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Full Length Research Paper

Phenotypic characterization of nosocomial bacterial species on contact surfaces of veterinary clinics and hospitals in Abuja, Nigeria

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Nosocomial bacterial infections are responsible for a variety of diseases in man and animals. 150 Samples were conveniently collected from contact surfaces of Veterinary Clinics and Hospital and analyzed using culture, microscopy, biochemical and in-vitro antibiotic testing. The results showed *Bacillus* species and *Escherichia coli* had highest prevalence of 14.7% each, followed by *Staphylococcus aureus* (13.5%), *Streptococcus uberis* and *Salmonella enterica* (12.0% each), *Listeria monocytogenes* (10.7%), *Corynebacterium diphtheria* and *Proteus vulgaris* (5.4 %) each. *Micrococcus luteus*, *Shigella*, *Klebsiella*, and *Yersinia enterocolitica* (2.7%). On premises, hospital premises 'A' had prevalence of 29.3% while hospital premise "B" had 17.3%. Contact surfaces showed prevalence of 7.3% in surgical tables to 2.7% in surgical equipment. Bacterial counts revealed that 6.5 x106 and 1.0 x 104. Chi-square showed Pearson CM-square of X2=2.529 (df2). P <0.05. Antibiotic resistance was Amoxicillin (17%), Trimethoprim (17%), Peflacin (33%) and Cefalexin (25%). Susceptibility was Gentamycin (67%), Ciprofloxacin (88%), Ofloxacin (58%), Streptomycin (50%), Ampicillin (67%) and Nalidixic acid (33%). Minimum inhibitory concentrations were 0.125, 0.1, 0.115, 0.10, 0.125, and 0.625 µg/ml). Questionnaire survey revealed that 36% respondents had no knowledge of nosocomial infection while 62% failed to sanitize their hands. Veterinary Clinics and Hospitals in Abuja are thus contaminated with nosocomial bacteria which are resistant to antibiotics. Awareness sensitization and hygienic measures are necessary in Nigerian veterinary clinics.

Keywords: Characterization, clinics, contact surfaces, nosocomial, phenotypic, veterinary clinics.

INTRODUCTION

Nosocomial or Hospital Acquired Infections (HAI) are any localized or systemic conditions that occurs in a patient as a result of the presence of infectious agents or its toxins that were not present but are being incubated or acquired at the time of hospital admission. They occur worldwide and are often acquired during hospitalization of the patient at the clinic or hospital (Horan *et al*., 2008). The incidence normally occurs at 48 hours or more after admission of the patient and within 30 days of discharge.

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Hospitalized patients have an unusually high risk of developing infections due to both intrinsic risk factors (endogenous infection) such as underlying disease conditions and extrinsic risk factors (exogenous infection) like lower immunity coupled with direct contact with bacteria contaminated surfaces during hospitalization (Emori & Gaynes, 1993).

The dissemination of health care associated infections often originates from cross contamination with the most common means of pathogens transference occurring between the hands of health professionals and patients (De-Oliveira and Damasceno, 2010). However, contact surface environments in the hospital contribute to the

spread of bacteria pathogens. These surfaces serve as vehicles for the retention, habitation, growth, multiplication and subsequent transmission of nosocomial infectious organisms (Oliveira and Damasceno, 2010). The environment may have greater effects on Intensive Care Units (ICU) because of the severe and unstable clinical conditions of patients in addition to factors such as poor cleaning, disinfection, physical structures, and surfaces in certain ICU's (Weber and Rutola, 2000). Some contact surfaces which were identified to harbour nosocomial bacteria pathogens includes; stethoscopes, endoscopes, computer keyboards, thermometers, multiple-dose vials, sponge pots, intravenous catheters and treatment tables of Veterinary Clinics and Hospitals (Van den Berg *et al*., 2000). Death has been reported in some animals that have become infected while been hospitalized (Dallap *et al*., 2010). An estimated morbidity and mortality rates of 41% and 11.1% respectively was reported in dogs in small animal hospitals. Also, 8.6-33% and 4.3- 13% respective mortality and morbidity rates were reported in cattle in large animal hospitals in some veterinary clinics in north eastern Nigeria (Benedict *et al*., 2008). The financial impact due to the nosocomial outbreaks in America was estimated to about $550,000 and approximately $4.12 million of lost revenue was incurred due to closure and renovations of hospitals (Dallap *et al*., 2010). Hospital acquired infections occur at a rate of 4.5% per 100 hospital admission and cost $6.65 billion in inflation-adjusted dollars for 2007 (around $25,000 per patient).

A survey of the American Veterinary Medical Association (AVMA) accredited Veterinary teaching hospitals by Benedict *et al*. (2008) revealed that 82% (31/38) of hospitals involved had identified outbreaks of nosocomial infections. Most veterinarians suffer bites from dogs and cats which become infected by aerobic and anaerobic bacteria. It is also very important to note that outbreaks of bacterial nosocomial infections can tarnish the reputation of most veterinary hospitals and may depreciate client confidence or morale problems amongst hospital staff (Morley and Weese, 2008).

Nosocomial infection rarely occurs if precautions are taken by the institutions involved. Infections are preventable and should not be interpreted as unavoidable (Morley and Weese, 2008). The issue thus gives serious concern as most nosocomial cases pass quietly unidentified, undiagnosed, untreated and unrecorded (Morley and Weese, 2008). As a working compromise, and to the best of our knowledge, there has been no single record of nosocomial outbreaks in Veterinary Clinics and Hospitals in the Federal Capital Territory (FCT), Nigeria. There is also no diagnostic plan or study that has been carried out in Abuja metropolis to characterize and ascertain the prevalence of nosocomial bacterial organisms. The aim and objectives of this study is to establish preliminary evidence of the presence of bacterial pheno species from five veterinary clinics premises and hospital facilities, and to determine their multi-drug antimicrobial susceptibilities, with a view to creating awareness on the control of nosocomial bacterial infections in hospital environments in Nigeria.

MATERIALS AND METHODS

Study area

This study covered five major veterinary clinics and hospitals within the Federal Capital Territory (FCT), Abuja. It is an 8,000 square kilometer land area centrally located and bound on the north by Kaduna State, on the east by Nassarawa State, on the west by Niger state and on the south/west by Kogi State. It lays between latitude 8.250 and 9.20 north of the equator and longitude 6.450 and 7.390 east of the Greenwich Meridian. Abuja is geographically located in the nerve centre of Nigeria (Benedict *et al*., 2008).

Sample collection and sampling

Samples were collected aseptically from five metropolitan veterinary premises which receive cases from different locations through visits of clients or via ambulatory services. Sampling was done by convenience based on its availability in the study areas. Commercially prepared sterile swabs were used to swab the various contact surfaces that are considered to be the most close to the patients. These surfaces included: surgical tables, hospital equipment and instruments, surgical packs, examination tables, surgical trays and drapes/towels. The original sample was collected into 5ml of transport medium (Carry Bliar Oxoid, containing sodium chloride 5g, sodium thioglycolate 1.5g, disodium phosphate 1.1g, calcium chloride 0.1g, agar 5g and demineralized water 1000ml) after swabbing an area of 100cm square of each surface and transported immediately to the laboratory in ice packs containers. The collection of samples lasted between January and June, 2015.

Experimental design

The experimental method adopted in this study involves designating each of the five sampling hospital premise as A-E while the sampling sites (contact surfaces) were designated as 1-30 such as A1-30,B 1-30, C 1-30, D 1-30, and E 1-30. Premises: A=Hospital premise 1 (Emman Veterinary Services), B=Hospital premise 2 (Agritech Veterinary Hospital), C=Hospital premise 3 (Veterinary World Hospital), D=Hospital premise 4 (Kings Veterinary Clinic), and E=Hospital premise 5 (Royal Veterinary Services). Sites: 1=surgical Table, 2=window, 3= examination Table, 4 weighing scale, 5= surgical equipment, 6 surgical equipment tray, 7= anaesthetic machine, 8 surgical drape, 9 stethoscope, 10= thermometer.

Laboratory analysis

Bacteria organisms from different premises and sampling sites were isolated with varying frequencies. All media were prepared based on their manufacturer's instructions. The laboratories used for this analysis were the Veterinary Microbiology laboratory, University of Abuja and the National Agency for Food and Drug Administration and Control (NAFDAC) reference Laboratory, Kaduna. A total of 1ml per sample collected was inoculated in pairs unto 5ml of their respective enrichment media; after which they were inoculated and streaked unto respective selective media in petri dishes. Samples that were enriched in peptone water were then inoculated unto petri-dishes containing blood agar base, Sorbitol MacConkey agar and Ethylene Methylene Blue (EMB) agar (Difco, Paisley, USA) respectively. In addition, samples in *Bacillus cerus* broth were inoculated into *Bacillus cerus* agar base (Difco, Paisley, USA); those in KL virulence enrichment broth were inoculated unto Columbia blood agar base (Difco, Paisley, USA). Those in Selenite-F broth and modified rappaport vissilialis medium were inoculated unto modified *Salmonella-shigella* agar (Difco, Paisley, USA). Those in Brain Heart Infusion Broth (BHI) were inoculated unto Oxford *Listeria* agar and those in modified tryptose soy broth were inoculated unto Baird Paker agar (Oxiod, London, UK). All enriched test tubes and inoculated Petri-dishes were incubated at 37^0C for 24 hours, and observed for characteristic colonies based on standard methods (Cowan and Steel, 1974). The isolated colonies were preserved on nutrient agar slants for staining and biochemical characterization.

A portion of each isolated colony was smeared on a glass slide and stained with Gram staining reagents and observed under a light microscope using an oil immersion objective (X100). The morphology and the gram reaction of the organisms were observed and samples were refrigerated for further biochemical tests. Biochemical tests were performed to confirm the isolates in accordance with the methods described by Cowan and Steel (1974); Bergy's (1992) and adopted by Bello *et al*., 2014, using the Microbact™ identification system kit (Oxoid). The Microbact™ is a commercial and computerized microsystem for the Identification and confirmation of common clinical isolates of *Enterebacteriaceae* and non-fermenting gram negative *Bacilli* and consists of dehydrated substrates distributed in wells of microtitre trays. The specified Microbact system used for this study was: Oxiod™ Microbact™ GNB 24E System kit, manufactured by Thermo–Fisher Scientific, Waltham, Massachusetts, USA. Biochemical tests conducted included: TSI **(**triple sugar iron agar), Citrate Utilization, Urease, Indole production (using kovacs reagent), Motility test, Hydrogen sulphide (H_2S) test, Methyl red test (MR), Vogues Proskuar (VP) test, Gelatin liquefaction test, Phenlanine test, Lysine decarboxylase test, Ornithine, Arginine hydrolysis test, Xylose test, Maltose test, Mannitol test, Rhamnose test, Sorbitol test, Arabinose test. Other tests included: Glucose, Lactose, Sucrcose, Nitrate, Oxidase, Phosphatase, Coagulase, Catalase, Haemolysis, Arabinose, Salicin, Trehalose, Aesculin, Hippurate hydrolysis, CAMP test, Motility and Growth in 7% Nacl.

Serial dilutions were used for colony counting of the isolates in duplicates plates. The dilution factor on a plate was taken as 10^5, the average counts were obtained using (12+10)12 and the product multiplied by the dilution factor. The estimated number of organisms in 5m1 was calculated as 5.5x 10 CFU/ml and total count per cm2 was calculated as 5.5 x 104 CFU/cm2. Bacterial counts exceeding 150 CFU/cm2 indicated inadequate sanitation (Morley and Weese, 2008).

Antimicrobial susceptibility testing

Kirby-Bauer modified disc diffusion technique was used to examine the antimicrobial susceptibility of the isolates. The antibiotic multiple disc (AbtekBiologicals Ltd-Lot HJ 03/P) was used and comprised of CN-Gentamycin 10mcg, AU-Amoxycillin 30mcg, CPX-Ciprofloxacin 10mcg, SXT Trimethoprim/Sulfamethoxazole 30mcg, C EP– Cefalexin 10mcg,S–Streptomycin 30mcg, PN – Ampicillin 30mcg, OFX – Ofloxacin 10mcg, NA – Nalidix acid 30mcg, PEF – Peflacine 10mcg, MIC – Minimum Inhibitory concentration, Each isolate was grown overnight on nutrient agar to obtain isolated colonies. Isolated colonies were transferred to a test tube of sterile saline (0.8% W/V Nacl) and vortexed thoroughly until the turbidity compared to the same with 0.5 McFarland turbidity standards of 1x108cells/ml. Within 15 minutes after standardizing the inoculum, a sterile cotton wool swab was dipped into the inoculum and excess liquid was removed by pressing the swab firmly against the inside wall of the tube just above the fluid level. The swab was used to streak the entire surface of Mueller –Hinton agar (Oxoid) plates. The plates were allowed to stand for 5 minutes. Antibiotics discs were aseptically placed firmly on the surface of the inoculated agar plates using sterile forceps, and the plates were incubated at 37^0C for 24 hours. Diameters of the zones of inhibition were measured with a rule to the nearest millimeter and isolates were characterized as susceptible or resistant. Sensitive zones were greater than 14mm while resistant zones were taken from 11 to 13mm according to manufacturer's instructions and the National Committee for Clinical and Laboratory Standards (NCCLS) interpretation chart (NCCLS, 2002). The minimum inhibitory concentration of the antibiotics to serial dilutions of the organisms was determined through standardized suspension (microbial rich broth) of the test organisms which were inoculated unto a series of test tubes containing two fold dilutions of the microbial suspensions. 0.5ml was pipetted unto 200µg/ml and serially diluted

Table 1. Dissipation of Array of Biochemical reactions of nosocomial bacterial strains isolated from FCT

ORGANISMS	SUBSTRATE																		
	Tsi	Citrate	Urease	Indole	Motility	H2s	MR	VP	Gelatin	Phanylanine	Lysine	Ornithine	Arginine	Xylose	Maltose	Mannitol	Rhannose	Sorbitol	Arabinose
E. coli	A/AG	-	-	+	+	-	+	-	-	-	+	D	D	D	+	+	D	D	+
Salmonella	ALK/AG+H2s	+	-	-	+	+	+	-	D	-	+	+		+	+	+	+		+
***Shigella* Species**	ALK/A	-	-	D	-	-	+	-	-	-	-	D		-	D	-	-	D	+
ProteusVulgaris	ALK/A	+	+	+	+	+	+	-	D	+	-	D		D	+	-	-		-
***Klebsiella* Species**	A/A	+	+	D	-	-	D	D	D	-	+	-		+	+	+	+	+	+
YeasiniaEnterotolitica	A/A	-	D	-	D	-	+	-	-	-	-	D	-	+	+	+	-	+	+

ORGANISMS	SUBSTRATE													
	Glucose	Lactose	Maltose	Mannitol	Sucrcose	xylose	Vp	Nitrate	Urease	Arginine	Oxidase	Phosphatase	Coagulase	Catalase
Staphaureus	+	+	+	+	+	+	+	+	+	+	-	+	+	+
MicrococcusLuteus	-	-	-	-	-	-	-	-	d	-	d	-	-	+

ORGANISMS	SUBSTRATE													
	Haemolysis	Arabinose	Lactose	Mannitol	Salicin	Sorbitol	Sucrose	Trehalose	Aesculin	Gelatine	Arginine	Hippuratehydrosis	CAMP test	
Strepuberis	Alpha	-	+	+	+	+	+	+	+	-	+	+	-	

ORGANISMS	SUBSTRATE															
	Motility	Catalase	Haemolysis	Glucose	Lactose	Maltose	Mannitol	Salicin	Sucrose	Trehalose	Xylose	VP	Nitrate	Gelatin	Urease	Argrinine
Corynebacteriumdiphtheria	-	+	D	+	-	+	-	-	-	-	-	-	+	-	-	-
Listeriamonocytogenes	+	+	+	+	(d)	+	-	+	(d)	+	-	+	-	-	-	

ORGANISMS	SUBSTRATE											
	Motility	Growth in 7% Nacl	Citrate	Glucose	Arabinose	Mannitol	Xylose	VP	Nitrate		Indole	Gelatin	Urease
Bacillus Substilis	+	+	+	+	+	+	+	+	+		-	+	d

Key: + = 85-100% strains positive (all, most many), D: = 16-84% strains positive (many, some), d: = different reactions given by lower taxa (genera, species, varieties) not known, A: = Acid production (yellow colour), ALK: = Alkaline reaction (orange colour), (d): = Delayed positive reactions and different reactions by different strains, -: = 0-15% positive strains (no, none, few, some).

with concentrations of 50, 25, 12.5, 6.25 and 3.13 µg/ml and incubated at 37^0C for 24 hours. The MIC was read as the least concentration that inhibited the growth of the microorganisms (Bergy's, 1992).

Questionnaire Survey

Fifty close ended structured questionnaires were

Table 2. Prevalence of Nosocomial bacteria in Different contact surfaces.

Premises	Number of Sample(s)	No +ve	%+ve	Bacterial Count (CFU/cm^2)
A	30	22	29.3	$1.5x10^5$
B	30	17	22.7	$2.5x10^4$
C	30	13	17.3	$3.4x10^3$
D	30	15	20.0	$4.3x10^2$
E	30	18	24.0	$1.5x10^4$
Total	**150**	**75**	**100**	

Key: A = Hospital premise 1, B = Hospital Premise 2, C = Hospital Premise 3, D = Hospital Premise 4, E = Hospital Premise 5
It was calculated as: 22/75 x 100 = 29.3 etc.

Table 3. Prevalence of Nosocomial bacteria in Different facilities (sites).

Sites	Number of Samples	No. +ve	% +ve	Bacterial Count (CFU/cm^2)
1	15	13	17.3	$6.5x10^6$
2	15	3	4.0	$3.4x10^3$
3	15	16	21.3	$2.0x10^2$
4	15	11	14.6	$1.0x10^4$
5	15	5	6.9	$3.4x10^2$
6	15	4	5.3	$3.1x10^1$
7	15	2	2.7	$2.3x10^2$
8	15	5	5.3	$2.0x10^4$
9	15	8	10.7	$3.4x10^2$
10	15	7	9.3	$4.5x10^2$
Total	**150**	**75**	**100**	
X^2=2.529 (df2)P <0.05				

Key: 1 = surgical table, 2 = window, 3 = examination table, 4 = weighing scale, 5 = surgical equipment, 6 = surgical equipment tray, 7 = anesthetic machine, 8 = surgical drape, 9 = stethoscope, 10 = thermometer.

administered randomly to clinicians and veterinary students of the Faculty of Veterinary Medicine, University of Abuja. Data was generated based on participant observations of the 50 respondents in order to obtain demographic information on nosocomial infections from clinicians and students of the Faculty of Veterinary Medicine and the University of Abuja. All the participants responded to the questionnaire administered to them promptly. Information obtained was based on: educational level, age, sex, geospatial inclination, marital status, vocation in relation to their general level of awareness of nosocomial infections among others. Statistical analysis was carried out using Chi-square test to attain a Pearson CM-square value as identified by NCCLS (2002).

RESULTS

From a total of 150 samples that were collected on 10 different contact surfaces in each of the 5 veterinary clinics and hospitals investigated across FCT, our study revealed that twelve bacteria organisms of different pheno species were isolated and characterized biochemically (Table 1). The prevalence of nocosomial bacteria from different contact surfaces, premises A had the highest prevalence of 29.3% while premises C had the lowest prevalence of 17.3%. Also the bacterial counts revealed colony counts in the various premises and sites that ranged between 1.5 x 10^6 to CFU/cm^2 to 4.3 x10^2 CFU/cm^2 (Table 2). Table 3 showed the prevalence of nosocomial bacteria in different facilities (sites), with the highest prevalence of 21.3% in site 3 while the lowest prevalence was 2.7% in site 7. The corresponding bacterial colony counts for the various sites showed that site 1 had the highest bacterial count of 6.5 x 10^6 CFU/cm^2 while site 6 had the lowest count of 3.1 x 10^1 CFU/cm^2. Statistical analysis of hospital contact surfaces with sites/premises on nosocomial spread of bacterial infections using Chi square showed Pearson cm-square of x2 = 2.529 (df2). P<0.05. Table 4 shows the microorganisms identified which included: *Streptococcus uberis* Constituted 12.0% (n=9) of the total (N=74) isolated organisms, *Staphylococcus aureus* constituted 13.3% (n=10), while *Corynebacterium diphtheria* formed 5.3% (n=4), *E. coli* was 14.7% (n=11) while *Proteus vulgaris* was 5.3% (n=4). *Micrococcus luteus, Bacillus subtillus, Salmonella enterica, shigella* species, *Kiebsiella* species, *Listeria monocytogenes* and *Yersinia enterocolitica* constituted 2.7% (n=2), 14.9% (n=11), 12.0%, 2.7%, 2.7%, 10.7%, 10.7%,

Table 4. Prevalence of Nosocomial Bacteria Organisms from Different Sites and Premises in FCT, Abuja.

Isolate Organisms	Number +ve	Percentage +ve
Streptococcus uberis	9	12.0
Staphylococcus aureus	10	13.3
Corynebacteria diphtheria	4	5.3
Escherichia coli	11	14.7
Proteus vulgaris	4	5.3
Micrococcus luteus	2	2.7
Bacillus subtilis	11	14.7
Salmonella enterica	9	12.0
*Shigella*species	2	2.7
Klebsiella species	2	2.7
Listeria monocytogenes	8	10.7
Yersinia enterocolitica	2	2.7
TOTAL	**74**	**100**

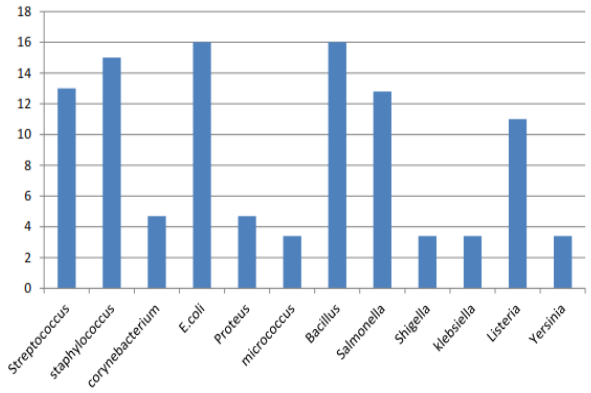

Figure 1. Chart Showing Isolation rate of Bacteria organisms from different premises.

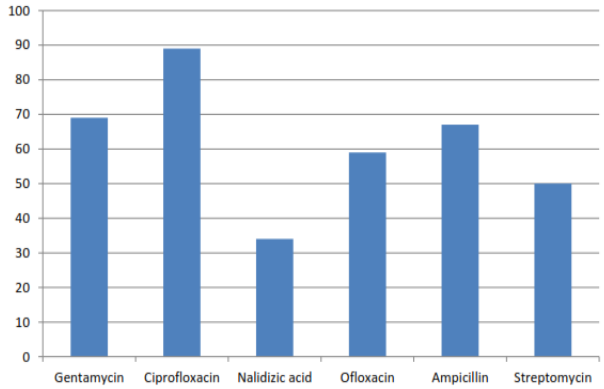

Figure 2. Chart Showing Percentage Susceptibility of Bacteria Organisms to some Antibiotics.

and 2.7% prevalence respectively. Figure 1 is a chart showing isolation rate of nosocomial bacteria from different sites and premises. *Bacillus* and *E. coli* had the highest prevalence rate (14.7%), while *Micrococcus luteus, Kiebsiella* species, *Shigella* species and *Yersinia enterocolitica* had the least isolation rate (2.7%). Table 5 shows the antibiotic sensitivity patterns dissipated by the isolated nosocomial bacterial organisms in response to different antimicrobial agents. All were resistant to Augumentin (Amoxicillin), Septrin (Trimethoprim), Peflacin and Cefalexin but susceptible to Gentamicin, Ciprofloxacin, Ofloxacin, Streptomycin, Ampicillin, and Nalidixic acid with minimum inhibitory concentration values ranging 0.125, 0.1, 0.115, 0.10, 0.125, and 0.625µg/ml respectively. Figure 2 is a Bar chart extrapolated for the susceptibility of nosocomial bacteria organisms to different commonly used antibiotics. The organisms were most susceptible to Ciprofloxacin (88%) while they were least susceptible to Nalidixic acid (33%). Table 6 shows the response of clinicians and students of the faculty of veterinary medicine university of Abuja on their level of awareness of nosocomial bacterial infection in Veterinary Clinics and Hospitals in Abuja. The subjected data revealed that more males (72%) were investigated than females (28%), most of which were fluent in English (65%) than Hausa (35%). Most (75%) of the respondents were between the age bracket of 20-25 years, while 600 level students constituted 73% of those investigated. More so, 36% do not know what nosocomial infection exist while 34% did not know what nosocomial zoonosis entailed. 40% experienced flu symptoms while 23% experienced a complex of symptoms and most (66%) did not consider visiting the hospital for treatment as several (31%) were not aware that nosocomial bacteria organisms can be treated. It further revealed that 62% failed to comply by washing their hands before retiring from the clinic everyday while only 46% of those who washed their hands attempt using soap and water.

DISCUSSION

The studies conducted on nosocomial infections in hospital environment and premises in Abuja found

Table 5. Percentages of antiobiotic sensitivity studies and minimum inhibitory concentration of twelve nosocomial bacterial organisms.

Zone diameter (Mg)				MIC µg/ml
Antibiotics	Samples tested	Sensitive	Resistant	
CN	12	8 (67%)	-	0.125
AU	12	-	2 (17%)	-
CPX	12	10 (88%)	-	0.1
SXT	12	-	2 (17%)	-
CEP	12	-	3 (25%)	-
S	12	6 (50%)	-	0.10
PN	12	8 (67%)	-	0.125
OFX	12	7 (58%)	-	0.115
NA	12	4(33%)	-	0.0625
PEF	12	-	4 (33%)	-

Key: CN – Gentamycin 10mcg, AU – Amoxycillin 30mcg, CPX – Ciprofloxacin, 10mcg, SXT Trimethoprim/Sulfamethoxazole, 30mcg, CEP – Cefalexin 10mcg, S – Streptomycin 30mcg, PN – Ampicillin 30mcg, OFX – Ofloxacin 10mcg, NA – Nalidix acid 30mcg, PEF – Peflacine 10mcg, MIC – Minimum Inhibitory concentration

potential nosocomial causers for diverse bacterial infections of humans and animals in areas covered by this study. During the course of this study, close agreement was depicted with the findings of previous workers carried out in related aspects (Weber and Rutola, 2000). The results of this study revealed that facilities in Veterinary Clinics and Hospitals within Abuja are heavily contaminated with pathogenic bacteria organisms. The identified organisms and their prevalence were found to correlate closely with that of previous workers (Tambuwal *et al*., 2009).

E.coli and *Bacillus subtilis* had the highest prevalence rate of 14.7% each as major sources of the major sources of infection. This may be due to the ubiquitous nature of *Bacillus* spores that are well known for rapid dissemination and ability to resist heat and are able to survive for a longer periods of time in hostile environment. The *Bacillus* spores multiplies rapidly at room temperature and survives on dry surfaces, leading to highly contagious and fatal disease known as anthrax (Emori and Gaynes, 1993; Centre for Disease Control, 2001). *E.coli* on the other hand is a normal commensal enteric flora and thus, faecal materials of animals presented for treatment may mess up the environment, releasing chums of the bacteria. This, coupled with the poor and unhygienic conditions of the hospital environment may be responsible for nosocomial spread of the disease to susceptible victims. *E.coli* infection may be responsible for severe gastrointestinal and diarrheal symptoms leading to subsequent dehydration in animals and humans especially immunocompromised infants) (Centre for Disease Control, 2001). Other risk factors that predispose nosocomial spread of infections may include malnutrition, malignancy, neutropenia, extensive burns and associated secondary infections.

Staphylococcus aureus constituted about 13.3% of the total isolates. This prevalence observed might be due to the fact that the organism occurs as a commensal on the skin and mucous membrane of animals and is comparatively stable in the environment especially under unhygienic conditions. The organism could be transmitted through the hands and clothes of veterinary personnel during clinical procedures that involve handling for medical examination, surgery or cleaning of hospital premises by workers. *Staphylococcus aureus* might produce latent enterotoxins which are the leading cause of gastroenteritis and other debilitating diseases such as osteomyelitis, endocarditis, mastitis and septicaemia in both animals and humans. The organism is responsible for human malignant carbuncles and abscesses found on the hand and skin. The disease is a worldwide problem often resulting from consumption, contact or handling of contaminated material (CAB, 2006). Therefore, physicians, veterinarians, animal handlers and hospital workers should ensure use of protective clothing, gloves when handling patients. More so, other devices such as stethoscopes, surgical tables and equipment should be kept clean. This will be useful to safeguard the populace from any nosocomial spread of diseases.

Streptococcus uberis and *Salmonella enterica* constituted 12.0 % each of the entire organisms isolated. *Streptococcus uberis* had a higher prevalence on the examination table, surgical table and thermometer. *Streptococci* are often commensals of the mucous membrane and are known to cause pyogenic infection in many animal and humans and are associated with abscess formation, suppurative conditions and septicemia. *Streptococcus* species cause strangles in horses, bovine mastitis, navel ill in foals, arthritis and pharyngeal abscess in swine and genital tract infection in dogs, horses and cows (Tambuwal *et al*., 2009). The prevalence of *Salmonella enterica* indicated in our study is as high as 12.0% which concurs with (Weese *et al*., 2000); *Salmonella* organisms are well known to be ubiquitous in the environment, more so, they are also known to inhabit the gastro-intestinal tract of humans and animals, causing variety of diseases including food poisoning and other diseases such as typhoid fever,

Table 6. Subjective responses of clinicians and clinical students of the Faculty of Veterinary Medicine, University of Abuja on their level of awareness on nosocomial infections in Veterinary Clinics and Hospitals.

Parameters		Percentage %
1.	Sex	
	Males	72
	Females	28
2	Age	
	Age 20-25 years	75
	Age over 25 years	25
3		
	Languages spoken	35
	Hausa	65
4	English	
	Educational level	73
	600L students	27
5	Non students	
	Knowledge of nosocomial infection	
	Yes	64
	No	36
6.	Knowledge of nosocomial zoonosis	
	Yes	66
	No	34
7.	Knowledge of nosocomial bacteria organisms	
	Yes	40
	No	60
8.	Frequency at the clinic	
	Frequently	98
	Seldom	2
9.	Hand washing after going to the clinic	
	Yes	38
	No	62
10.	Symptoms	
	Abdominal pain	10
	Diarrhea	2
	Vomiting	12
	Coughing	5
	Flu	40
	Headache	8
	All	23
11.	Requested for hospital treatment	
	Yes	34
	No	66
12.	Knowledge of economic and public health importance of nosocomial bacteria	
	Yes	38
	No	62
13.	Knowledge of treatment of nosocomial bacteria	
	Yes	69
	No	31
14.	Knowledge of nosocomial bacteria resistance to antibiotics	
	Yes	41
	No	59
15.	Hygiene knowledge	
	Yes	38
	No	62
16.	Hand washing	
	Water	40
	Soap and water	32

paratyphoid, fowl cholera which dissipates different symptoms in man, birds and livestock (Sterneroden *et al*., 2010). The implication of this finding is that humans stands risk of contracting infection via close contact or

handling of animals and animal or poultry products. The public needs to be enlightened and educated as the disease could lead to outbreak of salmonella-related diseases leading to millions of deaths annually. It was also observed that poor sanitation of contact surfaces within veterinary clinics and hospitals may have contributed to the high proliferation of the micro-organisms at high levels because it can grow and multiply within a wide range of environmental temperature. There is therefore need for use of dips, laboratory coats coupled with constant hand washing and adherence to adequate hygienic measures to prevent outbreaks of nosocomial bacterial infections in hospital environment.

Listeria monocytogenes was responsible for 10.7% of the total bacterial isolates and was most often isolated. The organism can be recovered from animal faeces and can grow and replicate in the environment at a wide range of temperatures (from 4^0C to 45^0C) is contributes to the strong presence of the organisms in our hospital premises. The organism is the causative agents of listeriosis, a disease characterized by abortion, septicemia, and endophthalmitis (De Oliveira and Damasceno, 2010). Other bacteria isolates incriminated in this study included *Proteus vulgaris* which constituted 5.3% of the entire isolated organisms. The organism causes nephritis and mastitis, pneumonia and serves as a secondary invader in most other bacterial infections in cattle worldwide. Proteus species is known to cause bacterial endocarditis in ruminants, coliform mastitis in goats, gangrenous dermatitis in chickens and turkeys and induces fleece rot in sheep (CAB, 2006). *Shigella* species formed 2.7% of the entire isolates. The organism causes shigellosis characterized by dysentery, colic and dehydration in man and animals. It can lead to death in severe cases especially in infants. *Kiebsiella* species formed 2.7% of the isolated organisms. *Klebsiella* is an important zoonotic pathogen which is widely distributed in soil, water and plant material. It survives well in sawdust and is a common source of nosocomial outbreaks in humans and animals. They have been isolated in acute infantile diarrhea in south-eastern Nigeria (Tambuwal *et al*., 2009). The gastrointestinal tract of warm blooded animals is the sole habitat of *Klebsiella* species which qualifies it as a faecal coliform that probably indicated that water used in cleaning veterinary clinics environment might have been contaminated. The organism is one of the causes of neonatal meningo encephalitis and sporadic cases of meningitis in adult horses (Merck Veterinary Manual, 2011). *Micrococcus luteus* belonging to the family *Micrococcaceae* constituted 2.7%, alongside *Yersinia enterocolitica* which also formed 2.7% of the entire isolated organisms. This is an important organism that survives in the hospital environment as a result of improper hygienic practices. They are responsible for diseases such as mastitis and diarrhea.

Statistical analysis using Chi square showed Pearson cm-square of $x2 = 2.529$ (df2). $P<0.05$. This established significant association between hospital contact surfaces with sites/premises and nosocomial spread of bacterial infections. The degree of contamination between the various premises was compared. The highest prevalence of bacteria isolates (29.3%) was premise A; this was followed by premise B which had a prevalence of 22.7%. This could be due to the large number of cases presented to these veterinary premises which is often characterized by constant movement of clients with their animals which troop such premises. Also, poor level of sterilization and disinfection of facilities within the premises could increase risks of disease spread. The second clinic sampled (Premise C) had the lowest prevalence (17.3%), which may be due to less number of cases received at such private clinic as compared to the other premises sampled. It may also be due to the fact that more sterilization /disinfection measures are employed in comparison with the other clinics. The frequency of occurrence of infectious agents in the contact surfaces of the facilities was also recorded and it was observed that examination tables had the highest prevalence of 21.3% followed by the surgical tables and weighing scale with 17.3% and 14.9% respectively. This is due to very high frequency of contact by patients and clinicians with the examination table and the surgical table thus permitting easy transmission of nosocomial bacteria organisms. Anaesthetic machine and windows had the lowest prevalence of 2.7% and 5.3% respectively. The low prevalence of anaesthetic machine may be due to the fact that only few animals require radiograph and thus there is limited contact by both clinicians and patients with the anaesthetic machine.

The results of the multidrug antimicrobial studies revealed that Nosocomial bacteria organisms are resistant to Augumentin (Amoxicillin), Septrin (Trimethoprim), Peflacin and Cefalexin. They were susceptible to Gentamycin, Ciprofloxacin, Ofloxacin, Streptomycin, Ampicillin, and Nalidixic acid this observation agrees with the findings of Abouzeed *et al*. (2000). The resistance may be due to the indiscriminate use of antibiotics by several clinicians and self-medication of patients by clients. The minimum inhibition rates of the respective drugs will therefore aid in the determination of the minimum dose required to treat the respective nosocomial bacterial infections.

From the responses of clinicians and students of the Faculty of Veterinary Medicine University of Abuja to the questionnaire on their level of awareness on nosocomial bacterial infection in veterinary clinics and hospitals, 72% were males, 78% were between the ages of 20-25, and 35% were Hausa, while 73% that were students were from 600 level. The demographic results observed are due to the fact that there are more students than clinicians in the faculty with a larger proportion being males who are still very young hence they are still single. Because the location of this study is in the north central part of Nigeria, we have more Hausa tribal persons

involved in this study. 36% do not know what nosocomial infection is while 64% do not know what nosocomial zoonoses entailed. 40% experienced flu symptoms while 23% experienced a complex of symptoms that warranted hospital treatment and most (66%) did not consider visiting the hospital for treatment as less several (31%) were not aware that nosocomial bacteria organisms can be treated. Further investigation revealed that 62% failed to wash their hands before retiring from the clinic everyday while only 32% of those investigated washed their hands with soap and water. This situation is further heightened by the unavailability of potent antiseptics and portable water in most Veterinary Clinics and Hospitals. Failure to curtail or control these bacteria organisms on contact surfaces within Veterinary Clinics and Hospital environments can cause potential outbreaks of devastating infections in susceptible patients and hospital exposed personnel resulting in morbidity, mortality and financial losses (Konkle *et al.,* 1997).

In conclusion, the study showed that the veterinary clinics and hospitals in Abuja are heavily contaminated with bacteria most of which are of public health significance. To minimize and control the risk of contracting and disseminating zoonotic diseases to practitioners and clients, and nosocomial infections to visiting animal patients, we recommend that specific guidelines should be made in place for animal owners and Veterinarians through the Veterinary Council of Nigeria and the Federal Livestock Department. Veterinarians should be encouraged to implement client oriented education programs that will contribute to good hospital hygiene practices, antibiotic usage, protection of clients and animals from contracting diseases. The use of improved techniques such as Polymerase Chain Reaction (PCR), Enzyme Linked Immunosorbent Assay (ELISA), Restricted Fragment Length Polymorphism (RFLP), will provide a better understanding of their microbiology which will lead to better diagnosis and improved control of nosocomial infections.

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