudomonas aeruginosa</i>	6	25
<i>Proteus mirabilis</i>	4	16.7
<i>Salmonella enterica typhi</i>	5	20.8
Total	24	100

Table 6. Susceptibility pattern of multidrug resistant isolates

Bacteria Isolates	NI	Pattern	OFX (%)	STR (%)	AU (%)	PEF (%)	SP (%)	SXT (%)	CN (%)	AM (%)	CH (%)	CPX (%)
<i>Klebsiella pneumoniae</i>	9	S	9(100)	0(0)	0(0)	1(11.1)	0(0)	0(0)	0(0)	0(0)	0(0)	9(100)
		I	0(0)	9(100)	0(0)	4(44.4)	1(11.1)	0(0)	1(11.1)	0(0)	0(0)	0(0)
		R	0(0)	0(0)	9(100)	4(44.4)	8(88.9)	9(100)	8(88.9)	9(100)	9(100)	0(0)
<i>Pseudomonas aeruginosa</i>	6	S	6(100)	0(0)	0(0)	1(16.67)	1(16.67)	0(0)	0(0)	0(0)	0(0)	6(100)
		I	0(0)	6(100)	0(0)	1(16.67)	1(16.67)	0(0)	1(16.67)	0(0)	0(0)	0(0)
		R	0(0)	0(0)	6(100)	4(66.7)	4(66.7)	6(100)	5(83.33)	6(100)	6(100)	0(0)
<i>Proteus mirabilis</i>	4	S	4(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	4(100)
		I	0(0)	4(100)	0(0)	0(0)	1(25)	0(0)	1(25)	0(0)	0(0)	0(0)
		R	0(0)	0(0)	4(100)	4(100)	3(75)	4(100)	3(75)	4(100)	4(100)	0(0)
<i>Salmonella enterica typhi</i>	5	S	5(100)	0(0)	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	5(100)
		I	0(0)	5(100)	0(0)	0(0)	0(0)	1(20)	1(20)	0(0)	0(0)	0(0)
		R	0(0)	0(0)	5(100)	4(80)	5(100)	4(80)	4(80)	5(100)	5(100)	0(0)

S= Sensitive, I= Intermediate, R= Resistant, OFX= Ofloxacin, STR= Streptomycin, SP= Sparfloxacin, CH= Chloramphenicol, SXT= Septrin, PEF= Pefloxacin, CPX= Ciprofloxacin, AU= Augmentin, CN= Gentamicin, AM=Ampicillin, NI= number of isolate.

The MAR index ranged from 0.61 to 0.7 in organisms and sampling sites between 0.62 and 0.63, indicating that the MAR values of all organisms and sites were from high-risk environments, as seen in Tables 6 and 7. *Proteus mirabilis* showed the highest MAR index (0.7), while *Pseudomonas aeruginosa* had the lowest (0.61) as seen in Table 6. The sampling sites of the MAR index revealed that wastewater from General Hospital and Standard Hospital had the highest MAR index of 0.63 while the University Health Service had the least value of 0.62 as seen in Table 7.

Table 6. MAR index value of multidrug-resistant Gram-negative bacteria from hospital wastewater

Organism Identified	MAR Index
<i>Klebsiella pneumoniae</i>	0.62
<i>Pseudomonas aeruginosa</i>	0.61
<i>Proteus mirabilis</i>	0.70
<i>Salmonella enterica typhi</i>	0.64

Table 7. MAR index value of wastewater from various sampling sites

Sampling Sites	MAR Index
General Hospital (n=6)	0.63
Standard Hospital (n=10)	0.63
University Health Service (n=8)	0.62

4.0 DISCUSSION

The high microbial count as observed in samples GH (2.15×10^7) can be attributed to the fact that high number of microbes are easily adapted to wastewater, possessing high content of organic nutrients, nitrogen compounds and suspended solids, which in turn encourages the high microbial count and this in turn favors high microbial populations in most wastewaters. In addition, poor or no enforcement of certain hygienic practices such as frequent treatment of hospital wastewater in most hospitals in developing countries like Nigeria, can also result to high microbial count. This current study agrees with the report of Moges et al. (2014) and Bapurao et al. (2022) which suggested that hospital wastewater as a hotspot for the selection of antibiotic-resistant bacteria. The high frequency of gram-negative enteric bacteria such as *Klebsiella pneumoniae* 9(37.5%), *Pseudomonas aeruginosa* 6(25%), and *Salmonella enterica* 5(20.8%) in hospital wastewater is primarily due to the fact that most hospital effluents receive waste from patients, wards, laboratories, and toilets. Thus, ensuring most hospital wastewater harbour so many intestinal Gram-negative bacteria. In addition, other intrinsic factors such as presence of outer membrane with lipopolysaccharide, biofilm formation, beta lactamase production and efflux pump abilities, help Gram-negative bacteria resist disinfectants and environmental stress, enabling persistence in hospital effluents. This in turn ensures they pose a high risk of environmental contamination. The findings of this research are in agreement with the study reported by Bapurao et al. (2022) and Wu et al. (2025). The high occurrence of *Klebsiella pneumoniae* among the three(3) hospital wastewater is based on the fact that *Klebsiella pneumoniae* is a normal human gut commensal which is continuously shed in the faeces and

urine of humans compared to *Pseudomonas aeruginosa*; which is an environmental commensal with minimal adaption and survival to wastewater and *Salmonella enterica typhi*; which is not a common faecal commensal, but occurs when there is an infection outbreak (Verburg et al., 2024; Karungamye et al., 2023; Shetty et al., 2025). However, the lowest occurrence of *Proteus mirabilis*, could be based on certain factors such as such low abundance in human microbiota and its poor survival capabilities in wastewater environment. This result is in agreement with the findings of (Shetty et al., 2025; Wu et al., 2025). In addition, the high bacterial isolates (10 bacterial isolates), observed in Standard hospital could be attributed to the fact that there is poor disposal of faecal matter practiced in the hospital. Similarly, other factors such as patient colonization, medical procedures, and inadequate infection control measures contribute to their presence in hospital wastewater, posing a risk of environmental contamination and potential transmission. The findings of this research are in agreement with the study reported by Bapurao et al. (2022).

All the isolates were found to be multidrug-resistant, with their multiple antibiotic resistance (MAR) index ranging from 0.61 to 0.7. Similarly, isolates associated with each sample locations had MAR index between 0.62 and 0.63. These high MAR index recorded among the isolates particularly *Proteus mirabilis* is a clear indication that this organism persist longer in hospital environments and as such engage in horizontal transfer of mobile genetic elements, which increases its potentials of developing high multiple antibiotic resistance index within the hospital environment especially the hospital waste water. In addition, the high MAR index recorded among the isolates and in the sampling sites, is a clear indication that the hospitals environment studied harbor high risk bacteria isolates that originated from other sources with high contamination of antibiotics and this in turn has rendered these studied sites reservoirs for persistence occurrence and dissemination of multi drug resistant organisms. This result agrees with the findings of Adefisoye & Okoh, (2017).

Conclusion

This study area revealed that three (3) samples of hospital waste water were highly contaminated with microorganisms such as *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella enterica typhi* *Pseudomonas aeruginosa*. However, all these bacterial contaminants are multi drug resistant, thus there is an imminent need for Government and health care providers to continuously ensure that: implementation of on-site wastewater treatment systems with tertiary treatment stages and regular monitoring of antibiotic resistance genes in hospital effluents are regularly employed to completely eradicate and prevent the spread of MDR bacteria in most hospital environment.

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Conflict of interest

Authors declare no conflict of interest

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