Journal of Biotechnology Research
ISSN(e): 2413-3256, ISSN(p): 2413-8878
Vol. 2, No. 7, pp: 49-54, 2016
URL: http://arpgweb.com/?ic=journal&journal=16&info=aims

Resistance Profiles of Bacteria Isolated from Wastewater in the University of Maiduguri Teaching Hospital

Adam Mustapha* Department of Microbiology, Faculty of Science University of Maiduguri, PMB 1069 Bama Road Maiduguri Nigeria

Isa Tijani Department of Microbiology, Faculty of Science University of Maiduguri, PMB 1069 Bama Road Maiduguri Nigeria

H. S. Bello Department of Microbiology, Faculty of Science University of Maiduguri, PMB 1069 Bama Road Maiduguri Nigeria

H. Y. Ismail Department of Microbiology, Faculty of Science University of Maiduguri, PMB 1069 Bama Road Maiduguri Nigeria

Abstract Antibiotics have always been considered one of the wonderful discoveries of the 20th century. Their use as effective antibacterial agents was however short-lived, as bacteria started developing resistance almost as soon as the agents were discovered. The worrisome growth of antibiotic resistance is blamed primarily on the misuse and overuse of the agents in human and agricultural settings. This study was carried out to identify common bacterial species in hospital wastewater and to determine their pattern of resistance to commonly used antibiotics. Grab samples were collected from the general wastewater channel of The University of Maiduguri Teaching Hospital, Maiduguri. The bacterial isolates identified were *E. coli, S. enterica, P. aeruginosa, Proteus mirabilis, K. pneumoniae, V. cholerae, M. morganii, Proteus vulgaris* and *C. fruendii*. Antibiotic susceptibility of the isolates was assayed using the Modified Kirby-Bauer disc diffusion method. Resistance was highest with Nalidixic Acid (100%) and lowest with Ciprofloxacin and Streptomycin (20% each). Others include Ceporex and Ampicillin (88% each), Tarivid, Gentamycin and Septrin (50% each), Reflacine (63%) and Augmentin (75%). Out of all the antibiotics used, *E. coli* showed 100% resistance whereas *M. morganii* was susceptible to all the antibiotics except Gentamycin and Erythromycin. It was found that, except for *M. morganii*, all the isolates were multi drug resistant suggesting that they have been well exposed to antibiotics and thus, developed multi resistance. This emphasizes the need for surveillance on trends in antibiotic resistance and development of alternative therapy to tackle antibiotic-resistant bacteria.

Keywords: Antibiotic-resistant; Resistant bacteria; Nosocomial infections; Wastewater.

1. Introduction

The widespread and often inappropriate administration of antibiotics in livestock, pets and humans is generally acknowledged as the primary pathway for proliferation of antibiotic-resistant bacteria in the environment and the major cause for changes in the ecology of bacterial infections and in the nosocomial infections [1]. After ingestion or parenteral administration of antibiotics, they are excreted mainly into wastewater. Some are completely changed on excretion, while others are only modified. These antibiotics in combination with high microbial biomass (which is readily abundant in hospital effluents) and an abundance of nutrients, makes wastewater a potential habitat for horizontal gene transfer and selection of antimicrobial resistant bacteria [2, 3]. Resistance could also spread to man through consumption of antibiotic-enhanced livestock, their products or from their waste. Environments exposed to the hospital and agricultural waste typically contain both antibiotic-resistant bacteria as well as moderately elevated levels of antibiotics, providing examples of ecosystems in which antibiotircs may show ability to select resistant strains since antibiotics at less than therapeutic concentrations are ideal for selecting resistant species. While some resistant bacteria exist naturally in the environment, pathogens and non-pathogens get into the environment by several means, contributing to a web of resistance that includes, humans and the environment [4] with the hospital being the major breeding ground or reservoir.

Many researches have shown that hospitals that are supposed to be a place where infectious diseases can be cured have become a breeding place for antibiotic resistant bacteria in Nigeria, other developing countries and even in developed nations with advanced systems of hospital surveillance. This is worrisome because the effectiveness of antibiotics for medical applications declines. Infections once easily curable are now regarded as a growing threat

*Corresponding Author

from the drug-resistant microbial agents of these diseases [4].Concurrent studies on antibiotic prescription quality in hospitals, antibiotic residue levels in their wastewater and resistant bacteria in the effluents of the same hospitals are few [4]. Such kinds of studies are therefore important as they provide data that are useful in checking the spread of resistance among bacterial species. On the other hand, the persistence in antibiotic resistance has remain one of the greatest challenges of the 21st century, and originates appeals from several international health organizations asking for regional data in bacterial susceptibility patterns, especially for strains of nosocomial circulation. Therefore, identifying microorganisms commonly isolated in the nosocomial environment and their susceptibility patterns to antimicrobial drugs could be useful in tracing the origins and determining the persistence of bacteria potentially associated to hospital infections. Moreover, data on resistance patterns of nosocomial environments in Nigeria are few [5]. And since there is a global appeal for such information, this research will provide information that will be useful in taking steps to reduce, and if possible eliminate the scourge of antibiotic resistance in the future.

2. Materials and Method

2.1. Experimental Design

The research design was carried out at The University of Maiduguri and University of Maiduguri Teaching Hospital between the months of April and June 2015. The university and University teaching hospital are both located in Maiduguri metropolis, capital of Borno State, Nigeria. The sampling point was a concrete well at which the wastewater (WW) from all departments and units of the University teaching hospital collect. A metal ladder attached to the wall of the well was used for support while collecting samples. A generating set by the well pumps WW from the point to a WW channel where it meets WW from the staff quarters and other sections of the institution. All practical work was done in the laboratory of the Department of Microbiology, University of Maiduguri.

2.2. Sample Collection and Serial Dilution

Grab sampling was employed as all samples were collected at the same period and without any special machinery. Samples were collected in four (4) 20ml sterile plastic bottles in the morning and transported to the laboratory within 2 hours. Two (2) samples (S1 and S2) were taken from the WW subsurface, and were clearer while the rest (S3 and S4) were surface samples and more particulate. Serial dilution was done based on the principle that when soil sample or water sample along with bacterial colonies are taken, the result obtained in the form of reduced bacterial colonies would be more appropriate in order to get pure colonies. 1ml of each sample was inoculated into 9ml of sterile distilled water and serially diluted from one tube (containing 9ml sterile distilled water) to the next in an 8-fold dilution (108).

2.3. Isolation of Bacteria

The bacteria were quantified by plating 1ml each, of the selected serial dilutions of the bacterial suspensions. The 5th and 6th (105 and 106) dilutions of S1 and S2, and the 7th and 8th (107 and 108) dilutions of S3 and S4 were plated. Using the pour plate method, each sample dilution was poured in nutrient agar and incubated at 370C for 24 hours. Colony count was done and distinct colonies from each nutrient plate were subcultured separately on MacConkey (MAC) agar and incubated at 370C for 24 hours. Lactose fermentation was quantified and each colony from the MAC agar was further subcultured onto nutrient slants, from which all inoculums for further tests were taken.

2.4. Bacteriological Analysis and Identification

The bacterial isolates were characterized based on morphological and biochemical tests and identified using the guidelines of Bergey's Manual of Determinative Bacteriology.

2.5. Antibiotic Resistance Profile Determination

Antibiotic sensitivity testing carried out based Modified Kirby-Baur disc diffusion method, using OPTUDISC (10-tipped) multiple susceptibility discs. The discs used contained Tarivid (OFX) 10µg; Reflacine (PEF) 10µg; Ciprofloxacin (CPX) 10µg; Augmentin (AU) 30µg; Gentamycin (CN) 10µg; Streptomycin (S) 30µg; Ceporex (CEP) 10µg; Nalidixic Acid (NA) 30µg; Septrin (SXT) 30µg and Ampicillin (PN) 30µg. Each of the identified isolate was spread on a separate nutrient agar plate, and antibiotic disc dropped on the plate and incubated at 370C for 24 hours. Zones of inhibition were measured in mm with a ruler and designated as resistant, intermediate or sensitive in accordance with the standard set by British society for antimicrobial chemotherapy [6].

3. Results

3.1. Colony Count and Bacterial Isolates Identified

Bacterial colonies in a mixed culture form were quantified by counting colonies. Colony count showed a higher number in the particulate samples (S1 and S2) than in the clear samples (S3 and S4). The colony forming units in the S1 and S2 were 6.0 ×107/ml and 1.24 ×108/ml respectively; while that of S3 and S4 were 4.4 ×107/ml and 3.2 ×107/ml colonies per plate. Results from bacteriological analysis were used to identify the isolates. The isolates

identified were Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Vibrio cholerae, Morganella morganii, Proteus vulgaris and Citrobacter fruendii.

3.2. Antibiotic Susceptibility Test

Antibiotic sensitivity test was done using the 10-Tipped Multiple Susceptibility discs (OPTUDISC). The zones of inhibition around each antibiotic disc on the isolates were measured and are represented in table 2.

Table-1. Zone of Inhibition Shown by the Bacterial Isolates

Identified Isolate	Zone of Inhibition (mm)									
	OFX	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
Escherichia coli	N	N	N	N	14	16	N	N	N	N
Proteus vulgaris	N	N	18	N	32	N	N	N	N	N
Salmonella enteric	24	26	32	N	N	18	N	N	16	N
Pseudomonas aeruginosa	32	19	30	26	26	24	24	N	20	14
Proteus mirabilis	28	N	14	N	16	20	N	N	N	N
Vibrio cholerae	26	28	32	18	18	22	N	N	16	N
Salmonella enteric	32	28	32	28	16	24	N	N	24	30
Citrobacterfruendii	24	N	26	N	30	16	N	N	14	N

OFX=Tarivid; PEF=Reflacine; CPX=Ciprofloxacin; AU=Augmentin; CN=Gentamycin; S=Streptomycin; CEP=Ceporex; NA=Nalidixic Acid; SXT=Septrin; PN=Ampicillin

Highest resistance was exhibited by E. coli while Morganella morganii showed the least resistance to the respective antibiotics used. E. coli resisted all antibiotics used; Proteus vulgaris and P. mirabilis were next in resistance as each of them showed resistance to 8 antibiotics; C. fruendii resisted 7 antibiotics, while S. enterica and K. pneumoniae showed resistance to 6 antibiotics each; V. cholerae showed resistance to 4 antibiotics; Pseudomonas aeruginosa was resistant to 3 antibiotics while the least resistant, Morganella morganii, was resistant only to 2 only as shown in Table 1. A detailed representation of the isolates as resistant, intermediate or susceptible to each antibiotic is given in Table 2. The percentage resistance to the 10 antibiotics tested against each isolate is given in Figure 1. Thus, all the isolates except Morganella morganii exhibited multi-drug-resistance.

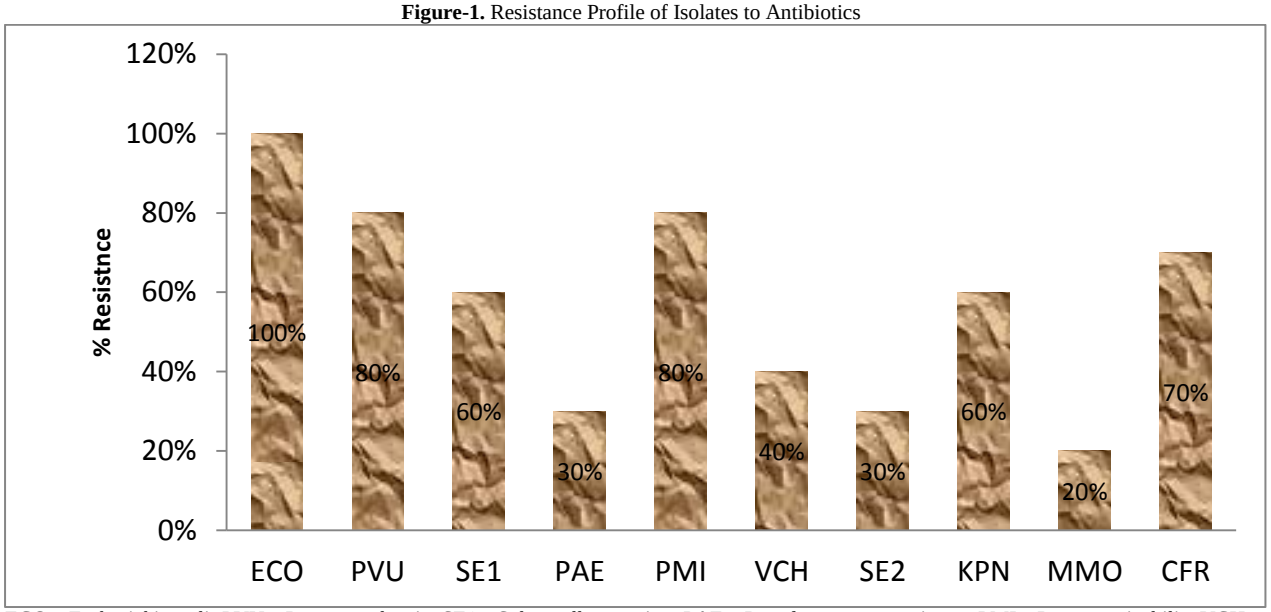
Table-2. Resistance Pattern of Isolates to the Respective Antibiotics used

Identified Isolates	Resistance Pattern									
	OFX	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
E. coli	R	R	R	R	R	R	R	R	R	R
Proteus vulgaris	R	R	I	R	S	R	R	R	R	R
Salmonella enterica	R	S	S	R	R	I	R	R	S	R
P. aeruginosa	S	R	S	S	S	S	S	R	S	R
Proteus mirabilis	I	R	R	R	R	S	R	R	R	R
Vibrio cholerae	I	S	S	R	I	S	R	R	S	R
Salmonella enterica	S	S	S	S	R	S	R	R	S	S
Citrobacter fruendii	R	R	S	R	S	I	R	R	R	R
Klebsiella pneumonia	NA	NA	S	NA	R	I	NA	NA	NA	NA
Morganella morganii	NA	NA	S	NA	R	S	NA	NA	NA	NA

R= resistant; I= intermediate; S= susceptible; NA= not applied

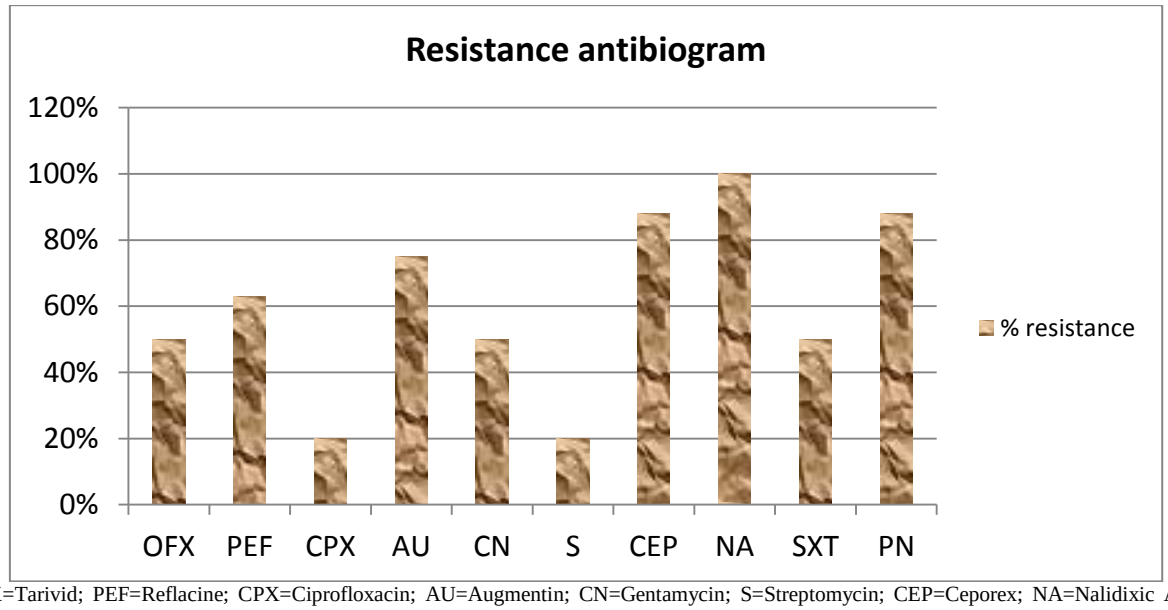
Figure 1**:** Resistance Profile of Isolates to Antibiotics

Figure-1. Resistance Profile of Isolates to Antibiotics



ECO= *Escherichia coli*; PVU= *Proteus vulgaris*; SE1= *Salmonella enterica*; PAE= *Pseudomonas aeruginosa*; PMI= *Proteus mirabilis*; VCH= *Vibrio cholerae*; SE2=*Salmonella enterica*; KPN= *Klebsiella pneumoniae*; MMO= *Morganella morganii*

Out of the antibiotics used, *K. pneumoniae* showed resistance to CN, AML, RD, E, CH and APX while *M. morganii* was susceptible to all the antibiotics except CN and E. Resistance to other antibiotics was highest with NA (100%) and lowest with CPX and S (20% each). CEP and PN were resisted by 88% of the isolates each while resistance to OFX, CN and SXT was 50%. Resistance to PEF was 63% while it was 75% against AU.



OFX=Tarivid; PEF=Reflacine; CPX=Ciprofloxacin; AU=Augmentin; CN=Gentamycin; S=Streptomycin; CEP=Ceporex; NA=Nalidixic Acid; SXT=Septrin; PN=Ampicillin;

4. Discussion

In the present study, a total of ten bacterial species were isolated and identified to be mostly members of the family Enterobacteria. Several studies have evaluated the microbiological content of hospital and urban wastewater and found that hospital wastewater contains pathogenic bacteria, with Bacillus spp., Staphylococci spp. and Streptococci spp. (all of which are Gram positive) being the most frequently encountered [7]. However, all the isolates identified in this research were Gram negative rods (GNRs). Studies have shown that GNRs are highly associated with nosocomial infections, have high prevalence in hospital wastewater (HWW) and possess resistance to most tested antibiotics [4, 7, 8]. The bacterial isolates identified were Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Vibrio cholerae, Morganella morganii, Salmonella enterica, Proteus vulgaris and Citrobacter fruendii. E. coli, K. pneumoniae, P. aeruginosa, Proteus mirabilis and C. fruendii (in decreasing order) have been shown to have the highest occurrence among Gram negative bacteria isolated in HWW studies [9]; [10, 11]. The highest prevalence of multiple drug resistance in this study was 100%. Multi drug resistance (MDR) has been shown to be high in Gram negative rods with Klebsiella spp., Enterobacter spp., E. coli and Pseudomonas spp. having significant MDR patterns [4, 10, 11]. This phenomenon is same as in the present

study where except for M. morganii, all isolates showed multi drug resistance with E. coli having the greatest resistance (100%) to the antibiotics tested against it. P. mirabilis and P. vulgaris were next with 80% resistance each. These were followed by C. fruendii (70%), S. enterica (60%) and K. pneumoniae (60%), V. cholerae (40%) while the least was Pseudomonas aeruginosa (30%). The resistance pattern of the P. aeruginosa isolate in this study was low (30%) but many other researchers have reported different results showing its high resistance to commonly used antibiotics [4, 7, 9].

Out of the antibiotics that were used against most of the isolates in this study, the highest resistance was shown against Nalidixic acid (100%) while the antibiotics with the highest activity (most efficacious) were Ciprofloxacin and Streptomycin (20% resistance). Others were Ceporex and Ampicillin (88% each), Augmentin (75%), Reflacine (63%), Tarivid, Gentamycin and Septrin (50%). This is in concert with previous findings in which low resistance to Ciprofloxacin was reported. Feleke, *et al.* [11] and Elmanama, *et al.* [9] reported similarly low resistance rates (12% and 2.4% respectively) to the antibiotic. However, resistance to Nalidixic acid observed in this study was higher than that reported by the later researchers. Among the other antibiotics used against K. pneumoniae and M. morganii only, Gentamycin and Erythromycin were resisted by both organisms; Amoxil, Rifampin, Chloramphenicol and Ampiclox were resisted by Klebsiella pneumoniae only while Norfloxacin and Levofloxacin were 100% active against both organisms. The present results were a little different from another study by Nigerian, where Enterobacteria were found to be 100% resistant to Septrin (30µg), Chloramphenicol (30µg), Amoxicillin (30µg) and Streptomycin (30µg); 90% resistance to Pefloxacin (10µg), Tarivid (30µg); 80% to Ciprofloxacin (10µg) and 70% resistance to Gentamycin (10µg) [4].

According to Kummerer [2], antibiotic concentrations calculated and measured in hospital effluents are in the same order of magnitude as the MICs for sensitive pathogenic bacteria. This phenomenon encourages selective pressure in bacteria that are able to survive. Although most of the human population does not come in contact with clinical wastewater, its effect would be adversely felt among populations close to final wastewater dumpsites (since antibiotics have been shown to have the ability to seep into groundwater which may be consumed) and with people who consume fruits and vegetables which have been irrigated with such wastewaters. The relatively high level of resistance to antibiotics recorded in this research is a pointer to the misuse and abuse of the agents in the environment. The notoriety of multidrug resistance has become a major global issue consuming a huge chunk of national budgets. Encountering multidrug resistance in this study is therefore worrisome and of public health concern most of the isolates identified here were common environmental and opportunistic pathogens.

5. Conclusion

This study showed that antibiotic resistant bacteria are present in hospitals WW in considerably large number. All isolates identified were Gram negative rods. The pattern of resistance shown by the isolates was daunting considering the fact that most of them are common pathogens and could come back to neighborhood through seepage. All the isolates except M. morganii showed multidrug resistance as they were resistant to more than two antibiotics used, which have different mechanisms of action. More studies and surveillance programs that would monitor developments and trends in antibiotic resistance in different environments should be given much more attention and sponsorship.

Conflict of Interests

The authors declare no conflict of interests.

Reference

[1] Olayemi, A. B. and Opaleye, F. I., 1990. "Antibiotic resistance among coliform bacteria isolated from hospital and urban wastewater." *World Journal of Microbiology and Biotechnology*, vol. 6, pp. 285-288.

[2] Kummerer, K., 2004. "Resistance in the environment." *Journal of Antimicrobial Chemotherapy*, vol. 54, pp. 311-320.

[3] Ottosson, J. R., Jarnheimer, P., Strenstrom, T., and Olsen, B., 2012. "A longitudinal study of antimicrobial resistant faecal bacteria in sediments collected from a hospital waste water system." *Infection Ecology and Epidemiology*, vol. 2, p. 7438.

[4] Bolaji, A. S., Akande, I. O., Iromini, F. A., Adewoye, S. O., and Opasola, O. A., 2011. "Antibiotic resistance pattern of bacteria spp. Isolated from hospital wastewater in Ede, Southwestern Nigeria." *European Journal of Experimental Biology*, vol. 1, pp. 66-71.

[5] Bouzada, M., Silva, V., Sa Moreira, F., Silva, A., and Diniz, C., 2010. "Antimicrobial resistance and disinfectants susceptibility of persistent bacteria in a tertiary care hospital." *Journal of Microbiology and Antimicrobials*, vol. 2, pp. xx-xx.

[6] British society for antimicrobial chemotherapy, 2012. "BSAC methods for antimicrobial susceptibility testing."

[7] Pandey, A., Afsheen, Ara, F., and Tiwari, S. K., 2011. "Isolation and characterization of multi drug resistance cultures from wastewater." *Journal of Pharmaceutical and Biomedical Sciences*, vol. 13, pp. 1-7.

[8] Schwartz, T., Kohnen, W., and Obst, U., 2003. "Detection of antibiotic resistant bacteria and their resistance genes in wastewater, surface water and drinking water biofilms." *FEMS Microbiology Ecology*, vol. 43, pp. 325-335.

[9] Elmanama, A. A., El kichariu, A. Y., and Mohsin , M., 2006. "Contribution of hospital wastewater to the spread of antibiotic resistance in comparison to non-health institution." *Journal of AL-Aqsa university*, vol. 10, pp. 108-121.

[10] Jim, B., 2011. "A perfect storm of antibiotic resistance." *British Food Journal*, vol. 108, pp. 1-119.

[11] Feleke, M., Mengistu, E., Yeshambel, B., and Walelegn, W., 2014. "Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia." *BMC Res Notes*, vol. 7, p. 21.