Original Article

Microbiological evaluation of health threat potentials of wastewater from different health-care categories in Lafia, Nigeria

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ABSTRACT

The increase in antibiotic resistance within clinical bacterial isolates is reducing the effort of antibiotic therapy in the treatment of infectious diseases. This study was aimed at evaluating the bacteriological health risks associated with wastewater effluents from different health-care outlets in Lafia, Nigeria. A total of 231 wastewater samples were collected in different units from three health-care outlets and analyzed for total bacterial counts and isolation of different bacterial species using standard microbiological techniques. Antibiotics were selected based on NCCLS criteria and susceptibility determined using the Kirby-Bauer disk diffusion method. Mean total heterotrophic bacteria count (THBC) of $1.37 \pm 1.96 \times 10^{11}$, $7.20 \pm 1.3 \times 10^{10}$, $1.00 \pm 1.64 \times 10^{11}$, 1.0×10^{11} CFU/ml was obtained from the laboratory, theater, in-patient wards, and labor room wastewater, respectively, from the various health-care outlets. There was no significant association ($P > 0.05$) between the THBC values from the different units and the different health-care outlets. Bacteria species isolated in wastewater from all outlets include *Staphylococcus aureus* (27.7%), *Escherichia coli* (26.5%), *Klebsiella* spp (13.2%), *Proteus* spp (10.3%), *Salmonella* spp (8.7%), *Pseudomonas aeruginosa* (5.4%), *Streptococcus* spp (4.8%), *Staphylococcus epidermidis* (1.7%), *Bacillus* spp (1.4%), and *Shigella* spp (0.3%). Isolates of most species were resistant to all the commonly used antibiotics with highest resistance obtained with amoxicillin. Wastewater from all outlets was found to harbor pathogenic bacterial species with multiple antibiotics resistance potentials. Effective management protocols of wastewater from the healthcare outlets need to be adopted before discharge to reduce the health risks, it may pose to the environment.

Keywords: Antibiotic resistance, bacterial pathogens, health risk, hospital effluents, microbial load

INTRODUCTION

Pathogenic bacteria are usually incriminated in nosocomial infections, especially in the environmental sites where poor hygiene exists.[1] These organisms can live and survive in lifeless surfaces for long period of time in the hospital domain. The bacteria can contaminate the meals and drinkable water of patients when exposed to them.[2]

In health-care facilities, water is used for several purposes and is being discharged as wastewater. This effluent usually contains countless numbers of microorganisms from different sources including laundering, sanitary wastes, dishes washings, laboratory, and human wastes. Depending on their sources, wastewater differs in their composition flow and volume.

Hospital effluents have been reported from the previous studies to comprise of antibiotics, radioactive element, anesthesia, heavy metals, disinfectants, and solvents. Human feces and antibiotics have been shown to harbor pathogenic *E. coli* and *S. aureus*.[3,4]

Wastewater from health facilities has been known to serve as a reservoir for harmful infectious pathogens. Potential health risks associated with wastewater include the transmission of infectious diseases by the microbial pathogens and the dissemination of antimicrobial resistance genes. Untreated hospital wastewater (HWW), therefore, possesses the risks of spreading antibiotic resistant bacteria in the environment. It has been considered a public health concern since it may contain not only pathogenic microorganisms but also many

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pollutants such as radioactive, chemical, and pharmaceutical wastes.[5] However, the main risk to public health is the fact that resistance genes carried by wastewater microorganisms can be transferred from environmental bacteria to human pathogen.[6-9]

Antibiotic resistance is now considered a serious threat to public health since it prevents the effective treatment of bacterial infections which can cause high mortality.[10] High consumption of antibiotics, their improper disposal, and excretion of drugs by humans and animals mainly account for the introduction of antibiotics and their metabolites into wastewater.[11]

In Nigeria, health-care facilities in many rural and semi-urban communities lack a functional medical wastewater treatment plant; hence, most HWW are channeled into open areas for natural remediation. Untreated wastewater effluents discharged into the environment directly or indirectly have been the possible cause of undocumented wastewater-related diseases in most communities. It has been assumed that HWW provides a favorable environment where multidrug resistant bacteria thrive due to their continuous exposure to low-level antibiotics.[12] However, direct evidence for the screening of resistant bacteria from this wastewater category has been lacking.[13] This study was, therefore, aimed at evaluating the pathogenic bacterial species associated with untreated wastewater from different health-care outlets in Lafia, Nigeria, and their multiple antibiotics resistance potentials.

MATERIALS AND METHODS

Study Area
The study area was Lafia, a semi-urban town located in North Central Nigeria and the capital city of Nasarawa State [Figure 1]. It has a total population of 330,712 inhabitants who are mainly farmers, businessmen, and artisans. Most settlements were usually congested with poor hygienic conditions. Few health facilities are available which are mostly primary and secondary health institutions. The health-care outlets used for this study were Primary Healthcare Centre Mararaba-Akunza, Medical and Diagnostic Hospital (MDH), and Dalhatu Araf Specialist Hospital (DASH), representing primary, secondary, and tertiary facilities, respectively, all located within the study area.

Sample Size Determination
The least sample size was determined using an average of 13.4% prevalence rate as reported by Ugwu *et al*.[14] The sample size was calculated as described by Thrusfield[15] using the formula

$$n = \frac{z^2 p(1-p)}{d^2}$$

where:
n = number of sample
p = prevalence rate of enteric pathogen of previous study =13.4% = 0.134
z = standard normal distribution at 95% confidence limit=1.96
d = absolute desired precision of 5% =0.05

Therefore: $n = \dfrac{3.84 \times 0.134 \times (1-0.134)}{0.0025} = 178.3$

Sample Collection
A facility-based and cross-sectional study was conducted at health-care facilities within Lafia Metropolis, Nigeria. Sampling was done thrice weekly (Mondays, Wednesdays, and Fridays) between the hours of 8 am and 12 noon. A total of 231 wastewater effluent samples were collected from four different units within the health-care facilities, namely, the theater ward, labor ward, inpatients ward, and laboratory. About 15 ml of each sample was collected into a sterile 20 ml universal container using a sterile syringe. All samples collected were transported in ice cold packs to the Microbiology Laboratory, Federal University of Lafia, within 6 h for analysis.

Sample Analysis
The effluents were agitated for even distribution of the bacterial cells. A 10-fold serial dilution of the samples was carried out as described by Torimiro *et al*.[16] An aliquot of 1.0 mL of each wastewater sample was dissolved in 9 mL of sterile distilled water to obtain 10^{-1} dilution. Further dilutions were performed using same diluent to 10^{-12}.

Determination of Total Heterotrophic Bacterial Counts (THBC) from Wastewater Samples
An aliquot of 1.0 mL from 10^{-11} and 10^{-12} sample dilutions was dispensed into petri dishes, pour plated into Nutrient Agar (Difco) and incubated at 37°C for 24 h. Colonies were counted and the results expressed as colony forming units per milliliter (cfumL^{-1}).

Identification of Isolates
Discrete colonies of all morphological types on nutrient agar plates were picked, sub-cultured, and maintained on nutrient agar slants at 8°C. The identity of all isolates was determined using standard conventional methods as reported by Cheesbrough.[17] The bacterial isolates were identified using morphological characteristics, cellular, and biochemical tests. Morphological characteristics were observed for each bacterial colony after resuscitation on nutrient agar plate at 35°C for 24 h. The appearance of the colony of each isolate on the media was studied and the characteristics observed include cell shape, elevation, edge, optical characteristics, consistency colony surface, and pigmentation. Biochemical tests carried out include catalase, hydrogen sulfide production
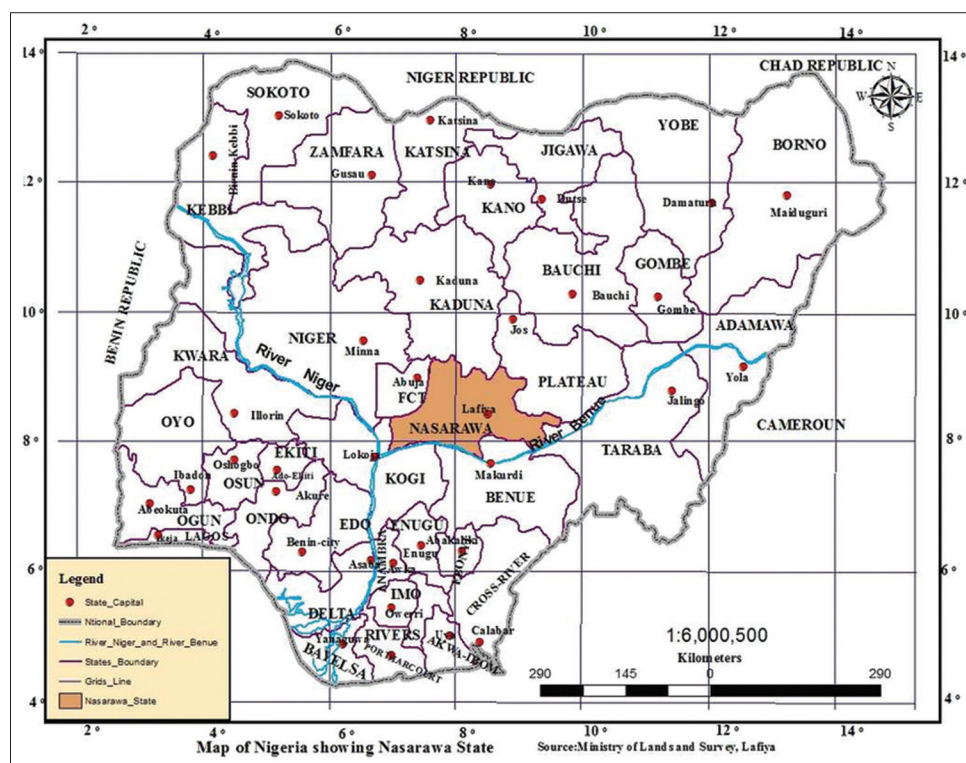
Figure 1: Map of Nigeria showing Nasarawa state and the study area (Lafia)

on triple sugar iron (TSI) agar (H_2S), indole, urease, methyl red, oxidase, coagulase, motility, citrate utilization, methyl red, voges-proskauer, starch hydrolysis, and sugar fermentation. The results were compared with those documented in Bergey's Manual of Determinative Bacteriology.[18]

Screening for *Salmonella* spp and *Shigella* spp

About 2.0 mL of each wastewater sample was transferred into 10 mL of selenite-F- medium (Difco) and incubated at $35°C$ for 24 h. Several loopful of the enriched sample was streaked on Deoxycholate Citrate agar (Difco) and incubated at $35°C$ for 48 h. Small colorless colonies were sub-cultured on nutrient agar and screened for *Salmonella* and *Shigella* species using biochemical tests such as oxidase, urease, growths on TSI agar slants, and motility medium as described by Udo and Nfongeh.[19]

Screening for *Vibrio* spp

About 1 mL of wastewater sample was transferred into 10 mL of sterile Alkaline peptone water (P H= 8.6) an incubated for 8 h at $8°C$ for enrichment. Several loopfuls of the enriched culture (taken from the surface) were streaked on Thiosulphate Citrate Bile Salt Sucrose agar (Difco) and incubated at $37°C$ for 24 h. Yellow and green colonies were sub-cultured on nutrient agar slants. Microscopic and biochemical tests such as Gram stain, oxidase, motility in peptone water, immobilization in distilled water, string test, growths in alkaline peptone water supplemented with 0%, 8%, and 10% NaCl, and slide agglutination tests were used to identify *Vibrio* species.[19]

Antibiotics Susceptibility Testing

Antibiotics susceptibility test of the identified bacteria was ascertained against 10 commonly used antibiotics adopting Kirby-Bauer disk diffusion method as described by Gosden *et al*.[20] The following 10 antibiotics were selected based on the National Committee for Clinical Laboratory Standard Assessment Criteria of 2012: Amoxicillin (10 µg), Cefuroxime (30 µg), Cefixime (30 µg), Erythromycin (15 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Cotrimoxazole (25 µg), Doxycycline (30 µg), Gentamicin (10 µg), and Amikacin (30 µg). Pure cultures of identified bacteria were inoculated in sterile nutrient broth and incubated at $37°C$ for 24 h. Two loopfuls (0.08 ml) of the suspension of each bacterial isolate (standardized by matching with $0.5 × 10^{-8}$ McFarland) were inoculated into 20 ml of sterile molten Mueller-Hinton agar in petri dishes. The disks impregnated with the required antibiotics were diligently placed on the Mueller-Hinton agar surface using sterile forceps and properly detached from each other to avoid protruding zones of inhibition. The diameter of the zone of inhibition around each disc was measured to the nearest millimeter and the results interpreted as Sensitive (S) or Resistant (R) based on standard susceptibility breakpoints as stated by CLSI.[21]

RESULTS AND DISCUSSION

HWW serves as a reservoir for nearly all clinically important antibiotics resistances. The importance of evaluating such environmental reservoirs for their bacteriological characteristics

is evident especially in situations where successive disease outbreaks could not be traced to an epidemiologic source as is the case in most underdeveloped communities.[12] The Global Action Plan on Antimicrobial Resistance drafted by the World Health Organization emphasizes on the need to understand the impact of human activities on the environment, particularly the spread of antimicrobial resistance among bacterial strains.[22]

Bacterial Counts in Wastewater from Different Health-care Categories

THBCs in wastewater samples from the different health-care categories are shown in Table 1. The mean THBC from the Primary Health Centre (PHC) ranged from $4.30 \times 10^{10} \pm 1.67$ to $7.20 \times 10^{11} \pm 1.88$ cfumL^{-1} while values obtained for the MDH and DASH were $4.74 \times 10^{10} \pm 1.31$–$2.30 \times 10^{11} \pm 2.90$ cfumL^{-1} and $7.25 \times 10^{10} \pm 1.13$–$1.93 \times 10^{11} \pm 2.43$ cfumL^{-1}, respectively. However, no significant association was observed in the THBC between the sampling units and the health-care categories. The high THBC values obtained in this study are a clear indication of the unhygienic conditions that prevailed in the health-care facilities. This may also be an indication of poor and inefficient management of wastewater in the health-care facilities. The absence of facilities for wastewater treatment before releasing it to the environment may contribute to the

dissemination of heavy microbial load from the health facilities to the environment similarly, Amouei *et al*.[23] obtained a value of 2.6×10^{10} cfumL^{-1} in a HWW from an Iranian University. Furthermore, a THBC range of 1×10^{2}–4.7×10^{9} cfumL^{-1} has been reported for industrial waste effluent.[24] However, lower range values of 1.6×10^{5}–8.3×10^{6} cfumL^{-1}[25] and 2.0×10^{6}–1.36×10^{7} cfumL^{-1}[26] in HWW have been reported. Contrary values for THBC have also been obtained from HWW in Kwara State, Nigeria with maximum mean values of $2.7 \pm 0.24 \times 10^{6}$ cfumL^{-1} during the wet season and $2.8 \pm 0.17 \times 10^{7}$ cfumL^{-1} during the dry season.[27] The differences in the results relative to that of this study may be due to possible differences in the hygienic conditions of the health-care facilities in the different locations. Furthermore, seasonality was not taken into consideration during this research.

Occurrence of Bacterial Species in Wastewater from Various Health-care Categories

The percentage occurrence of bacterial species in sections of all the three health-care categories is shown in Table 2. *S. aureus* had highest overall percentage of 27.7% followed by *E. coli* with 26.5%. Other species isolated include *Klebsiella* spp (13.2%), *Proteus* spp (10.3%), *Salmonella* spp (8.7%), *P. aeruginosa* (5.4%), *Streptococcus* spp (4.8%), *S. epidermidis*

Table 1: Mean total heterotrophic bacterial count (cfumL^{-1}) of sample collection points in all sampled hospitals

Sampling units	Mean counts (±SD)			Mean total	*F*	*P*
	PHC	MDH	DASH			
Laboratory	$9.43 \times 10^{10} \pm 1.38$	$1.10 \times 10^{11} \pm 1.89$	$2.10 \times 10^{11} \pm 2.37$	$1.37 \times 10^{11} \pm 1.96$	2.033	0.140
Labour room	$4.30 \times 10^{10} \pm 1.67$	$8.16 \times 10^{10} \pm 1.39$	$9.14 \times 10^{10} \pm 1.37$	$7.25 \times 10^{10} \pm 1.13$	1.025	0.365
In-patient ward	$1.20 \times 10^{11} \pm 1.88$	$2.30 \times 10^{11} \pm 2.90$	$2.10 \times 10^{10} \pm 2.37$	$1.93 \times 10^{11} \pm 2.43$	1.321	0.275
Theater	NA	$4.74 \times 10^{10} \pm 1.31$	$1.62 \times 10^{11} \pm 2.20$	$1.00 \times 10^{11} \pm 1.64$	5.154	0.029

PHC: Primary Health Center, MDH: Medical and Diagnostic Hospital; DASH: Dalhatu Araf Specialist Hospital, NA: Not available, SD: Standard deviation

Table 2: Percentage occurrence of bacterial species in wastewater from all health-care categories

Bacterial isolates	Healthcare categories				Total, *n* (%)	χ^2
	N	PHC, *n* (%)	MDH, *n* (%)	DASH, *n* (%)		
Staphylococcus aureus	175	51 (29.1)	63 (36.0)	61 (34.9)	175 (27.7)	0.090
Staphylococcus epidermidis	11	3 (27.3)	5 (45.5)	3 (27.3)	11 (1.7)	0.517
Streptococcus spp	30	6 (20.0)	10 (33.3)	14 (46.7)	30 (4.8)	0.000
Bacillus spp	9	1 (11.1)	1 (11.1)	7 (77.8)	9 (1.4)	0.046
Escherichia coli	167	53 (31.7)	57 (34.1)	57 (34.1)	167 (26.5)	0.132
Klebsiella spp	83	27 (32.5)	24 (28.9)	32 (38.6)	83 (13.2)	0.002
Proteus spp	65	22 (33.8)	21 (32.3)	22 (33.8)	65 (10.3)	0.000
Salmonella spp	55	13 (23.6)	14 (25.5)	28 (50.9)	55 (8.7)	0.000
Shigella spp	2	0	1 (50)	1 (50)	2 (0.3)	0.641
Pseudomonas aeruginosa	34	4 (11.8)	14 (41.2)	16 (47.1)	34 (5.4)	0.000
Total					631 (100)	-

PHC: Primary Health Center, MDH: Medical and Diagnostic Hospital, DASH: Dalhatu Araf Specialist Hospital, *N*: Total number of positive isolates, *n*: Number of positive isolates from the health-care category

(1.7%), *Bacillus* spp (1.4%), and *Shigella* spp (0.5%). A statistically significant difference was observed in the rate of occurrence of *Salmonella* spp, *Klebsiella* spp, *Proteus* spp, *P. aeruginosa,* and *Streptococcus* spp among the three health-care categories ($P < 0.05$). Most of these bacterial species have been shown to be associated with the gastrointestinal tract and the human body. Majority are also considered to be notorious pathogens with established disease-conditions common to developing countries. The isolation of these bacterial species may, therefore, serve as an indication of the human origin of the wastewater effluents and the health risks they pose to the environment. Similar bacterial species have been isolated in wastewater from different health-care environments.[28-32]

Antibiotic Resistance Profile of Various Bacterial Species from Wastewater Effluents

The antibiotics resistance patterns of the various bacterial isolates from the wastewater effluents are shown in Table 3. *E. coli*, *S. aureus,* and *Proteus* spp had highest percentage resistance values of 74.4%, 66.3%, and 53.4%, respectively, to amoxicillin. However, less than half each of all other isolates were resistant to all the antibiotics, they were subjected to. All *Shigella* species were resistant to cotrimoxazole and susceptible to all other antibiotics. Amoxicillin had highest percentage resistance value of 58.6% against all the isolates. In general, no statistically significant difference ($P > 0.05$) in the percentage resistance values was observed among the various isolates. Similarity in the antibiotic resistance pattern of the various isolates may be due to similarity in the wastewater characteristics from the various health-care outlets which must have ensured equal exposure of the pathogens to the antibiotics. Similar reports on high resistance to amoxicillin by wastewater isolates from hospital environment have been proposed.[31,33] This study also agrees with the results of some researchers though with slight differences in the antibiotics combinations.[25,26,28-30,34,35]

Cumulative Resistance Pattern of Bacterial Isolates from Various Health-care Categories

The overall antibiotics resistance pattern of all bacteria species isolated from the different health-care categories are shown in Figure 2. In general, all isolates had highest resistance to amoxicillin with values of 66.7%, 58.1%, and 50.6% obtained from PHC, MDH, and DASH isolates, respectively. The overall resistance values of isolates from the health-care outlets to the various antibiotics had no significant difference ($P > 0.05$). Uzoije *et al*.[35] also obtained similar cumulative resistance pattern using isolates from different hospital environments in Umuahia, Nigeria. Wastewater effluents from the various health-care outlets may, therefore, be considered to be of equal health risk to the receiving environments. The persistence of large amounts of antibiotics in health-care environments serves as a serious threat to the ecosystem since it could encourage resistance in microbes, which may result in an increase in

Table 3: Antibiotics resistance profile of various bacterial isolates from wastewater effluents

Bacterial isolates	N	AMX, n (%)	CEF, n (%)	CFX, n (%)	ERY, n (%)	CPR, n (%)	LEV, n (%)	COT, n (%)	DOX, n (%)	GEN, n (%)	AMI, n (%)
Staphylococcus aureus	175	116 (66.3)	54 (30.9)	47 (26.9)	62 (35.4)	62 (35.4)	51 (29.1)	44 (25.1)	45 (25.7)	53 (30.3)	60 (34.3)
Staphylococcus epidermidis	11	4 (36.4)	3 (27.3)	2 (18.2)	3 (27.3)	1 (9.1)	2 (18.2)	5 (45.5)	4 (36.4)	3 (27.3)	3 (7.3)
Streptococcus spp	30	11 (35.7)	10 (33.3)	9 (30.0)	9 (30.0)	9 (30.0)	9 (30.0)	8 (26.7)	10 (33.3)	13 (43.3)	16 (53.3)
Bacillus spp	9	4 (44.4)	1 (11.1)	2 (22.2)	3 (33.3)	2 (22.2)	0	3 (33.3)	2 (22.2)	0	2 (22.2)
Escherichia coli	167	127 (76.4)	53 (31.7)	45 (26.9)	50 (29.9)	51 (30.5)	44 (26.3)	45 (26.9)	51 (30.5)	50 (29.9)	50 (29.9)
Klebsiella spp	83	35 (42.2)	29 (34.9)	26 (31.3)	28 (33.7)	25 (30.1)	27 (32.5)	23 (27.7)	25 (30.1)	23 (27.7)	33 (39.8)
Proteus spp	65	36 (53.4)	18 (27.7)	19 (29.2)	22 (33.8)	18 (29.7)	27 (41.5)	27 (41.5)	19 (29.2)	19 (29.2)	17 (26.2)
Salmonella spp	55	18 (32.7)	20 (36.4)	18 (32.7)	20 (36.4)	24 (43.6)	23 (41.8)	16 (29.1)	16 (29.1)	18 (32.7)	17 (30.9)
Shigella spp	2	0	0	0	0	0	0	2 (100)	0	0	0
Pseudomonas aeruginosa	34	12 (35.3)	11 (32.4)	6 (17.6)	9 (26.5)	14 (41.2)	7 (20.6)	12 (35.3)	17 (50.0)	11 (32.4)	14 (41.2)
Total	631	370 (58.6)	199 (31.5)	174 (27.6)	209 (33.1)	206 (32.6)	186 (29.5)	185 (29.3)	189 (30.0)	190 ((30.1)	212 (33.6)

AMX: Amoxicillin, DOX: Doxycycline, CEF: Cefuroxime, GEN: Gentamicin, CFX: Cefexime, AMI: Amikacin, ERY: Erythromycin, COT: Cotrimoxazole, CPR: Ciprofloxacin, LEV: Levofloxacin, N: Total number of isolates screened, *n*: Number of resistant isolates
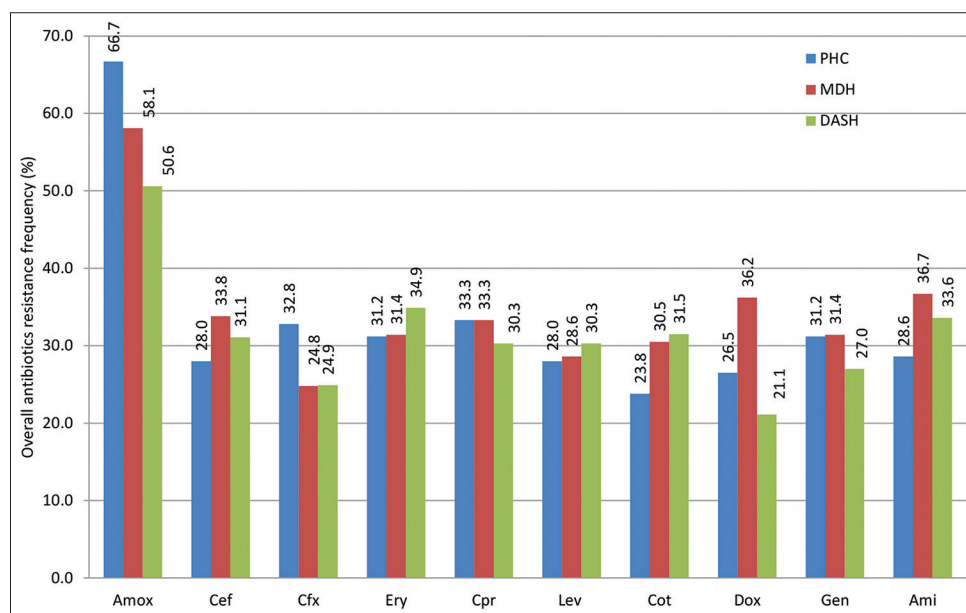
Figure 2: Overall antibiotics resistance pattern of bacterial isolates in wastewater effluents from various health-care categories. Amx: Amoxicillin, Cef: Cefuroxime, Cfx: Cefixime, Ery: Erythromycin, Cpr: Ciprofloxacin, Lev: Levofloxacin, Cot: Cotimoxazole, Dox: Doxycycline, Gen: Gentamicin, Ami: Amikacin

disease burden and also a change in the structure of the microbial community.

CONCLUSION

This study has shown that wastewater effluents from the various health-care categories have high microbial load. Bacterial species isolated are common in wastewater from all outlets. Most of the isolates are pathogenic and possibly from human origin. The existence of multidrug resistance among the isolates has been well established. Discharge of untreated wastewater from the various health-care outlets to the environment will certainly have a severe health risk implication.

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CONFLICT OF INTEREST

All items used in this research were obtained locally and mainly used in our area of research. There is, therefore, no conflict of interest between the authors and producers of such items.

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