



Research Article

Bacteriological Analysis and Antibiotic Susceptibility Profile of Wastewater Effluent, From Alex Ekwueme Federal Teaching Hospital, Abakiliki, Ebonyi State Nigeria

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Abstract

This research paper was conducted to determine the antimicrobial resistance patterns of bacterial isolates which were obtained in the hospital wastewater of Alex Ekwueme Federal University Teaching Hospital, Abakiliki (AE-FETHA). The bacterial isolates were identified using selective culture media, Gram staining, and biochemical tests. The findings revealed that the most common pathogens in the hospital wastewater were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp, and *Shigella* spp. The resistance of *Staphylococcus aureus* was the highest and all the isolates were resistant to pefloxacin, 60% to gentamicin, and 80 percent to rifampicin. The isolates of *Escherichia coli* had 100% resistance to pefloxacin, Ampiclox, and Amoxicillin and 75% resistant to ciprofloxacin. *Salmonella* spp. had 100% resistance to Ampiclox and Amoxicillin, 66.7% resistance to Zithromax and 100% resistance to rifampicin. *Shigella* spp. was 100 percent resistant to Ampiclox, Amoxicillin, rifampicin and ciprofloxacin and half of the isolates were resistant to Zithromax. These results revealed that multidrug resistance in hospital wastewater is very high and thus there is a call to enhance infection control, antimicrobial stewardship, and wastewater management behaviors in hospitals and antimicrobial resistance patterns should be monitored regularly to reduce the diffusion of the resistant pathogen.

Keywords

Bacteriology, Antibiotic, Susceptibility, Profile, Wastewater

1. Introduction

AMR is one of the most urgent problems in the healthcare system of the contemporary world [1]. The commonplace use of antibiotics in the medical facility has resulted in the occurrence and spread of resistant forms of bacteria, which are dire health dangers to the population [2]. The overuse or misuse of antibiotics is a global issue since the sub-lethal doses once introduced into the environment can hasten the evolution of the

resistance in hospitals [3]. As a result, hospital wastewater (HWW) has been defined as a highly important yet underestimated reservation and carrier of antimicrobial-resistant bacteria (ARB) [4]. HWW represents a region of ARB because of the presence of antibiotics and other pharmaceuticals that cause the emergence of resistant pathogenic bacteria [5]. The daily generation of wastewater is estimated at 200–400 L per

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capita in the low-income nations, and 400–1200 L in the high-income countries [6]. Nevertheless, certain production at the facility level is frequently dependent on the beds capacity and services that are offered. The topicality of the issue is especially high in developing countries such as Nigeria where health outcome indicators are still quite high, with a maternal mortality ratio of 814 per 100,000 and under-five mortality rate of 104 per 1,000 live births [6]. In such areas, the release of untreated HWW into water systems is a major cause of epidemics of such diseases as cholera, typhoid, and dysentery [7]. The resistance rate of bacteriology in HWW has been documented in previous regional studies between 30.1 and 33.3% indicating the fertility of such environments to breed the resistant pathogens [8, 9]. There is still a major research gap because the data on the resistance patterns of strain-specific bacteria obtained in the environmental sources are not thoroughly examined in a variety of geographic areas [8]. Although the historical drug failures were usually explained by the presence of falsified or out-of-date drugs, the recent data indicates that the unsuccessful treatments might be associated with the possibility of bacteria in wastewater to become resistant [8]. The paper seeks to fill these gaps by carrying out a rigorous bacteriological study and antibiotic susceptibility of the waste water effluent in Alex Ekwueme Federal University Teaching Hospital (AEFUTHA) in Abakaliki, Nigeria.

2. Methodology

2.1. Study Area and Design

The study employed a cross sectional and experimental research design to assess bacteriological profile and antimicrobial resistance of hospital wastewater in the Alex Ekwueme Federal University Teaching Hospital (AEFUTHA) in Abakaliki, Nigeria. AEFUTHA is a tertiary health center and national referral center founded on the principles of providing long-range medical services, diagnostic laboratories, and surgical procedures. The hospital is a critical study location because of the large number of patients it receives and the fact that it is likely to experience excessive consumption of antibiotics, which in turn help to increase the occurrence of resistant strains of bacteria [8].

2.2. Collecting Wastewater Samples

Eighty (80) samples of the analytical wastewater were taken in hospital septic reservoirs. According to the recommendations of the Environment Protection Authority and the American Public Health Association, a grab sampling method was used to guarantee the collection of representative specimen [8]. The samples were manually collected in a pre-sterilized container towards the middle of the flow channel, 10 to 15 cm of water depth. To keep specimens biologically intact, collection was followed by placing the specimen in the coolers with a cold chain and transporting the specimen to the laboratory

within 2 hours of collection [8].

2.3. Bacterial Identification

Isolation of bacteria was done by a 10-fold serial dilution technique, which is a standard procedure. A thorough shaken mixture was created, 1 ml of wastewater was added to 99 ml of 0.85 percent sodium chloride. Subsequently, 0.1 ml of the diluted sample was smeared on blood agar and kept in 37 degrees Celsius under the standard atmospheric conditions. The further sub-culture of colonies with specific morphology was performed on selective and differentiated media, such as Mannitol Salt Agar, MacConkey Agar, and Salmonella-Shigella Agar. Colony color, Gram staining, using 100x magnification and a series of biochemical tests (indole production, citrate utilization, catalase, oxidase, and triple sugar iron tests) were used to identify isolates [8].

2.4. Testing of Antibiotic Susceptibility

The susceptibility of selected microbes to antimicrobials was identified by the Kirby-Bauer disk diffusion technique on the Mueller-Hinton Agar according to the Clinical and Laboratory Standards Institute instructions. A suspension of 3–5 separate colonies was made in normal saline and standardized to the 0.5 McFarland turbidity standard. The swabs of the agar plates were inoculated with standardized samples and ampicillin, chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole, erythromycin, and ceftazidime zones were inoculated with antibiotic disks [8]. Following incubation, zones of inhibition were determined in millimeters and under 37 degrees Celsius 18 to 24 hours.

2.5. Quality Control

Standard operation was used at all the study stages in order to make the findings reliable. Sample processing was observed in the Biosafety Level 2 cabinets in aseptic conditions and wastewater samples were gathered following the sterilization of the manhole area to avoid external contamination. The culture mediums were checked in terms of sterility by incubating a percentage of every batch without inoculation. The concentration of antimicrobial disks and media performance was confirmed with the help of the American Type Culture Collection standard reference strains, namely *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922) [10].

2.6. Data Analysis

The statistical package of the social sciences was used to enter and analyze data. The frequency and percentages were computed as descriptive statistics to demonstrate the distribution of the bacteria isolates in the wards of the hospitals and their pattern of antibiotic susceptibility after that. This stringent measure allowed the precision of the resistance profile determined in the hospital wastewater effluents.

3. Results

Escherichia coli was determined as pink colonies on MacConkey agar which is an indicator of lactose fermentation. Gram-negative rods were observed under microscopy, which is characteristic of enteric coliform bacteria. The isolate was catalase positive and oxidase negative (biochemical) which validates its Family Enterobacteriaceae. The positive indole test and negative citrate test were additional features which differentiated it with other enteric organisms. According to Triple Sugar Iron agar, the Acid/Acid reaction featuring gas production with no sulfide production confirmed the presence of glucose and lactose fermentation in a high-salt environment, which is characteristic of *E. coli*. *Staphylococcus aureus* was identified based on its growth on Mannitol Salt Agar where yellow colonies signified mannitol fermentation in a high-salt environment. Gram staining revealed Gram-positive cocci in clusters, which is staphylococcal morphology. The isolate was catalase positive with oxidase negative which distinguished it among streptococci. Expected biochemical behavior was in agreement with negative indole and citrate reactions. Carbohydrate fermentation without the formation of sulfide on TSI agar was supported by the production of acids in the absence

of hydrogen sulfide, and this is typical of *Staphylococcus* species.

The isolates of *SaAalmonella* spp. were characterized by growth in Salmonella-Shigella agar in colorless colonies with black centers, which indicated fermentation of the non-lactose sugars and production of hydrogen sulfide. Gram-negative rods were seen under microscopy. The isolate was catalase positive and oxidase negative as expected of enteric pathogens. Offensive indole and positive citrate test results were additional indications of *Salmonella*. The presence of hydrogen sulfide formation was confirmed as the TSI reaction of alkaline slant and acid butt with fermentation on glucose with the formation of the colorless colonies without black centers on Salmonella-Shigella agar, which signified non-lactose fermentation and the lack of hydrogen sulfide generation. On microscopy, the organisms were Gram-negative rods. The isolate was catalase negative and oxidase negative biochemically with negative indole and citrate reactions distinguishing it among *Salmonella* and other enteric bacteria. Its identity was further determined to be *Shigella* spp. on TSI agar with the alkaline slant and acid butt devoid of gas and hydrogen sulfide (Table 1) on TSI agar.

Table 1. Biochemical test result, culture and morphological characteristics of bacterial isolates.

Isolated microorganism	Selective / differential culture characteristics (as used in this study)	Gram reaction & morphology	Catalase	Oxidase	Indole	Citrate	Triple Sugar Iron (TSI)
<i>Escherichia coli</i>	MacConkey agar: pink colonies due to lactose fermentation	Gram-negative rods	+	–	+	–	Acid/Acid (A/A), gas, no H ₂ S
<i>Staphylococcus aureus</i>	Mannitol Salt Agar: yellow colonies due to mannitol fermentation	Gram-positive cocci in clusters	+	–	–	–	Acid production (no H ₂ S)
<i>Salmonella</i> spp.	Salmonella-Shigella agar: colorless colonies with black centers	Gram-negative rods	+	–	–	+	Alkaline/Acid (K/A) with H ₂ S
<i>Shigella</i> spp.	Salmonella-Shigella agar: colorless colonies without black centers	Gram-negative rods	–	–	–	–	Alkaline/Acid (K/A), no gas, no H ₂ S

The highest percentage of the isolates was *Salmonella* spp. at 35 percent. *Escherichia coli* was a contributing factor to 26% of the isolates. Being a widespread fecal coliform, the appearance of *E. coli* in hospital wastewater may indicate potential problems with hygiene or sanitation in a given hospital. Its occurrence may be an indication of fecal contamination. The *Staphylococcus aureus* was the cause of 18% of the isolates.

This organism causes various types of infections as skin infections, respiratory, and bloodstream infections. Its significant concentration in the wastewater can be indicative of hospital-acquired infections, and this is a cause of concern in health facilities. *Shigella* spp. was 21 percent of the bacterial isolates. *Shigella*, a dysentery causative agent, implicates the possibility of the prevalence of enteric diseases in the hospital setting (Figure 1).

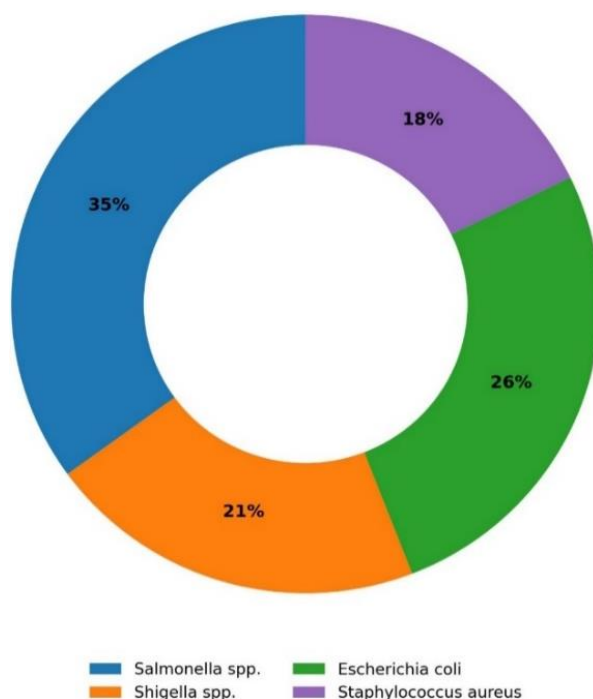


Figure 1. The proportion of bacterial isolates recovered from wastewater across selected departments of Federal Teaching Hospital Abakaliki (FETHA).

T2. A total of 35 *Salmonella* spp. isolates were obtained, the largest percentage of which was recorded in the Accident and Emergency department of 12 isolates (34.3%). The Surgery department had the next highest number of 7 isolates (20.0%), then Pediatrics and Medicine departments had 5 each (14.3% and 17.1%, respectively). The Outpatient Department (OPD) and the Obstetrics and Gynecology had 5 isolates (14.3% and zero isolates respectively). Overall, fifty percent of the isolates were *Salmonella* spp. In the case of *Shigella* spp, there were 21 isolates in total. The highest proportion was in the Accident and Emergency department with 7 isolates (33.3%), and then Surgery and Medicine had 4 isolates (19.0% and 19.0% respectively). The highest count of 3 isolates was registered by Pediatrics (14.3%), followed by Obstetrics & Gynecology (14.3%). The isolates of *Shigella* spp. constituted

21% of the total isolates of the Outpatient Department (OPD).

The total count of *Escherichia coli* isolates was 26 where Surgery had the highest proportion of 6 isolates (23.1). The Pediatrics and Medicine departments had 5 (19.2% each) and Obstetrics and Gynecology and OPD had 4 (15.4% each), respectively. The lowest proportion was in the Accident & Emergency department which had only 2 isolates (7.7%). *Escherichia coli* was 26% of the total isolates. In the case of *Staphylococcus aureus*, the number of the isolates amounted to 18 and 4 isolates were obtained in Surgery, Pediatrics, Medicine, and Accident and Emergency departments (22.2% each). Obstetrics & Gynecology and OPD both had 3 isolates (16.7%). *Staphylococcus aureus* constituted 18 percent of the overall isolates (Table 2).

Table 2. Distribution of bacterial isolates recovered from hospital wastewater across departments of Federal Teaching Hospital Abakaliki (FETHA).

Department	<i>Salmonella</i> spp. n (%)	<i>Shigella</i> spp. n (%)	<i>Escherichia coli</i> n (%)	<i>Staphylococcus aureus</i> n (%)
Surgery	7 (20.0)	4 (19.0)	6 (23.1)	4 (22.2)
Pediatrics	5 (14.3)	3 (14.3)	5 (19.2)	4 (22.2)
Medicine	6 (17.1)	4 (19.0)	5 (19.2)	-
Obstetrics & Gynecology	-	3 (14.3)	4 (15.4)	3 (16.7)
Outpatient Department (OPD)	5 (14.3)	-	4 (15.4)	3 (16.7)
Accident & Emergency	12 (34.3)	7 (33.3)	2 (7.7)	4 (22.2)

Department	<i>Salmonella</i> spp. n (%)	<i>Shigella</i> spp. n (%)	<i>Escherichia coli</i> n (%)	<i>Staphylococcus aureus</i> n (%)
Total	35 (100)	21 (100)	26 (100)	18 (100)

Key:

n = number of isolates; (%) = proportion within each organism column; - = isolate not detected.

The case of the *Staphylococcus aureus* (n = 5) was also observed to have high percent resistant to pefloxacin (100). The amoxicillin (100 percent), Rifampicin (80 percent) and ampiclox (100 percent) were also resistant. Eighty percent of the isolates were ciprofloxacin resistant; 60 percent were erythromycin resistant. The other isolates were also sensitive to gentamicin, azithromycin and levofloxacin and 60 percent sensitive to the three said antibiotics. It was mentioned that the *Escherichia coli* (n = 4) had complete resistance to pefloxacin, ampiclox, amoxicillin and rifampicin with all of them having a hundred percent resistance to ampiclox and amoxicillin. Nevertheless, the isolates were resistant to gentamicin and ciprofloxacin resistance only in a half and three out of four respectively. They were resistant to azithromycin and levofloxacin and not resistant to erythromycin with 25 percent

of them being susceptible to the antibiotic.

The 100 per cent resistance in *Salmonella* spp. was to ampiclox and amoxicillin (n = 3). All the isolates were resistant to pefloxacin (100%). Moreover, 66.7 percent of the isolates were also resistant to zithromax, and only 33.3 percent of the isolates had a resistance to gentamicin and azithromycin. Not all the isolates were resistant to erythromycin and levofloxacin. *Shigella* spp. (n=2) were totally ampiclox and amoxicillin resistant. The isolates were also resistant to rifampicin and ciprofloxacin at 100 percent. It was also identified that the isolates were resistant to gentamicin and azithromycin and levofloxacin and gentamicin at 50 and 62.5 percent respectively. To his astonishment they were not resistant to erythromycin on *Shigella* spp (Table 3).

Table 3. Antimicrobial resistance profile of bacterial isolates recovered from hospital wastewater at Alex Ekwueme Federal University Teaching Hospital, Abakaliki.

Bacteria isolated	Antibiotics tested, n (%) resistant	PEF	CN	APX	Z	AM	R	CPX	AZ	LEV	E
<i>Staphylococcus aureus</i> (n = 5)		5 (100)	3 (60.0)	5 (100)	4 (80.0)	5 (100)	4 (80.0)	-	4 (80.0)	2 (40.0)	3 (60.0)
<i>Escherichia coli</i> (n = 4)		4 (100)	2 (50.0)	4 (100)	-	4 (100)	3 (75.0)	1 (25.0)	-	1 (25.0)	2 (50.0)
<i>Salmonella</i> spp. (n = 3)		-	3 (100)	3 (100)	2 (66.7)	3 (100)	3 (100)	1 (33.3)	2 (66.7)	-	-
<i>Shigella</i> spp. (n = 2)		-	1 (50.0)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50.0)	-	0 (0.0)	1 (50.0)

Key - PEF = pefloxacin; CN = gentamicin; APX = ampiclox; Z = zithromax; AM = amoxicillin; R = rifampicin; CPX = ciprofloxacin; AZ = azithromycin; LEV = levofloxacin; E = erythromycin.

The resistance to four antibiotics namely ampiclox (APX), amoxicillin (AM), rifampicin (R) and zithromax (Z) was the most prevalent mode of resistance in *Staphylococcus aureus* with the resistance pattern being shared by 40 percent of the isolates (2 out of 5). The other 20 percent of the isolates (1 out of 5) resistant to the combination of APX, AM, R, azithromycin (AZ) and erythromycin (E) and 20 percent (1 out of 5) resistant to APX, AM and Z. Eighty percent of the total isolates were multidrug resistant (MDR) i. e. are resistant to three or

more antibiotic groups. The proportion of the resistant isolates of APX, AM, R and Z were 25% (1 in 4) in the case of *Escherichia coli*. One quarter (25 percent) of them also opposed APX, AM, AZ and E. thus the presentation of the vigorous opposition to the majority of classes of antibiotics. In the case of *Salmonella* spp. (n = 3), 33.3% (1 out of 3) was resistant to APX, AM, R and Z and remaining 33.3% (1 out of 3) resistant to APX and AM. Overall, 66.7 percent of the *Salmonella* spp. isolates were noted to be multi-drug resistant whereby at least

three classes of antibiotics were found to be resistant. According to Schisangaraj (2020), the case of *Shigella* spp. was reported to be multi-drug resistant ($n = 2$), and one out of every two was resistant to the combination of APX, AM, R and Z.

The other half, 50 percent (1/2) was not susceptible to the same mix and this increased the prevalence of MDR in *Shigella* spp. to 50 percent (Table 4).

Table 4. The multidrug-resistant (MDR) patterns of the bacterial isolates recovered from hospital wastewater at Alex Ekwueme Federal University Teaching Hospital, Abakaliki.

Bacteria	Resistance antibiotic combinations	No. of antibiotic classes	Frequency n (%)	MDR n (%)
<i>Staphylococcus aureus</i> (n = 5)	APX, AM, R, Z	4	2 (40.0)	4 (80.0)
	APX, AM, R, AZ, E	5	1 (20.0)	
	APX, AM, Z	3	1 (20.0)	
<i>Escherichia coli</i> (n = 4)	APX, AM, R, Z	4	1 (25.0)	3 (75.0)
	APX, AM, AZ, E	4	1 (25.0)	
	APX, AM, R	3	1 (25.0)	
<i>Salmonella</i> spp. (n = 3)	APX, AM, R, Z	4	1 (33.3)	2 (66.7)
	APX, AM, R	3	1 (33.3)	
<i>Shigella</i> spp. (n = 2)	APX, AM, R, Z	4	1 (50.0)	1 (50.0)
Total			10 (—)	10 (58.8)

4. Discussion

Multidrug resistance (MDR) of the samples of hospital wastewater to be used in this study is presented in the antimicrobial resistance patterns. The results are in line with the growing concerns regarding the problem of antibiotic resistance in hospitals, particularly in low-resource facilities. The occurrence of resistance among the bacterial isolates in Federal Teaching Hospital Abakaliki (FETHA) implies that misuse or overuse of antibiotics in the healthcare facilities might be helping to increase the number of resistance pathogens as is the case with the present study [10].

Most antibiotics used were resistant to *Staphylococcus aureus* and 80% of all the isolates were multidrug resistant. This is in accordance with other reports that have reported increasing resistance against β -lactams, aminoglycosides, and macrolides to hospital-acquired infections caused by *S. aureus* [11, 12]. The combination of ampiclox (APX), amoxicillin (AM), rifampicin (R), and zithromax (Z) in specific cases appeared to be as common as possible because the *S. aureus* has been persistently resistant to these treatments. This means that the hospital sanitation protocols might not be sufficient to prevent the transmission of these resistant strains, and this has been a problem in these hospitals [13].

Three-fourths of all *Escherichia coli* isolates carried multidrug resistance, the common ones being β -lactam and fluoroquinolone. This is particularly worrying, as *E. coli* is among

the major causes of gastrointestinal infections and urinary tract infections (UTIs), whose resistance to fashionable antibiotics can make the treatment challenging [14]. It is also concerning that ciprofloxacin and amoxicillin also have resistance to them due to their use as a first-line intervention not only in UTI but also gastrointestinal infections [15]. *E. coli* resistance to fluoroquinolones in the current study is consistent with the increased resistance rates of *E. coli* across the globe, which has been reported in several hospital settings, particularly in low-resource nations [16].

The level of multidrug resistance was also high in *Salmonella* spp. and *Shigella* spp. with 66.7% of *Salmonella* isolates and 50% *Shigella* isolates resistant to at least three classes of antibiotics. The given observation is consistent with the recent surveys that show the alarming emergence of the resistance of enteric pathogens, i. e. *Salmonella* and *Shigella*, to the most popular antibiotics, i. e. ampicillin and chloramphenicol [17, 18]. The most concerning trend is the emergence of MDR *Salmonella* and *Shigella* strains because those are typically linked to foodborne illnesses, and the resistance to the medications against them might result in a prolonged duration of the disease, the increase in hospitalization cases, and fatalities [19].

The current study reported the high prevalence of multidrug resistance, which implies the increased risk of antimicrobial resistance of wastewater in a hospital [20, 21]. The wastewater discharge in the hospital is a major source of environmental contamination and can be a carrier of the dissemination of resistant bacteria to the external environment [22]. It reveals that

more efforts and activities are needed in the hospitals to improve infection control through adopting tough antimicrobial stewardship programs, controlling the trends of resistance regularly, and improving the use and use of hospital waste in order to reduce the spread of the resistant pathogens [23].

5. Conclusion

The findings of the study revealed that the threat posed by the emergence of multidrug-resistant (MDR) bacterial agents in the wastes of the hospital of the Alex Ekwueme Federal University Teaching Hospital, Abakaliki (FETHA) is alarming. The most prevalent pathogens were found to be *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. and extremely resistant to the commonly used antibiotics. This resistance has been witnessed particularly to ampicillin, amoxicillin, and fluoroquinolones which is a grave issue to the population health as these pathogens may develop serious infections. The paper shows that there is an imminent need to maximize the quality of infection control, the management of antimicrobial stewardship, and appropriate wastewater management towards preventing the further release of resistant bacteria into the environment. The fact that hospital wastewater is a potential source and a transport vehicle of the spreading the resistance of the pathogens also contributes to the need of effective sanitation and regular monitoring of antimicrobial resistance in the health facilities. The data is an excellent source of information on the extent of antibiotic resistance in the hospital and the potential danger to the surrounding society. In the light of the rising incidences of antimicrobial resistance, the issue of spread of resistant bacteria should be resolved at once.

Abbreviations

MDR	Multidrug-Resistant
AE-FETHA	Federal University Teaching Hospital, Abakaliki
UTIs	Urinary Tract Infections
PEF	Pefloxacin
CN	Gentamicin
APX	Ampiclox
Z	Zithromax
AM	Amoxicillin
R	Rifampicin
CPX	Ciprofloxacin;
AZ	Azithromycin
LEV	Levofloxacin
E	Erythromycin

Authors Contributions

Onu Euslar Nnenna: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Visualization,

Writing – original draft, Writing – review & editing

Ilang Donatus Chukwuma: Data curation, Supervision, Validation

Eze Blessing: Resources

Onu Henrrienta Chinaemerem: Formal Analysis, Project administration

Obaji Ezekiel Amuche: Methodology, Software

Conflicts of Interest

The authors declare no conflicts of interest.

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