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ANTIBIOTIC RESISTANCE PROFILE OF AEROBIC BACTERIA FROM ENVIRONMENTAL SURFACES OF SOME VETERINARY CLINICS IN ENUGU STATE, NIGERIA

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ABSTRACT

This study was conducted to determine the prevalence and antibiotic resistance profile of aerobic bacterial organisms from environmental surfaces in veterinary clinics in Enugu State, Southeast, Nigeria. Surface swabs of some equipment and floors in some units were collected from 4 selected veterinary clinics consisting of 3 government–owned clinics and a private clinic. The swabs were cultured and aerobic bacterial organisms identified by standard microbiological methods. Antibiogram of the isolates was determined by disc diffusion procedure. A total of 56 aerobic bacteria were isolated from 46 swab samples cultured. The bacteria belonged to 7 genera namely: Bacillus (39.3%), Staphylococcus (32.5%), Pseudomonas (8.9%), Klebsiella (5.4%), Escherichia coli (3.6%), Citrobacter (3.6%) and Proteus (1.8%). Sixty percent of the isolates were obtained from University of Nigeria Veterinary Teaching Hospital, 14.3% from Zonal Veterinary Clinic Nsukka and 12.5% each from Enugu State Veterinary Clinic and Eva Veterinary Clinic. Out of 43 Gram-positive isolates, 55.8% were resistant to ampicillin and ceftazidime, 39.5% to tetracycline, 27.9% to erythromycin, 20.9% to cefoxitin and streptomycin, 9.3% to ceftriaxone and 2.3% to ciprofloxacin and gentamicin. Out of 13 Gram-negative isolates, 84.6% were resistant to ampicillin, 61.5% to cefoxitin, 53.8% to ceftazidime, 46.2% to tetracycline, 38.5% to ceftriaxone, 30.8% to streptomycin, and 7.7% to imipinem and ciprofloxacin. This study has shown that antibiotic-resistant aerobic bacteria contaminate surfaces in veterinary clinics in Enugu State, Nigeria.

***KEY WORDS*:** Veterinary clinics, Environment, Nosocomial infection, Antibiogram.

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INTRODUCTION

Nosocomial infections or hospital associated infections are infections acquired by a patient and/or client on visitation to a hospital and/or on hospitalization. This type of infection is caused by microorganisms harboured by the hospital patients, hospital workers and those contaminating the environmental surfaces

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in the hospital [1]. These organisms have been found to survive for long periods on many different surfaces in the hospital environment and they thrive better in hospitals with poor biosecurity measures [2-4]. Reports have shown that because these organisms are continually exposed to antibiotics in the hospital environment, they often evolve resistance mechanisms against these drugs [1,5]. Development of resistance in these bacteria often results in difficulty in treating infections caused by these organisms and such infections often have fatal outcome [6].

There have been increasing reports of antimicrobial-resistant aerobic bacteria in animals and veterinary professionals which has made these bacteria a potential emerging problem in veterinary hospital environments [7-11]. Sources of these antimicrobial-resistant environmental surfaces-contaminating organisms in veterinary hospitals include those carried on the body of the clients, veterinary professionals and animals, and those that are shed by animals in their discharges and faeces [12]. Environmental surface contamination by these antimicrobial-resistant organisms has been implicated as sources of zoonotic and nosocomial infections in veterinary clinics [3, 11]. Environmental surfaces from which these resistant organisms can be contracted include medical equipment such as the weighing balance, washing hand basins, examination table, stethoscope, thermometer, cages and clinic floors [3]. Thus, animal owners, clients, veterinary professionals, and animals contract these potential nosocomial organisms when they have direct contact with contaminated surfaces in the veterinary clinics [5,13].

Increase in incidence of nosocomial infections within veterinary settings, has necessitated the conduct of surveillance studies on environmental surface contaminations in veterinary hospitals in countries such as Canada [3], United Kingdom [14], Japan [13], United States [9, 15] and Malaysia [5] with a view to devise control measures. No such study has been conducted in any veterinary clinics in Nigeria. The objective of this study was therefore to determine the prevalence and antibiotic resistance profile of aerobic bacteria isolated from environmental surfaces of selected veterinary clinics in Enugu State, Southeast Nigeria.

MATERIALS AND METHODS

Sampling

Surface swabs from wash hand basins (WHB), weighing balance (WB), treatment tables (TT), floor of treatment rooms (FTR), operating table (OT), cages in out-patient units (COPU) and floor in out-patient units (FOPU) were collected from 4 purposively-selected veterinary clinics in Enugu State. These clinics consisted of 3 government-owned clinics (University of Nigeria Nsukka Veterinary Teaching Hospital [UNVTH], Zonal Veterinary Clinic, Nsukka [ZVCN] and Enugu State Veterinary Clinic [ESVC]) and a private clinic (Eva Veterinary Clinic, Emene Enugu). The samples were collected between July, 2011 and November, 2011. Each of the clinics was visited once to avoid re-sampling. The samples were collected using sterile swab sticks moistened with sterile normal saline by rolling over the surfaces. The swabs were transported aseptically in ice packs to the Microbiology Laboratory of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka within 2 hours of collection.

Isolation and phenotypic identification of aerobic bacteria

The swabs were inoculated into nutrient broth (Oxoid®) and incubated at 37°C for 24 hours aerobically. A loopful of each broth culture was streaked on nutrient agar (Oxoid®), Mac Conkey agar (Oxoid®) and 7.5% salt agar, and incubated at 37°C for 24 hours. Morphologically distinct colonies were purified by sub-culturing on fresh media and incubating at 37°C for 24 hours. Purified colonies were used for Gram staining and stocked on nutrient agar slant at 4°C until needed for further identification. Colonies that were Gram-negative rods were sub-cultured on eosin methylene blue agar (Oxoid®), incubated at 37°C for 18 hours and observed for greenish metallic sheen appearance. They were further subjected to biochemical tests such as citrate, urease, oxidase, and triple sugar iron agar test, while colonies that were Gram-positive were subjected to catalase test.

Determination of antibiogram of isolates

This was carried out using disc diffusion method [16]. The isolates were sub-cultured on nutrient agar, incubated at 37^{o}C for 24 hours. Then colonies for each of the isolate were adjusted to 0.5 McFarland's turbidity standard (equivalent to $1x10^{8}$ colony forming unit/ml) in sterile phosphate buffered saline. The standardized broth culture was used to inoculate sterile Mueller-Hinton agar plate using sterile swab stick.

Ten antibiotics discs (Oxoid®) which included: gentamicin (10µg), streptomycin (5µg), erythromycin (15µg), ciprofloxacin (5µg), imipenem (10µg), ampicillin (10µg), cefoxitin (30µg), ceftazidime (30µg), ceftrioxone (30µg) and tetracycline (30µg) were placed strategically on each inoculated Mueller-Hinton agar plate and the plates were incubated at 37^{o}C for 18 hours. After incubation, the zone of inhibition around each disc was measured with a meter rule. Each test was performed in triplicate and the mean inhibitory zone diameter (IZD) was calculated for each isolate and each antibiotic to the nearest whole millimetres. The mean IZD was interpreted as resistant or susceptible according to the Clinical and Laboratory Standards Institute (CLSI) [17] criteria.

Data presentation

Data generated were analyzed descriptively and expressed in percentages

RESULTS

Isolation rates of aerobic bacteria from veterinary clinics in Enugu State

A total of 46 surface swabs were collected and processed for isolation and identification of aerobic bacteria. From the 46 swab, 56 aerobic bacterial isolates belonging to 7 genera namely: *Bacillus* (22/56, 39.3%) *Staphylococcus* (21/56, 37.5%), *Pseudomonas* (5/56, 8.9%), *Klebsiella* (3/56, 5.4%), *Escherichia coli* (*E. coli*) (2/56, 3.6%), *Citrobacter* (2/56, 3.6%) and *Proteus* (1/56, 1.8%) were isolated (Table 1). Thirty-four (60.7%) of the isolates were obtained from the UNVTH; 8 (14.3%) from ZVCN, and 7(12.5%) from each of ESVC and EVC.

Table 1: Isolation rates of aerobic bacteria from veterinary clinics in Enugu State

Clinic	Number (Percent) of isolates obtained							
	Staph.	***Bacillus***	***Pseudo.***	***Kleb.***	***E. coli***	***Citro.***	***Proteus***	**Total (%)**
UNVTH	13 (22.3)	10 (17.9)	5 (8.9)	3 (5.4)	2 (3.6)	1 (1.8)	0 (0)	34(60.7)
ZVCN	0 (0)	8 (10.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8(14.3)
ESVC	4 (7.1)	2 (3.6)	0 (0)	0 (0)	0 (0)	1 (1.8)	0 (0)	7(12.5)
EVC	4 (7.1)	2 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	7(12.5)
Total	21 (37.5)	22 (39.3)	5 (8.9)	3 (5.4)	2 (3.6)	2 (3.6)	1 (1.8)	6(100)

Keys: UNVTH = University of Nigeria Veterinary Teaching Hospital; ZVCN = Zonal Veterinary Clinic Nsukka; ESVC = Enugu State Veterinary Clinic, Enugu; EVC = Eva Veterinary Clinic, Enugu; *Staph = Staphylococcus; Pseudo = Pseudomonas*; *Kleb. = Klebsiella; Citro. = Citrobacter*.

Distribution of aerobic bacteria from various surfaces in veterinary clinics in Enugu State

In the UNVTH, out of the 8 surface swabs cultured, all (100%) gave positive culture of aerobic bacteria. Of the 34 aerobic bacterial isolates obtained, the highest isolation (9/56, 16.1%) was obtained from the floor of operating unit (FOPU), while the least (2/56, 3.6%) was obtained from the operating table (OT) and cages in out-patient units (COPU) (Table 2). In the ZVCN, out of the 8 surface swabs cultured, 3(37.5%) gave positive culture of aerobic bacteria. The highest (4/56, 7.1%) and the least (1/56, 1.8%) number of isolates was obtained from wash hand basin (WHB) and floor of treatment room (FTR), respectively (Table 2). In ESVC, out of the 8 surface swabs cultured, 3(37.5%) yielded positive culture of

aerobic bacteria. The highest (4/56, 7.1%) and the least (1/56, 1.8%) number of isolates was obtained from the FTR and WHB, respectively (Table 2). In EVC, out of the 8 surface swabs cultured, 3(37.5%) yielded positive growth of aerobic bacteria. The highest (4/56, 7.1%) and least (1/56, 1.8%) number of aerobic bacteria isolates were obtained from the FTR and WHB, respectively (Table 2).

Table 2: Distribution of aerobic bacteria isolated from surfaces of veterinary clinics in Enugu State

Site	**Number of isolates obtained**							
	Staph	***Bacillus***	***Pseudo***	***Kleb.***	***E. coli***	***Citro.***	***Proteus***	**Total (%)**
University of Nigeria Veterinary Teaching Hospital								
WHB	1	1	1	0	0	0	0	3(5.4)
WB	2	1	0	0	0	0	0	3(5.4)
TT	2	0	1	0	0	1	0	4(7.1)
FTR	2	0	3	0	1	0	0	6(10.7)
OT	1	1	0	0	0	0	0	2(3.6)
FOR	1	4	0	0	0	0	0	5(8.9)
FOPU	4	2	0	2	1	0	0	9(16.1)
COPU	0	1	0	1	0	0	0	2(3.6)
Total (%)	13(22.3)	10(17.9)	5(8.9)	3(5.4)	2(3.6)	1(1.8)	0(0)	34(60.7)
Zonal Veterinary Clinic Nsukka								
WHB	0	1	0	0	0	0	0	1(1.8)
TT	0	3	0	0	0	0	0	3(5.4)
FTR	0	4	0	0	0	0	0	4(7.1)
Total	0(0)	8(10.7)	0(0)	0(0)	0(0)	0(0)	0(0)	8(10.7
Enugu State Veterinary Clinic								
WHB	0	0	0	0	0	1	0	1(1.8)
TT	1	1	0	0	0	0	0	2(3.6)
FTR	3	1	0	0	0	0	0	4(7.1)
Total (%)	4(7.1)	2(3.6)	0(0)	0(0)	0(0)	1(1.8)	0(0)	7(12.5)
Eva Veterinary Clinic, Enugu								
WHB	1	0	0	0	0	0	0	1(1.8)
TT	1	1	0	0	0	0	0	2(3.6)
FTR	2	1	0	0	0	0	1	4(7.1)
Total (%)	4(7.1)	2(3.6)	0(0)	0(0)	0(0)	0(0)	1(1.8)	7(12.5)

Keys: % = percent; UNVTH = University of Nigeria, Veterinary Teaching Hospital; ESVC = Enugu State Veterinary Clinics; ZVCN = Zonal Veterinary Clinics, Nsukka; EVC = Eva Veterinary Clinic, Enugu; WHB = wash hand basin; WB = Weighing balance; TT = treatment table; FTR = floor in treatment room; OT = operating table; FOR = floor in operating room; FOPU = floor in out-patient units, COPU = cage in out-patient units; *Staph = Staphylococcus;Pseudo = Pseudomonas.*

Antibiogram of aerobic bacterial isolates from surfaces in veterinary clinics in Enugu State
All (100%) the Gram-positive aerobic bacteria isolates were susceptible to imipinem. Twenty-four (55.8%) of the isolates were resistant to ampicillin and ceftazidime, 17 (39.5%) to tetracycline, 12 (27.9%) to erythromycin, 9 (20.9%) to cefoxitin and streptomycin, 4 (9.3%) to ceftriaxone and 1(2.3%) to ciprofloxacin and gentamicin (Table 3).

All (100%) the Gram-negative aerobic bacterial isolated were susceptible to gentamicin. Eleven (84.6) were resistant to ampicillin, 8 (61.5%) to cefoxitin and erythromycin, 7 (53.8%) to ceftazidime, 6 (46.2%) to tetracycline, 5 (38.5%) to ceftriaxone, 4 (30.8%) to streptomycin, and 1 (7.7%) to imipinem and ciprofloxacin (Table 3).

Table 3: Antibiogram of Gram-positive and Gram-negative aerobic bacteria isolated from veterinary clinics in Enugu State

Antimicrobial agent	Number (Percentage) of aerobic bacteria			
	Gram-positive (n = 43		Gram-negative (*n = 13*)	
	Resistant	Susceptible	Resistant	Susceptible
Ampicillin	24(55.8)	19(44.2)	11(84.6)	2(15.4)
Imipinem	0(0)	43(100)	1(7.7)	12(92.3)
Ciprofloxacin	1(2.3)	42(97.7)	1(7.7)	12(92.3)
Cefoxitin	9(20.9)	34(79.1)	8(61.5)	5(38.5)
Ceftazidime	24(55.8)	19(44.2)	7(53.8)	6(46.2)
Ceftriaxone	4(9.3)	39(90.7)	5(38.5)	8(61.5)
Erythromycin	12(27.9)	31(72.1)	NT	NT
Gentamicin	1(2.3)	42(100)	0(0)	13(100)
Streptomycin	9(20.9)	34(79.1)	4(30.8)	9(69.2)
Tetracycline	17(39.5)	26(60.5)	6(46.2)	7(53.8)

NT = Not Tested

DISCUSSION
In the present study, 56 aerobic bacteria belonging to 7 genera were isolated from various surfaces in the veterinary clinics indicating gross contamination of surfaces in veterinary clinics in Enugu State. Recent studies showed that the environment in veterinary clinics may be potential source of aerobic bacteria [5, 11]. Isolation of both Gram-positive and Gram-negative bacteria suggests that both types of bacteria constitute environmental surface contaminants in veterinary settings. Muhammad *et al*. [18] also reported isolation of both types of bacteria from human hospitals in northern Nigeria. The two Gram-positive bacteria genera isolated had the highest isolation prevalence of 39.3% for *Bacillus* and 37.5% for *Staphylococcus*. This finding agrees with the report of Inweregbu *et al*. [1] that Gram-positive organisms are the most frequently isolated organisms from hospital environmental surfaces. The ability of these Gram-positive bacteria to survive longer than their Gram-negative counterparts on environmental surfaces has been related to their ability to tolerate adverse environmental conditions [19]. *Bacillus* species form spores in the environment and *Staphylococcus* species are resistant to desiccation [19, 20]. However, the two organisms are established environmental contaminants and may not be pathogenic. The 37.5% *Staphylococcus* isolation rate in this study is higher than the 12% and 27% respectively reported by Hoet *et al*. [11] and Hamilton *et al*. [15] from veterinary hospital environment in USA.

Pseudomonas which was isolated at the rate of 8.9 % in this study has been reported by Yetkin *et al*. [21] to be an established nosocomial pathogen. The fact that all the Gram-negative organisms (i.e. *E. coli,*

Klebsiella, *Citrobacter* and *Proteus*) isolated belonged to the family Enterobacteriaceae implies that the organisms survived on the dry habitats. Reports have shown that enteric organisms can survive on dry hospital surfaces for very long periods up to 16 months [22, 23]. Presence of the enteric organisms on the sampled surfaces could have been as a result of improper cleaning of faecal materials discharged by the animals within the clinics. It could also be that the organisms were carried on the foot of animals or clients into the clinics. Moreover, isolation of these enteric organisms from other surfaces apart from the floors indicates that the hands of the veterinary professionals were inadequately cleaned following contamination by faecal material. Also cross-contamination by wind, cloth of the veterinary professionals and animals are possible sources of the organisms.

The fact that isolation rate of aerobic bacteria was highest (60.7%) in the UNVTH, suggests that the environmental surfaces in the teaching hospital were contaminated more than the other veterinary clinics. This is further supported by the fact that all the surface swabs cultured yielded positive growth of aerobic bacteria. This heavy contamination in UNVTH may be because it is a tertiary veterinary hospital and therefore records more human and animal traffic than the other clinics. Thus, with these individuals and animals serving as possible vehicles for bacterial transmission, the hospital surfaces become more contaminated than the others. The highest isolation frequency from FOPU in the UNVTH may be related to the fact that attending personnel and animals tread on the floors thereby introducing organisms. It might also be attributed to the fact that admitted animals defecate and urinate on the FOPU, thereby contaminating the surfaces. There is therefore the need to improve biosecurity measures in the hospital in order to reduce the level of contamination of surfaces.

The low prevalence of aerobic bacteria from the OT may be because the OT is usually disinfected after each surgical procedure with the operating room tightly closed. In ZVCN, the highest prevalence of aerobic bacteria from the WHB may suggest that it is either disinfectant is not added in the water in the WHB or that the dilution used is not effective against the contaminating organisms. The reverse might be the case in ESVC and EVC where the least prevalence was obtained from the WHB. The least prevalence from the FTR suggests low contamination. This low FTR contamination may be as a result of minimal visit by clients and animals to the clinic. It might also be that the biosecurity measures taken in the clinic was able to reduce the contamination. The reverse might be the case in ESVC and EVC where the highest prevalence was from the FTR.

The high rate (55.8%) of resistance to ampicillin among the Gram-positive isolates, suggest that the organisms have developed resistance to the drug. However, among the Gram-negative bacteria, resistance rate to ampicillin was high (84.6%). This high ampicillin resistance in both types of bacteria may have been mediated by the production of beta-lactamase which is the commonest mechanism of beta-lactam resistance [24]. The 55.8% and 53.8% resistance rates to ceftazidime among the Gram-positive and Gram-negative bacteria, respectively, suggest that the isolates could have produced extended spectrum beta-lactamases (ESBLs). This resistance to ceftazidime in this study may have resulted due to acquisition of ESBLs genes. The resistance rates of 61.5% and 38.5% resistance to cefoxitin and ceftriaxone, respectively, among the Gram-negative bacteria were higher than 20.9% and 9.3% rates, respectively, among their Gram-positive counterparts. This variation in cefoxitin and ceftriaxone resistances indicates that the Gram-negative bacteria isolated may have produced ESBLs more than the Gram-positives. Ceftazidime, cefoxitin and ceftriaxone are third-generation oxyimino-cephalosporin produced to counter the high level resistance of bacterial organisms to first- and second-generation beta-lactams [25]. These organisms resistant to extended spectrum antibiotics could be contracted by humans and/or animals, thereby transferring the ESBLs resistance genes to other organisms in the individual - this would consequently confer resistance to many other antibiotics thus, portending health risks.

The low rates of resistance to imipenem (0% and 7.7%) among the Gram-positives and Gram-negative bacteria respectively, suggest that the organisms were highly susceptible to the drug. This high

susceptibility may be because imipenem is not a commonly used drug in both human and veterinary medicine in Enugu State. This low usage of imipenem may have resulted to low selection pressure exerted against it by the bacterial isolates. Similarly, low resistance rates to ciprofloxacin by both types of bacteria may be related to low usage of fluoroquinolones in Enugu State.

The low rates of resistance to gentamicin by both types of bacteria may not be unconnected to the fact that gentamicin is a banned drug and therefore may no longer be in use in Enugu State. This could have resulted to minimal exposure of the isolates to gentamicin and hence low selection pressure. The higher rate of resistance to erythromycin among the Gram-negatives (61.5%) indicates that the Gram-negative isolates exerted selection pressure to the drug more than the Gram-positive isolates (27.9%). This may also explain the higher rate of resistances to streptomycin and tetracycline among the Gram-negative bacteria than among their Gram-positive counterparts.

In conclusion, this study has shown that antibiotic-resistant Gram-positive and Gram-negative aerobic bacteria contaminate environmental surfaces in veterinary clinics in Enugu State, Nigeria. This heavy bacterial surface contamination is probably due to poor biosecurity measures. Therefore, there is need to step up biosecurity measures in these veterinary clinics in order to minimize the risk of nosocomial infections and cross-contaminations.

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