

Veterinary clinic surfaces as reservoirs of multi-drug- and biocide-resistant Gram-negative bacteria

Chinedu A. Akwuobu^{1,*}, Emmanuel O. Ngbede¹, Levi M. Mamfe¹, Ekene V. Ezenduka² and Kennedy F. Chah³

Abstract

This cross-sectional study was carried out to determine the common Gram-negative bacteria (GNB) contaminating veterinary clinic environments, and to evaluate the susceptibility of the isolates to commonly used antibiotics and biocides. A total of 62 swab samples were collected from different frequently touched surfaces in the 4 veterinary clinics visited. The samples were processed for isolation and identification of GNB using standard microbiological procedures. The susceptibility of the isolates to disinfectants and antibiotics was determined using agar dilution and disc diffusion techniques, respectively. A total of 114 GNB were isolated from the 4 clinics with isolation rates of 21.9, 22.8, 23.7 and 31.6% in clinics A, B, C and D, respectively. The surfaces of treatment tables were more contaminated (16.7%) than receptionist/clinician desks (15.8%), weighing balances (10.5%), door handles (7.9%), drip stands (7.9%), handwashing basins (7.0%) and client chairs (7.0%). The surface-contaminating isolates were distributed into 20 genera, with members of *Enterobacteriaceae* predominating ($n=97$). Fifty-nine per cent of the isolates were resistant to the disinfectant Septol, while 5.3 and 0.9% were resistant to Purit and Dettol disinfectants, respectively. Multiple drug resistance was observed among 99% of the isolates with approximately 100% resistance to beta-lactams. Phenotypic expression of extended-spectrum (3.5%) and AmpC beta-lactamase (38.6%) production was detected. These findings highlight the role of clinic environments in serving as reservoirs for potential pathogens and sources for the spread of multi-drug resistant GNB.

INTRODUCTION

Contaminated hospital surfaces and equipment, if not cleaned properly between patient use, can be sources of infection. This is because in human healthcare settings and veterinary medicine, micro-organisms from the skin or faeces of a patient can contaminate hospital surfaces and equipment and be spread to other patients if proper disinfection is not performed between patient use [1–4]. Thus, environmental contamination by bacteria can be a source of hospital-acquired infections in human and veterinary hospitals [3–6]. Although the prevalence of hospital-acquired infections in privately owned veterinary practices remains largely unknown [3, 4], the results of a study by Benedict *et al.* [7] indicated that ~82% of veterinary teaching hospitals reported outbreaks of hospital-acquired infections.

There has been an increased emergence of multidrug-resistant (MDR) bacteria in recent decades [8, 9]. Environmental contamination by bacteria such as methicillin-resistant *Staphylococcus aureus* [6] and MDR *Escherichia coli* [10] has been reported to be a source of infections in human and veterinary hospitals. Further, as a result of extensive use of biocides, El-Mahmood and Doughari [11] reported that a significant proportion of the pathogens have not only developed resistance, but also grow in solutions of these biocides. Misuse, such as frequent exposure to sub-lethal concentrations of biocides, could result in the development of resistance in bacteria to biocides and also to antibiotics [9, 12–14]. The use of sub-inhibitory concentrations of biocides can also select for strains that are tolerant to these chemicals [9, 15].

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Author affiliations: ¹Department of Veterinary Microbiology, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria; ²Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria; ³Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

***Correspondence:** Chinedu A. Akwuobu, akwuobu.chinedu@uam.edu.ng

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Abbreviations: AML, amoxicillin; AmpC, AmpC beta-lactamase; C, chloramphenicol; CN, gentamicin; CPD, cefpodoxime; ENR, enrofloxacin; ESBL, extended-spectrum beta-lactamase; FOX, ceftiofur; GNB, gram-negative bacteria; IPM, imipenem; MBC, minimum bactericidal concentration; MDR, multidrug resistant; MHA, Mueller-Hinton agar; MIC, minimum inhibitory concentration; P, penicillin; S, streptomycin; SXT, sulphamethoxazole/trimethoprim; TE, tetracycline.

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Data on the frequency and potential virulence factors of these bacteria within veterinary environments in Nigeria are lacking despite the fact that the hospital environment (surfaces and equipment) can be a reservoir for pathogens if they are not cleaned and disinfected properly. Such contaminated environments could also be a source of nosocomial infections in domestic/pet animals, as well as an important source of antimicrobial-resistant bacterial infections in animals. Given the possibility of developing resistance to routinely used biocides, which may contribute to a potential risk for the development of antibiotic resistance by these potential pathogens due to misuse, documented reports on the susceptibility profile of these pathogens, with regard to biocides, are lacking for the veterinary hospital environment in Nigeria. The study, therefore, aimed to determine the prevalence of Gram-negative bacterial pathogens on frequently touched surfaces in veterinary clinics, and to evaluate their antibiotic and biocidal susceptibility profiles.

METHODS

Study area

The study was conducted in Makurdi, Benue State, in north-central Nigeria. The geographical coordinates of Makurdi are longitude 8° 32'00' and latitude 7° 44'00' [16]. Makurdi is mainly inhabited by civil servants, paramilitary personnel, military officers, traders, fishermen, farmers and craftsmen. It is known for its warm, humid and tropical climate, which favours the growth of many micro-organisms.

Study sites and design

Four veterinary clinics were identified in Makurdi and subsequently selected for sample collection for this study. The research was a survey study that involved cross-sectional study of the prevalence and characterization of bacterial isolates from veterinary clinic environments.

Sample collection and culturing for isolation of bacteria

Sampling was performed at close of work after routine environmental cleaning using disinfectants (Purit, chlorhexidine, Z Germicide) at arbitrary concentrations/dilutions. Surfaces in veterinary clinic environments were swabbed with sterile wooden handle swabs moistened in sterilized peptone water. After surface swabbing, the tip of the swab stick was cut off and put into a bijoux bottle containing sterilized peptone water (pre-enrichment broth), appropriately labelled for identification, and transported to the laboratory. The surfaces swabbed included examination/treatment tables, clinic floors, animal waiting areas, clinician desks, receptionist desks, door handles, drug cabinet/tables, chair handles, etc.

Isolation and identification of Gram-negative bacterial isolates

In the laboratory, the inoculated pre-enrichment broths were incubated at 37 °C overnight. The broth cultures were subsequently streak-inoculated on MacConkey and blood

agar for discrete colonies. The inoculated plates were incubated aerobically at 37 °C for 24–48 h. After incubation, the resultant colonies were examined visually and different distinct colonies were subsequently streak-purified on nutrient agar and stock-cultured on nutrient agar slants for further processing. The resultant colonies from the purified cultures, after incubation, were picked and examined visually and microscopically following the procedure described by Parija [17]. All isolates were identified to generic/species level using standard biochemical procedures as described by Parija [17] and the Microbact 24E Gram-negative system (Oxoid).

Biocide susceptibility test

The susceptibility of the bacterial isolates to commonly used biocides (disinfectants) was tested using the agar dilution method [17]. The following disinfectants were tested.

- (1) Dettol: formula – chloroxylenol BPC w/v, *Oleum pini aromaticum*, isopropyl alcohol, *Sapo vegetalis*, *Saccharum ustum* qs, aqua.
- (2) Septol: formula – 2.3% pine oils and 1.1% 5-chloro 2-hydroxyl methane.
- (3) Purit: formula – chlorhexidine gluconate BP w/v, cetrimide BP 3.0% w/v, aromatic pine oil, isopropyl alcohol, brown, deionized water.
- (4) Tetmosol: formula – chloroxylenol (4.85%), castor oil, sodium hydroxide, isopropyl alcohol, fragrance, colour, water.
- (5) Z Germicide: 7% tar acid phenol and 2% cresylic creosote.

Minimum inhibitory concentration (MIC) of biocides

The agar dilution method, as described by Parija [17], was used to determine the MICs of the disinfectants against the test isolates. Briefly, using sterile micro-pipettes, the varying dilutions (concentrations) of the respective disinfectants were prepared in 50 ml of molten Mueller–Hinton agar after autoclaving and allowing the agar to cool to about 55–50 °C, viz 2.5 ml/50 ml (50 µl ml⁻¹), 1.25 ml/50 ml (25 µl ml⁻¹), 0.625 ml/50 ml (12.5 µl ml⁻¹), 0.3125 ml/50 ml (6.25 µl ml⁻¹), 0.156 ml/50 ml (3.13 µl ml⁻¹), 0.078 ml/50 ml (1.56 µl ml⁻¹), 0.039 ml/50 ml (0.78 µl ml⁻¹), 0.0195 ml/50 ml (0.391 µl ml⁻¹), 0.0098 ml/50 ml (0.195 µl ml⁻¹) and 0.0049 ml/50 ml (0.098 µl ml⁻¹). The mixtures were shaken well to obtain homogenous suspensions and then dispensed into sterile Petri dishes that were labelled appropriately for each concentration. The plates were allowed to solidify. Using permanent marker, the plates were numbered 1 to 30 on the bottom, representing the number/identity of the bacterial isolates to be inoculated on the surface of the medium. Using a wire loop, test isolates were inoculated on the surface of the medium against the numbers marked on the bottom. Inoculated plates were incubated at 37 °C overnight. The test plates were read for the presence or absence of growth. The concentration at which growth was completely inhibited was considered to be the MIC of the bacteria.

Table 1. Isolation rate for potentially pathogenic bacteria from the veterinary clinic environment

Isolates	Clinics (%)*				Total (%)†
	A	B	C	D	
<i>Enterobacteriaceae</i>	20 (20.6)	20 (20.6)	27 (27.8)	30 (30.9)	97 (85.1)
Non-enteric Gram-negative bacteria	5 (29.4)	6 (35.3)	7 (41.2)	6 (35.3)	17 (14.9)
Total	25 (21.9)	26 (22.8)	27 (23.7)	36 (31.6)	114

*% of row total.

†% of row total.

Minimum bactericidal concentration (MBC) of biocides

The MBC was determined using the method described by Parija [17]. Inoculated surfaces of MIC plates without growth were scratched with a sterile wire loop and transferred onto the surface of appropriately labelled Mueller–Hinton agar plates without disinfectants (unsupplemented Mueller–Hinton agar) and incubated at 37 °C for 24 h. The test plates were read for the presence or absence of growth. The concentration at which there was no growth was considered to be the MBC of the bacteria.

Antimicrobial susceptibility test

The sensitivity of the bacterial isolates to 12 different commonly used antimicrobials was determined using the Kirby–Bauer disc diffusion method as described by Parija [17] with Mueller–Hinton agar. The antibacterial agents tested included enrofloxacin (5 µg), tetracycline (30 µg), penicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), amoxicillin (10 µg), gentamicin (10 µg), streptomycin (25 µg), chloramphenicol (10 µg), sulphamethoxazole/trimethoprim (25 µg), imipinem (10 µg), ceftiofur (30 µg) and cefpodoxime (10 µg). The inhibition zone diameters were interpreted as susceptible, intermediate or resistant according to the Clinical Laboratory Standard Institute (CLSI) criteria for aerobic bacterial isolates [18]. Extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase (AmpC) producers were tentatively determined using Oxoid combination disc method comprising cefpodoxime/clavulanic acid (10/1 µg) and cefpodoxime (10 µg). An organism was interpreted as producing an ESBL if there was an increase in zone size of ≥5 mm between the cefpodoxime/clavulanic acid disc compared to that of the cefpodoxime disc alone. Isolates producing AmpC β-lactamase were tentatively determined if there was a zone difference of ≤5 mm.

RESULTS

A total of 114 Gram-negative bacterial organisms were isolated aerobically from the 62 swab samples collected from different surfaces in the 4 veterinary clinics visited in the study (Tables 1–3). Surface swabs from clinics A, B, C and D, respectively, yielded 25 (21.9%), 26 (22.8%), 27 (23.7%) and 36 (31.6%) isolates (Table 1). High isolation rates, as shown in Fig. 1, were obtained from the surfaces of treatment tables,

Table 2. Frequency of isolation of *Enterobacteriaceae* from the veterinary clinic environment

Isolates	Frequency (%)
<i>Escherichia coli</i>	11 (11.3)
<i>Enterobacter cloacae</i>	19 (19.6)
<i>Enterobacter agglomerans</i>	5 (5.2)
<i>Enterobacter amnigenus</i> biogp I	1 (1.0)
<i>Hafnia alvei</i>	4 (4.1)
<i>Klebsiella pneumoniae</i>	20 (20.6)
<i>Klebsiella oxytoca</i>	1 (1.0)
<i>Proteus mirabilis</i>	12 (12.4)
<i>Proteus vulgaris</i>	1 (1.0)
<i>Serratia rubidaea</i>	9 (9.3)
<i>Stenotrophomonas maltophilia</i>	2 (2.1)
<i>Acinetobacter baumannii</i>	3 (3.1)
<i>Tatumella ptyseos</i>	1 (1.0)
<i>Citrobacter freundii</i>	1 (1.0)
<i>Budvicia aquatica</i>	1 (1.0)
<i>Providencia</i> sp.	1 (1.0)
Others	5 (5.2)
Total	97

%=percentage of total number of isolates of *Enterobacteriaceae*

receptionist/clinician desks, weighing balances, door handles and drip stands, handwashing basins and client chairs.

Sixteen species, belonging to 12 genera in the family *Enterobacteriaceae*, were identified from the 97 isolates, while 5 isolates of the family were unidentified (Table 2). *Enterobacter* and *Klebsiella* species were the most prevalent. In the case of non-enteric Gram-negative bacilli (Table 3), nine species, belonging to eight genera, were identified, with *Moraxella* and *Pseudomonas* species being the most prevalent.

Out of the 114 Gram-negative bacterial isolates subjected to biocide susceptibility testing, 46 (40.4 %) were susceptible to

Table 3. Frequency of isolation of non-enteric Gram-negative bacilli from the veterinary clinic environment

Isolates	Frequency (%)
<i>Moraxella</i> species	5 (29.4)
<i>Pseudomonas fluorescens</i>	4 (23.5)
<i>Pseudomonas aeruginosa</i>	1 (5.9)
<i>Myroides odoratus</i>	1 (5.9)
<i>Vibrio alginolyticus</i>	1 (5.9)
<i>Pasteurella</i> species	1 (5.9)
<i>Actinobacillus</i> species	1 (5.9)
<i>Weeksella zoohelcum</i>	1 (5.9)
<i>Aeromonas hydrophila</i>	1 (5.9)
Others	1 (5.9)
Total	17

%, percentage of total number of isolates of non-enteric Gram-negative bacilli.

all biocides, while 62 (54.4%) were resistant to only 1 biocide and 6 (5.3%) were resistant to 2 of the disinfectants tested (Table 4). As shown in Table 5, all the isolates were susceptible to Tetmosol and Z Germicide disinfectants, while more than half of the isolates were resistant to Septol disinfectant. It was observed that some isolates, viz *Klebsiella pneumoniae* (one), *Proteus mirabilis* (three) and *S. rubidaea* (one) exhibited resistance to both Purit and Septol disinfectants. The only isolate that was resistant to Dettol was identified as *Tatumella ptyseos*. The only isolate of *Weeksella zoohelcum* was resistant to both Purit and Septol, while the other non-enteric Gram-negative bacilli were only resistant to Septol.

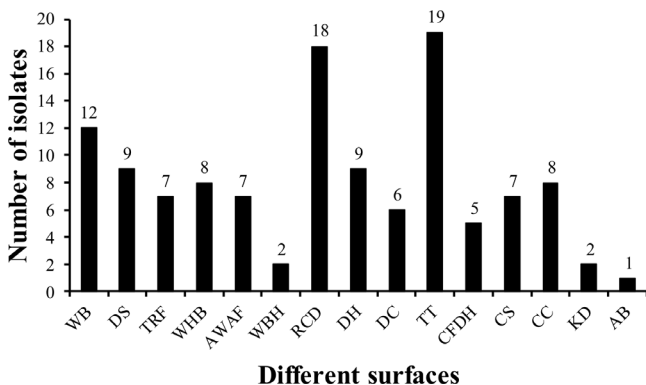


Fig. 1. Summary of distribution of Gram-negative bacteria isolated from surfaces of veterinary clinics in Makurdi, Benue State. Weighing balance, WB; drip stand, DS; treatment room floor, TRF; handwashing basin, HB; animal waiting area floor, AWAf; waste bin handles, WBH; receptionist/clinician desks, RCD; door handles, DH; drug cabinet, DC; treatment tables, TT; clinic fridge door handles, CFDH; clinic switches, CS; client chairs, CC; kidney dish, KD; animal bath, AB.

The biocide MIC results for the 114 Gram-negative bacilli isolates are shown in Table 6. Different ranges of MICs were recorded for the isolates for the different disinfectants tested. Out of the 114 Gram-negative bacilli tested, 113 (99.1%) were inhibited by Dettol. The majority of the isolates (57.0%) had an MIC of 6.25 $\mu\text{l ml}^{-1}$. Of the 114 Gram-negative bacilli, 47 (41.2%) were inhibited by Septol at different concentrations (Table 6). For Purit disinfectant, 108 (94.7%) of the 114 Gram-negative bacteria tested were inhibited at varying concentrations (Table 6). All the isolates tested were inhibited respectively by Tetmosol and Z Germicide at a concentration of 25 $\mu\text{l ml}^{-1}$.

The biocide MBC results for the Gram-negative bacilli are shown in Table 7. MBCs ranging from 1.56 to 50 $\mu\text{l ml}^{-1}$ were observed for Dettol and Purit[®], while the MBCs for Tetmosol ranged from 1.56 to 25 $\mu\text{l ml}^{-1}$. The isolates recorded upper MBC values of 12.5, 25 and 50 $\mu\text{l ml}^{-1}$ with Septol, and 25 and 50 $\mu\text{l ml}^{-1}$ with Z Germicide.

The *Enterobacteriaceae* displayed high rates of resistance (33–99%) to all 11 antibiotics tested, as shown in Table 8. All of the *Escherichia coli* and *Klebsiella* species isolates were resistant to penicillin, amoxicillin and streptomycin. The *E. coli* isolates also demonstrated high resistance rates (36–80%) to cefoxitin, cefpodoxime, tetracycline, enrofloxacin and sulphamethoxazole/trimethoprim, while 55% of the isolates displayed intermediate resistance to gentamicin. Low resistance rates of 18 and 27% to imipenem and chloramphenicol, respectively, were recorded for *E. coli*. The *Klebsiella* species isolates also demonstrated high resistance rates (33–75%) to imipenem, cefoxitin, cefpodoxime, gentamicin, tetracycline, chloramphenicol, enrofloxacin and sulphamethoxazole/trimethoprim. *Enterobacter* species isolates displayed high resistance rates, ranging from 29–95%. All the tested isolates of *Serratia rubidaea* were resistant to penicillin, amoxicillin and streptomycin. The isolates displayed high resistance rates, ranging from 33–83%, to other antibiotics. The *Acinetobacter baumannii* isolates demonstrated a resistance rate of 100% to penicillin, amoxicillin, cefoxitin, cefpodoxime and chloramphenicol. *A. baumannii* showed high resistance rates (33–50%) to gentamicin, streptomycin, tetracycline, enrofloxacin and sulphamethoxazole/trimethoprim, while they displayed 100% rate of intermediate resistance to imipenem. Similarly, all the tested *Proteus* species isolates were resistant to penicillin, amoxicillin, streptomycin and tetracycline. *Proteus* species displayed high resistance rates to imipenem (77%), cefoxitin (38%) and chloramphenicol (31%), while low rates (8–15%) were recorded for cefpodoxime, gentamicin and sulphamethoxazole/trimethoprim. The *Proteus* species isolates demonstrated a high rate (92%) of susceptibility to enrofloxacin. Other *Enterobacteriaceae* were resistant to almost all the antibiotics tested. The results of the antibiogram for non-enteric Gram-negative bacilli are presented in Table 9. High rates of resistance (45–100%) to penicillin, amoxicillin, cefpodoxime, streptomycin, tetracycline, chloramphenicol and sulphamethoxazole/trimethoprim were recorded. The non-enteric Gram-negative bacilli displayed low resistance rates (9–27%) to imipenem, cefoxitin, gentamicin and enrofloxacin.

Table 4. Frequency of biocide susceptibility/resistance for bacterial isolates

Isolates	No. susceptible to all biocides (%)*	Resistant to		Total (%)†
		One biocide (%)*	Two biocides (%)*	
<i>Enterobacteriaceae</i>	36 (37.1)	56 (57.7)	5 (8.2)	97 (85.1)
Non-enteric Gram-negative bacilli	10 (58.8)	6 (35.3)	1 (5.9)	17 (14.9)
Total	46 (40.4)	62 (54.4)	6 (5.3)	114

*% of row total.

†% of total bacterial isolates (114).

Table 5. Frequency of biocide resistance for bacterial isolates

Bacteria	No. of isolates (%)*				
	Dettol	Septol	Purit	Tetmosol	Z Germicide
<i>Enterobacteriaceae</i> (n=97)	1 (1.0)	60 (61.9)	5 (5.2)	–	–
Non-enteric Gram-negative bacilli (n=17)	–	7 (41.2)	1 (5.9)	–	–
Total (n=114)	1(0.9)	67 (58.8)	6 (5.3)	–	–

*% of total isolates for each bacterial group.

Table 6. Frequency of biocide MIC for Gram-negative bacteria

MIC ($\mu\text{l ml}^{-1}$)	No. of isolates (%)*				
	Dettol	Septol	Purit	Tetmosol	Z Germicide
50	–	35 (30.7)	7 (6.1)	–	–
25	6 (5.3)	2 (1.8)	25 (21.9)	1 (0.9)	10 (8.8)
12.5	15 (13.2)	6 (5.3)	29 (25.4)	1 (0.9)	97 (85.1)
6.25	65 (57.0)	4 (3.5)	28 (24.6)	45 (39.5)	7 (6.1)
3.13	24 (21.1)	–	13 (11.4)	62 (54.4)	–
1.56	3 (2.6)	–	3 (2.6)	4 (3.5)	–
0.78	–	–	3(2.6)	1(0.9)	–
0.391	–	–	–	–	–
0.195	–	–	–	–	–
0.098	–	–	–	–	–
Total	113 (99.1)	47 (41.2)	108 (94.7)	114 (100)	114 (100)

*Percentage of total isolates tested.

Of the 97 isolates of Gram-negative bacteria, none were susceptible to all the antimicrobials tested, and only 1 (1%) was resistant to 2 antimicrobial agents, while 29 (30%) isolates were resistant to 3–5 antibiotics, and 67 (69%) were resistant to more than 5 antibiotics (Table 10). A total of 56 resistance patterns, as shown in Table 10, were recorded for the 97 isolates tested, with IPM+ENR+P+S+CN+AML+TE+SXT+FOX+CPD and IPM+P+S+AML+TE+FOX being the predominant patterns. A wide spectrum of multiple drug resistance was observed among 96 (99 %) out of the

97 isolates used for the antibiogram. Only four (3.5%) of the Gram-negative bacilli were ESBL-producers, and all four were members of the *Enterobacteriaceae*, viz. *Enterobacter cloacae*, *K. pneumoniae*, *S. rubidaea* and *A. baumannii*. Forty-four (38.6%) isolates were AmpC producers. Of the 44 AmpC producers, 38 were *Enterobacteriaceae* with *E. cloacae* and *K. pneumoniae* predominating, while the remaining 6 AmpC-producing isolates were non-enteric Gram-negative bacilli.

Table 7. Frequency of biocide MBC for Gram-negative bacteria

MBC ($\mu\text{l ml}^{-1}$)	No. of isolates (%)*				
	Dettol	Septol	Purit	Tetmosol	Z Germicide
50	3 (2.6)	35 (30.7)	24 (21.1)	–	1 (0.9)
25	77 (67.5)	3 (2.6)	70 (61.4)	17 (14.9)	111 (97.4)
12.5	11 (9.6)	3 (2.6)	10 (8.8)	75 (65.8)	–
6.25	20 (17.5)	–	–	19 (16.7)	–
3.13	–	–	1 (0.9)	–	–
1.56	1 (0.9)	–	1 (0.9)	1 (0.9)	–
0.78	–	–	–	–	–
Total	112 (98.2)	41 (36.0)	102 (89.5)	112 (98.2)	112 (98.2)

*Percentage of total isolates tested.

Table 8. Summary of antibiogram of *Enterobacteriaceae*

Antibiotics	No. of isolates (%)*			
	S	I	R	Total
Penicillin	0	1 (1)	75 (99)	76
Amoxicillin	3 (4)	0	82 (96)	85
Imipenem	17 (20)	27 (33)	40 (48)	84
Cefoxitin	26 (31)	10 (12)	47 (57)	83
Cefpodoxime	22 (28)	18 (23)	38 (49)	78
Gentamicin	11 (13)	33 (40)	39 (47)	83
Streptomycin	1 (1)	2 (3)	72 (96)	75
Tetracycline	21 (26)	2 (2)	62 (73)	85
Chloramphenicol	37 (43)	15 (17)	34 (40)	86
Enrofloxacin	34 (40)	24 (28)	28 (33)	86
Sulphamethoxazole/trimethoprim	30 (35)	5 (6)	51 (59)	86

*% of row total.

DISCUSSION

All surfaces sampled in this study recorded at least one bacterial isolate. The high isolation rate for treatment tables (TTs) might result from the fact that many kinds of animals with different conditions are examined and treated on TTs, leading to contamination of these tables. These microbial contaminants may persist due to inadequate cleaning and disinfection of the tables after each treatment and at the close of work. This finding was contrary to the report of Anyanwu *et al.* [19], where clinic floors recorded the highest isolation rate.

During physical examination and treatment of animals, clinicians wear hand gloves to touch animals most of the time. There is a very strong tendency for clinicians to touch and contaminate the surfaces of their tables, chairs and door handles, as well as other surfaces, as they walk around

performing their duties. This could account for the high isolation rate of bacteria from receptionist/clinician desks (RCDs) observed in this study, since these surfaces are rarely disinfected. Weighing balances are used frequently to determine the weight of animals either by directly placing the animal on them or indirectly by someone carrying the animal and stepping on the balance. These animals, their owners and attending personnel tread on the floor, thereby introducing these bacteria to the scales. Thus, the high isolation frequency from weighing balances (WBs) may also be due to inadequate cleaning and disinfection of the contaminated weighing scales after each use and at the close of work. Similarly, door handles in the clinics recorded high isolation frequency. This is not really surprising because door handles are neglected for cleaning and disinfection despite being one of, if not the

Table 9. Antibiogram of non-enteric Gram-negative bacilli

Antibiotics	No. of isolates (%)*			
	S	I	R	Total
Penicillin	0	0	8 (100)	8
Amoxicillin	0	0	11 (100)	11
Imipenem	10 (91)	0	1 (9)	11
Cefoxitin	5 (63)	2 (25)	1 (13)	8
Cefpodoxime	2 (25)	2 (25)	4 (50)	8
Gentamicin	6 (55)	2 (18)	3 (27)	11
Streptomycin	0	1 (13)	7 (88)	8
Tetracycline	5 (45)	0	6 (55)	11
Chloramphenicol	1 (9)	1 (9)	9 (82)	11
Enrofloxacin	7 (64)	2 (18)	2 (18)	11
Sulphamethoxazole/trimethoprim	6 (55)	0	5 (45)	11

*% of row total.

most, frequently touched objects in the clinics by both staff and visitors to the clinics. This result corroborates the report of Bhatta *et al.* [20] concerning a similar study conducted in a tertiary care hospital in Nepal in which a high isolation rate (25.1%) of bacteria from door handles was recorded. Interestingly, this study also revealed that the isolation frequency for potential pathogens from client chairs was high. Client chairs are among the frequently touched surfaces that are neglected for cleaning and disinfection. This ignores the fact that the clients are directly in contact with their animals while bringing them to the clinic, contaminating their hands and other parts of their bodies in the process. Clients consequently contaminate their chairs while waiting for their animals to be attended to.

Other surfaces that recorded significantly high isolation frequencies for bacteria in the present study included the treatment room floor (TRF), the drug cabinet (DC), the drip stand (DS), the animal waiting area floor (AWAF), the handwashing basin (HB), clinic switches (CSs), and clinic fridge door handles (CFDHs). One would have thought that the floors of treatment rooms and animal waiting areas would record a larger number of different bacteria isolates than any other surface sampled, but that was not the case in this study. This could be attributed to the fact that animals brought for examination and treatment stand, rather than sit, for long periods on the floors of these units before being attended to. This reduces the surface area contact between the animal and the floor. This is unlike what happens on examination/treatment tables, where the animals are made to lie down most of the time, establishing wide surface area contact between the bodies of animals and the surfaces of treatment tables. This enhances the contamination of the tables by the animals. However, the large number of bacterial isolates from the floors sampled in this study agrees with the report by Anyanwu *et*

al. [19] that recorded a high isolation rate for bacteria from the floor of treatment rooms in the veterinary clinics visited in their study in Enugu. The DCs, DSs, CSs and CFDHs are all frequently touched surfaces, especially by personnel at the clinics, and are neglected for cleaning and disinfection. Negligence regarding these surfaces accounted for the high isolation frequencies for bacteria.

The large number of bacterial isolates from handwashing basins was not unexpected, since this is the point where clinic personnel wash their hands after attending to patients. This finding agrees with that of Anyanwu *et al.* [19], who reported the isolation of bacteria from handwashing basins in the clinics visited. The low isolation frequency recorded for waste bin handles (WBHs) was surprising because waste bins are one of the most frequently used units in the clinics that are not cleaned and disinfected. This finding suggests that users of these bins were probably not touching the handles while using them. The low isolation frequencies for microbial contaminants from the other surfaces may be attributed to: (1) the sampled items not being among the frequently touched surfaces and thus being less exposed to microbial contaminants and (2) sampled items not being found in all the veterinary clinics visited. For example, kidney dishes (KDs), among other items, were only sampled in clinic C, while animal baths (ABs) were only sampled in clinic A.

Unlike in previous reports [19, 20], common pathogens of fish, *Aeromonas hydrophila* and *Vibrio alginolyticus*, were isolated in this study. This may likely have resulted from the common practice in the study area of feeding dogs and cats raw fish that may have been contaminated or infected with these fish pathogens.

The different biocide activities of bacterial isolates observed in this study agree with the reviews of Maillard [21] that suggest biocide activity varies greatly between different types of micro-organisms and it might also differ between different

Table 10. Antibiotic resistance patterns of the Gram-negative bacilli

S/no.	Pattern	Frequency (%)
1.	P+AML	1
2.	S+C+SXT	1
3.	P+S+AML	3
4.	CN+AML+CPD	1
5.	P+C+AML	1
6.	P+C+AML+SXT	2
7.	P+S+AML+CPD	3
8.	P+S+AML+TE	1
9.	P+S+CN+AML	1
10.	P+CN+AML+CPD	1
11.	IPM+P+S+AML+FOX	1
12.	IPM+P+S+CN+AML	1
13.	P+S+AML+TE+SXT	2
14.	IPM+P+S+AML+TE	3
15.	P+C+AML+FOX+CPD	1
16.	ENR+P+S+AML+SXT	1
17.	P+S+AML+TE+CPD	3
18.	P+S+C+AML+TE	3
19.	ENR+P+S+AML+TE+SXT	1
20.	P+S+C+AML+TE+SXT	1
21.	P+S+CN+AML+FOX+CPD	1
22.	P+S+C+AML+FOX+CPD	1
23.	P+S+C+AML+SXT+CPD	1
24.	IPM+P+S+CN+AML+FOX	1
25.	IPM+P+S+AML+TE+SXT	1
26.	P+S+CN+AML+TE+SXT	1
27.	IPM+P+S+AML+TE+FOX	5
28.	P+S+C+AML+TE+CPD	2
29.	IPM+P+S+C+AML+TE	3
30.	IPM+P+S+CN+AML+TE+SXT	1
31.	P+S+C+CN+AML+TE+SXT	3
32.	P+S+C+CN+AML+SXT+CPD	1
33.	IPM+P+S+AML+TE+FOX+CPD	2
34.	IPM+P+S+AML+TE+SXT+FOX	1
35.	ENR+P+S+AML+TE+SXT+FOX	2
36.	P+S+C+AML+TE+SXT+CPD	2
37.	ENR+P+S+C+CN+AML+SXT	1
38.	IPM+ENR+P+S+CN+AML+FOX	1

Continued

Table 10. Continued

S/no.	Pattern	Frequency (%)
39.	IPM+ENR+P+S+AML+TE+SXT+CPD	1
40.	IPM+P+S+CN+AML+TE+SXT+FOX	1
41.	P+S+C+CN+AML+SXT+FOX+CPD	1
42.	IPM+P+S+C+CN+AML+FOX+CPD	1
43.	ENR+P+S+C+CN+AML+TE+SXT	3
44.	P+S+C+CN+AML+TE+SXT+CPD	2
45.	P+S+C+AML+TE+SXT+FOX+CPD	2
46.	ENR+P+S+CN+AML+TE+SXT+CPD	1
47.	IPM+P+S+C+CN+AML+TE+SXT+FOX	1
48.	ENR+P+S+CN+AML+TE+SXT+FOX+CPD	1
49.	ENR+P+S+C+CN+AML+TE+SXT+FOX	1
50.	P+S+C+CN+AML+TE+SXT+FOX+CPD	2
51.	IPM+ENR+P+S+AML+TE+SXT+FOX+CPD	4
52.	IPM+ENR+P+S+CN+AML+TE+SXT+FOX+CPD	6
53.	IPM+P+S+C+CN+AML+TE+SXT+FOX+CPD	2
54.	IPM+ENR+P+S+C+CN+AML+TE+SXT+FOX	1
55.	ENR+P+S+C+CN+AML+TE+SXT+FOX+CPD	3
56.	IPM+ENR+P+S+C+CN+AML+TE+SXT+FOX+CPD	2

strains of the same species. Maillard [22] and SCENIHR [23], in their review reports, pointed out that some biocides currently used in hospitals have been found to be ineffective against bacteria that produce biofilms. The biocide-resistant isolates in this study comprised both biofilm-producing and non-biofilm-producing bacteria. This means that biofilm production was not the only resistance mechanism deployed by bacterial strains in the study area.

This study revealed that all the isolates of the Gram-negative bacteria tested were inhibited by Tetmosol and Z Germicide disinfectants at 25 $\mu\text{l ml}^{-1}$, while at concentrations of 0.391 and 3.13 $\mu\text{l ml}^{-1}$ all isolates were resistant to Tetmosol and Z Germicide, respectively. Going by the dilutions recommended by the manufacturers of these disinfectants for domestic cleaning, which were 27 $\mu\text{l ml}^{-1}$ for Tetmosol and 200 $\mu\text{l ml}^{-1}$ for Z Germicide, these biocides were very effective against Gram-negative bacteria even at a lower concentration. The high susceptibility rate (100%) of bacteria to Tetmosol and Z Germicide was probably due to the composition of these biocides. Chloroxylenol, sodium hydroxide and isopropyl alcohol are the active components in Tetmosol. These agents are collectively bactericidal with broad-spectrum antimicrobial activities. Chloroxylenol is bactericidal and exerts its pharmacological action by the denaturation of proteins and inactivation of enzymes in the micro-organisms, altering the permeability of the cell membrane, which results in the uncoupling of oxidative phosphorylation, inhibition of active transport and cytoplasmic membrane damage. Sodium

hydroxide is effective against many bacteria by saponifying the lipids in the enveloping membrane, leading to destruction of the superficial structure. Isopropyl alcohol exhibits broad-spectrum antimicrobial activity against vegetative bacteria via denaturation of proteins. The active agents in Z Germicide are tar acid phenol and cresylic creosote, both of which are derived from coal tar or petroleum. These phenolic compounds are effective against both Gram-negative and Gram-positive bacteria, in which they act specifically on the cell membrane and inactivate intracytoplasm enzymes by forming unstable complexes, denaturing the bacterial proteins, leading to lysis of the cell membrane. The combined activities of the different active components of these biocides were demonstrated in the high rates of bactericidal action against the Gram-negative bacteria.

Resistance to Dettol (1/114, 0.9%), Purit (6/114, 5.3%) and Septol (67/114, 58.8%) was observed despite using the manufacturers' recommended maximum concentrations for domestic cleaning: Dettol, 50 $\mu\text{l ml}^{-1}$; Septol, 6.75 $\mu\text{l ml}^{-1}$; and Purit, 13.5 $\mu\text{l ml}^{-1}$. The isolates were resistant to these biocides, particularly Septol and Purit, even at a concentration of 50 $\mu\text{l ml}^{-1}$, which was far above the recommended concentrations for these disinfectants. These findings are of public health importance because these disinfectants are among the commonly used ones in human and animal clinics, and also in households for domestic cleaning. The only isolate (*Tatumella ptyseos*) resistant to Dettol was susceptible to other disinfectants tested. This could suggest that the

organism exhibits a different resistance mechanism than the other biocide-resistant Gram-negative bacilli. The major difference in the composition of Dettol and Tetmosol is the presence of sodium hydroxide in Tetmosol. This could also suggest that the Dettol-resistant isolate may be susceptible to sodium hydroxide. The resistance rate (5.3%) observed for Purit in this study should not be ignored because Purit inhibited all the susceptible isolates at a concentration ($50 \mu\text{l ml}^{-1}$) far above the recommended concentration ($13.5 \mu\text{l ml}^{-1}$). This implies that if the dilution recommended by the manufacturer is adhered to, the resistance rate will be up to 33%. The resistance to Purit may be attributed to the substitution of chloroxylenol with chlorhexidine gluconate in the disinfectant. The staggeringly high resistance rate (58.8%) recorded for Septol could be due to the composition (pine oils and 5-chloro 2-hydroxyl methane) of the disinfectant. None of the active agents found in the other disinfectants is a component of Septol. Also, if the dilution recommended by the manufacturer for domestic cleaning ($6.75 \mu\text{l ml}^{-1}$) is adhered to, the resistance rate will be up to 96%. Based on our findings, disinfectants containing only pine oils and 5-chloro 2-hydroxyl methane as their active agents are not effective against Gram-negative bacteria. In a study by El-Mahmood and Doughari [11] that analysed five frequently used disinfectants (Dettol, Purit, Septol, Z Germicide and parazone) for bacterial contamination in Specialist Hospital, Yola, Nigeria, all the in-use diluted disinfectants were found to be contaminated. They attributed this to non-adherence to the manufacturers' recommended dilution values. From our findings, this may not be true for disinfectants such as Dettol, Purit and particularly Septol, where resistance was observed at concentrations far above the recommended dilutions.

Four biocide resistance patterns observed in this present study suggest that approximately four mechanisms of resistance to biocides may be involved in the resistance demonstrated by the bacteria isolated in the study area.

Members of the family *Enterobacteriaceae* were highly resistant to penicillin (99%) and amoxicillin (96%), while all the isolates of non-enteric Gram-negative bacilli were resistant to these antibiotics. This result was not surprising because penicillin and amoxicillin are generic β -lactam antibiotics that inhibit cell wall synthesis of bacteria; the predominant mechanism of resistance to β -lactams in Gram-negative bacteria is the production of β -lactamases that destroy the amide bond of the β -lactam ring, rendering the antimicrobial ineffective [24]. The rate (48%) of resistance to imipenem by members of the family *Enterobacteriaceae* was high, as this drug belongs to the class of carbapenem antibiotics, the most potent β -lactams available in clinical practice [24]. This suggests that strains of *Enterobacteriaceae* in the study area have developed resistance to carbapenems. This observation did not agree with the result of Anyanwu *et al.* [19], which recorded a very low resistance rate (7.7%) to imipenem in Gram-negative bacteria. Members of the family *Enterobacteriaceae* demonstrated high resistance rates to cefoxitin (57%) and cefpodoxime (49%). These antibiotics are respectively second- and third-generation cephalosporins that target bacterial cell wall synthesis. This is

a pointer to the fact that Gram-negative bacteria in the study area are seriously developing (or acquiring) mechanisms for resistance to new β -lactam compounds with a wider spectrum of activity and less susceptibility to penicillinases (such as ampicillin). According to Munita and Arias [24], the development of newer generations of β -lactams has systematically been followed by the rapid appearance of enzymes capable of destroying any novel compound that reaches the market, in a process that is a prime example of antibiotic-driven adaptive bacterial evolution. Among the cefpodoxime-resistant Gram-negative bacteria, only 4 isolates produced extended-spectrum beta-lactamase (ESBL) and 44 produced AmpC beta-lactamase (AmpC) enzymes. This implies that these bacteria have acquired the ability to produce enzymes that hydrolyze third generation cephalosporins and aztreonam.

The high level of resistance to gentamicin in the Gram-negative bacteria is alarming because this drug is not commonly used in veterinary medicine as it is generally found in injectable forms and thus does not form a component of the common oral drug preparations used indiscriminately. This result is contrary to the report of Anyanwu *et al.* [19] in which low rates of resistance to gentamicin were recorded. The high level of resistance to streptomycin and tetracycline in this study agrees with the report Anyanwu *et al.* [19]. The high resistance to streptomycin and tetracycline may be associated with the extensive use of these antibiotics.

The resistance rates to chloramphenicol in bacteria observed in this study were significantly high, particularly in non-enteric Gram-negative bacilli (82%), despite the fact that this antibiotic is not commonly used in veterinary medicine. Many ESBL producers are additionally multi-resistant to non- β -lactam antibiotics, including fluoroquinolones, aminoglycosides, trimethoprim, tetracyclines, sulfonamides and chloramphenicols [25–27]. This may suggest that AmpC β -lactamase producers are also multi-resistant to these antibiotics. This may partly explain the high level of resistance to chloramphenicol in bacteria isolated in the study area. The high resistance to chloramphenicol recorded in this study was similar to the resistance level reported by Moawad *et al.* [25] among members of the family *Enterobacteriaceae* isolated from apparently health broilers. Also in a cross-sectional study on the use of antimicrobials in the veterinary clinics and antibiotic resistance, Yehualaeshet [28] reported high susceptibility level to chloramphenicol in *Enterobacteriaceae* and a high level of resistance in *Pseudomonas* species.

The resistance level (31%) to enrofloxacin in Gram-negative bacteria is relatively high and worrying considering the fact that fluoroquinolones are not among the common drugs for veterinary use in Nigeria; probably because they are expensive. In contrast to the observation made in this study, Anyanwu *et al.* [19], in a similar study in Enugu, reported low resistance rate to ciprofloxacin (a fluoroquinolone) in Gram-negative bacteria (7.7%).

The present study revealed high resistance to sulphamethoxazole/trimethoprim in spite of the fact that it is a broad-spectrum sulfonamide. This could be due to indiscriminate use of sulfonamides for prophylaxis and treatment in both humans and animals in Nigeria. Bhatta *et al.* [20] and Sserwadda *et al.* [29] similarly

recorded high resistance rates to sulphamethoxazole/trimethoprim in bacteria isolated from contaminated surfaces in human hospitals in Nepal and Uganda respectively.

Multiple drug resistance to at least three antimicrobial agents was observed in 99% of the Gram-negative bacteria tested in this study. This is bothersome and very high compared with similar studies that reported MDR bacteria from the hospital environment. In a study by Bhatta *et al.* [20] to determine the bacterial contamination of common hospital objects frequently touched by patients, visitors and healthcare workers in a tertiary care hospital, *Acinetobacter* species (52.6%) and *E. coli* (46.6%) were found to be ESBL producers. A wide-spectrum resistance pattern was recorded for the Gram-negative (56) bacteria isolated in this study, which suggests that several pressures may be involved in the induction of drug resistance

It is important to note that 98% of the biocide-resistant isolates exhibited multiple drug resistance to 3 to 10 different classes of antimicrobial agents. The resistance demonstrated by these isolates to both biocides and antimicrobial agents could be explained by the report of SCENIHR [23] indicating that many similarities exist in the mechanisms of action of biocides and antibiotics. Both agents diffuse into bacteria, can modify or destroy the bacterial membrane, and can disrupt key steps in bacterial chemical reactions. The report also pointed out that genes that confer resistance to antibiotics can also be involved in biocide resistance, such as efflux pump genes, so bacteria that acquire resistance genes sometimes become resistant to both types of antimicrobials at the same time. Our findings further give credence to reports that MDR bacteria have the propensity to develop resistance to biocides, as there are similarities in the modes of action of these antimicrobial agents.

Conclusions

The isolation of many important Gram-negative bacteria in the present study reveals that veterinary clinic environments in Makurdi, Benue State are reservoirs of wide varieties of potential pathogens of veterinary and public health importance. The findings should be of concern to veterinarians and other public health officers because the members of the *Enterobacteriaceae* identified have been implicated in hospital-acquired infections in companion animals [4, 30–32]. Similarly, the non-enteric Gram-negative bacilli isolated in this study are important animal pathogens and have been reported in many disease conditions, such as wooden tongue in cattle, fatal pneumonia in pigs and septicaemia in neonatal foal caused by *Actinobacillus* species [33], infectious bovine keratoconjunctivitis [34, 35] and pulmonary abscesses in zoo herbivores [36] caused by *Moraxella* species.

The high isolation frequency for bacteria from these surfaces points to a high risk of transmission of the contaminating potential pathogens. Also, the high rate of bacterial contamination of the surfaces sampled in this study, particularly clinician desks, door handles, client chairs, light and fan switches, and fridge door handles,

reflects poor hand hygiene among the clinic personnel and visitors, as contamination of these surfaces mainly occurs through contaminated fingers.

Based on the findings of this present study, it is imperative to emphasize consistent hand hygiene and also sanitization of the frequently touched surfaces and environments in veterinary clinics. Disinfectants containing chloroxylenol, castor oil, sodium hydroxide and isopropyl alcohol, and tar acid phenol and cresylic creosote are recommended for decontamination of clinic environments with biocidal solutions applied at recommended concentrations by the manufacturer. Adherence to these recommendations will reduce the risk of the development of biocide tolerance by bacteria in the study area, which will invariably reduce the incidence of nosocomial infections. Due to the high level of multiple drug resistance among the bacterial isolates, the use of antimicrobial drugs should be based on the results of sensitivity tests. There is a need to monitor veterinary clinic environments for potential pathogens to facilitate the control of possible nosocomial infections.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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