Evaluation of Post-Operative Antibiotic Administration on Phenotypic Antibiotic Susceptibility and Resistance Profiles of Gram-negative Bacterial Flora of Healthy Local Experimental Dogs Undergoing Partial Gastrectomy

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Abstract

Reliable data that can serve as platform for policy formulation on the use of antimicrobial drugs are compulsory for every country. But baseline data to judge effects of long-term post-operative antibiotic administration in clinical and surgical canine health conditions are currently lacking in Nigeria. This study aimed at providing vital baseline antibiotic profiles of canine bacteria of veterinary and public health importance. Phenotypic antibiotic susceptibility and resistance profiles of some easily recoverable Gram-negative bacterial species of canine origin were determined towards the most commonly prescribed and administered antibiotics in veterinary practices, using agar disk- and modified agar well-diffusion methods. Overall resistance of the bacteria against antibiotics (discs) were- tetracycline (25.0-57.1%), cotrimoxazole (80.0-100%), nitrofurantoin (20.0-100%), nalidixic acid (0.91-60.0%), gentamicin (18.2-70.0%), ofloxacillin (20.0-42.9%) and augmentin / amoxicillin (100%), except among *Klebsiella pneumoniae* and *Proteus mirabilis* (60.0-100), with percentage multiple antibiotic resistance (%MAR) of 25.0 - 100%. Overall percentage resistance rates towards antibiotic drugs were- amoxicillin (9.1-100%); oritaxim / oxytetracycline (20.0-100%) and nitaxim (30.0-100%), with %MAR mostly between 50.0 and 100%. Twenty eight antibiotic resistance profiles were exhibited against antibiotic (discs), commonest profiles being – augmentin-amoxicillin-cotrimoxazole; augmentin-tetracycline-amoxicillin-cotrimoxazole-nitrofurantoin-nalidixic acid-gentamicin-ofloxacillin; augmentin-tetracycline-amoxicillin-cotrimoxazole and augmentin-amoxicillin-cotrimoxazole-nitrofurantoin. Twelve antibiotic resistance profiles were exhibited against the antibiotic drugs, with most-resisted / commonest profile being – oritaxim-nitaxim. These were also the most-resisted nitaxim (47.3%) and oritaxim (52.7%) antibiotic drugs. Only

29.1% of the Gram-negative bacteria were totally susceptible to the four antibiotic drugs, while a total of 70.9%, including mono-resistance (21.8%) and multi-resistance (49.1%), were recorded. This preliminary baseline report indicated significant phenotypic antibiotic resistance among easily-recoverable, indigenous Gram-negative bacterial species of canine origin, which is of veterinary and human public health significance, and indicative of therapeutic treatment failure.

Keywords: antibiotic resistance, dogs, veterinary public health, veterinary surgery

Introduction

Antimicrobial prophylaxis is only one relatively minor effort among numerous preventive measures against microbial infections, and although it is not a substitute for good surgical techniques, the efficacy and impact of pre-operative and post-operative antimicrobial prophylaxis has been demonstrated to be significant (Stinner *et al.*, 1998; Bowater *et al.*, 2009) but timing of antibiotic administration is critical for efficacy (Wilcke, 1990; Dunning, 2003). In certain gastrointestinal procedures, oral and intravenous administration of antimicrobial agents with activity against Gram-negative and anaerobic bacteria is warranted. Whereas, unwarranted antimicrobial administration, inappropriate antimicrobial prophylaxis, such as inappropriate selection of antimicrobial agents, inappropriate dosing regimen or extended duration of antibiotic administration can increase prevalence of antibiotic resistant strains (Holmberg, 1978; Classen *et al.*, 1992; Bratzler and Houck, 2004; Smet *et al.*, 2010).

Several studies have demonstrated efficacy with antibiotic spectrum usually directed at Gram-negative aerobic and anaerobic bacteria (Clarke *et al.*, 1977; Baum *et al.*, 1981; Kaiser *et al.*, 1983; Solla and Rothenberger, 1990; Sullivan *et al.*, 2001; Solomkin *et al.*, 2010); still, colorectal procedures have very high intrinsic risk of infection and therefore, warrant strong recommendation for prophylaxis. The crisis of antimicrobial drug resistance in human medicine has brought into question every aspect of use of these drugs in animals (Prescott *et al.*, 2002), even with often fragmented data on antimicrobial drug resistance in bacteria of food animal origin (Van den Bogaard and Stobberingh, 1999; Wegener, 1999; Ogunshe *et al.*, 2011; **Geser** *et al.*, 2012). By contrast however, the effect of antimicrobial drug use in companion animals like dogs or the acquisition of resistant bacteria by humans from companion animals has not been explored in any significant way (Pleydell *et al.*, 2012), most especially in developing countries, like Nigeria.

As reported by Prescott *et al*. (2002), pet owners may be at risk when their companion animals like dogs are treated with antimicrobial drugs that are prone to be resisted by bacterial species of canine origin. In addition, the natural commensal, and especially the beneficial Gram-negative bacteria resident in the GIT will ultimately be affected by such resistance effect. In Nigeria, in addition to the fact that prescription / administration of antibiotics were mostly empiric, and usually not matching antibiotics that would have been recommended by microbiological results, data on quantities of antimicrobial drugs used in companion animal practices, especially during surgical procedures, as well as reliable data on antibiotic resistance in canine pathogens are currently lacking. Similarly, there are no baseline data against which to judge any effect of antibiotic resistance in surgical interventions. The objective of this study therefore, was to determine the antibiotic susceptibility and resistance profiles of indigenous Gram-negative bacterial flora isolated from rectal contents of healthy experimental dogs that had partial gastrectomy, as a preliminary baseline data in Nigeria.

Materials and Methods

Ethical approval

Experimental protocols and ethical approval were sought and obtained from the Faculty of Veterinary Medicine Ethical Committee, Faculty of Veterinary Medicine, University of Ibadan, Nigeria (Akinrinmade *et al*., 2013).

Experimental animals

Twelve local adult Mongrel dogs, consisting of six males and six females with body weight ranging from 11-15kg were used in this study. The experimental animals, clinical condition and housing, stabilisation of the experimental animals, surgical procedures and collection of rectal (faecal) specimens for microbiological analyses were as reported in Akinrinmade *et al*. (2013). The experimental animals were fed on same diet and maintained under same pre-surgical, surgical and post-surgical conditions throughout the experimental period. The dogs whose age compositions were 18-24 months were randomised into three treatment groups, each consisting of 3 dogs, while an additional group served as control. All the experimental animals were clinically examined prior to the experiments and declared healthy. Laparotomy was performed on each of the experimental animals after aseptic preparation according to the method of Hedlund (2002).

Bacterial isolation and identification

Bacterial flora assayed for antibiotic profiles in the current study were aseptically isolated from the experimental animals and identified using standard phenotypic taxonomic tools as reported in the study of Akinrinmade *et al.* (2013).

Antibiotic susceptibility determination (discs)

Using agar disk-diffusion method (Bauer *et al.*, 1966), bacterial isolates from rectal contents of local, healthy experimental dogs were screened against the most-commonly available and used antibiotic discs, manufactured by ABTEK, Biologicals Ltd. (Liverpool, UK). The discs were – amoxycillin (AMX 25µg); augmentin (AUG 30µg); cotrimoxazole (COT 25µg); gentamicin (GEN 10µg); nalidixic acid (NAL 30µg); nitrofurantoin (NIT 300µg); ofloxacillin (OFL 30µg) and tetracycline (TET 30µg). Zones of inhibition were measured and recorded in millimetre diameter, while zones less than 10.0 mm in diameter or absence of inhibition zones were recorded as resistant (negative).

Antibiotic susceptibility determination (antibiotic drugs)

Inhibitory activities of three intramuscular antibiotic drugs- amoxicillin, cefotaxime (nitaxim) and intramuscular oxytetracycline (250 mg) used in the study of Akinrinmade *et al.* (2013) and oritaxim were assayed for in this study, using a modification of agar well-diffusion method of Tagg *et al.* (1976). The modification was incorporation of the antibiotic drugs into sterile semi-solid, Mueller-Hinton agar before dispensing the drugs into the agar wells to avoid spreading of the antibiotics from the agar wells to the agar surfaces. The seeded agar plates were then incubated at 35^0C for 24-48 hours and zones of inhibition were measured and recorded in millimetre diameter, while zones less than 10.0 mm in diameter or absence of inhibition zones were recorded as resistant (negative). Statistical method used was means of triplicates of recorded zones of inhibition in the antibiotic susceptibility determination.

Results

In the current study, 55 Gram-negative bacterial strains isolated from rectal contents of healthy local experimental dogs before and after partial gastrectomy (Figs. 1-5) were all resistant to amoxicillin and augmentin antibiotics (discs), except among *Klebsiella pneumoniae* and *Proteus mirabilis* in which

60.0% and 90.0% resistance were recorded respectively against amoxicillin (discs). Least-resisted antibiotic by the Gram-negative bacteria was ofloxacillin (0.0-42.9%), while, cotrimoxazole (89.1%), amoxicillin (96.4%) and augmentin (100%) were the most-resisted antibiotics (discs), with percentage multiple antibiotic resistance (%MAR) and percentage antibiotic resistance profiles (%ARP) of 25.0-100% recorded (Tables 1 & 2).

In general, the overall resistance profiles of the Gram-negative bacteria against the test antibiotic (discs) in this study were- augmentin (100%), tetracycline (25.0-57.1%), amoxicillin (60.0-100%), cotrimoxazole (80.0-100%), nitrofurantoin (20.0-100%), nalidixic acid (0.91-60.0%), gentamicin (18.2-70.0%) and ofloxacillin (20.0-42.9%). None of the Gram-negative bacteria from rectal contents of the experimental local dogs that had partial gastrectomy in this study was totally susceptible to all the eight antibiotics (discs), while a total of 28 different multiple antibiotic resistance profiles were exhibited. Commonest multi-resistance profiles were - augmentin-amoxicillin-cotrimoxazole (7.3%), augmentin-tetracycline-amoxicillin-cotrimoxazole-nitrofurantoin-nalidixic acid-gentamicin-ofloxacillin (7.3%), augmentin-tetracycline-amoxicillin-cotrimoxazole (9.1%) and augmentin-amoxicillin-cotrimoxazole-nitrofurantoin (12.7%) (Table 2).

Lower resistance rates of 0.0-40.0% against intramuscular amoxicillin drug and 0.0-50.0% against intramuscular oxytetracycline drug were exhibited by the Gram-negative bacteria, while as high as 50.0-100% and 72.7% -100% resistance rates were recorded against intramuscular nitaxin and oritaxim drugs respectively. In general, overall resistance profiles of the Gram-negative bacteria were - amoxicillin (9.1-100%); oritaxim (20.0-100%); nitaxim (30.0-100%) and oxytetracycline (20.0-100%). Recorded MAR were mostly 50.0 - 75.0% and 50.0-100%, while percentage antibiotic resistance profiles were mostly between 25.0 and 100%.

A total of 13 different antibiotic profiles were exhibited by the Gram-negative bacteria against the four intramuscular antibiotic drugs. Profile 1 indicated that just 29.1% of the 55 Gram-negative bacterial strains were totally susceptible to the four antibiotic drugs, while only one bacterial strain was totally resistant. Commonest antibiotic resistance profile was that of oritaxim-nitaxim, which was displayed by 29.1% of the Gram-negative bacteria, while nitaxim (47.3%) and oritaxim (52.7%) were the most-resisted intramuscular antibiotic drugs. Recorded mono- and multi-resistance rates were 21.8% and 49.1% respectively (Table 3), with very low overall resistance rates recorded for oxytetracycline (18.2%) and amoxicillin (20.0%). A total of 70.9% of the Gram-negative bacterial strains exhibited antibiotic resistance against the intramuscular antibiotic drugs, while 49.1% of the Gram-negative bacterial strains exhibited multiple antibiotic resistance.

The Gram-negative bacterial species exhibited the widest zones of inhibition against nalidixic acid disc (22.0-30.0 mm) and nitrofurantoin disc (22.0-25.0 mm) but significantly wider zones of inhibition of between 24.0 and 40.0 mm were exhibited by the Gram-negative bacteria against the intramuscular antibiotics drugs (Table 1).

Table 1: Overall *in vitro* antibiotic resistance patterns / zones of inhibition profiles of Gram-negative bacterial species isolated from the rectal contents of healthy local dogs

% Resistance / susceptibility zones of inhibition

Bacterial species	Antibiotic discs (µg-1/ mg l-1)									Intramuscular antibiotic drugs (µg-1/ mg l-1)					
	AUG	TET	AMX	COT	NIT	NAL	GEN	OFL	%MAR	% ARP	AMX	ORTX	NTX	OXYT	% MAR	% ARP
Citrobacter aerogenes [8]	100	37.5%	100%	87.5%	62.5%	50.0%	37.5%	25.0%	25.0-100%	25.0-100% **[1]	25.0%	75.0%	50.0%	-	50.0-100%	25.0-100% *[2]
	-	(11.0-21.0)	-	(23.0)	(20.0-25.0)	(12.0-24.0)	(10.0-12.0)	(12.0-27.0)			(13.0-25.0)	(13.0-24.0)	(12.0-25.0)	(11.0-32.0)		
E. coli [2]	100	50.0%	100%	100%	-	50.0%	-	-	50.0%		-	-	-	-	-	
	-	(13.0)	-	-	(15.0-18.0)	(22.0)	(11.0-13.0)	(26.0-30.0)			(25.0-30.0)	(11.0-30.0)	(12.0-31.0)	(27.0-30.0)		
Enterobacter aerogenes [1] ^	100	-	100%	100%	-	-	-	-	37.5%		100%	100%	-	100%	75.0%	
	-	(17.0)	-	-	(17.0)	(26.0)	(15.0)	(28.0)			-	-	(15.0)	-		
Kleb. pneumoniae [5]	100	40.0%	60.0%	80.0%	20.0%	60.0%	-	40.0%	25.0-75.0%	25.0-75.0%	40.0%	20.0%	-	20.0%	50.0%	25.0-50.0% *[2]
	-	(11.0-26.0)	(12.0)	(23.0)	(10.0-25.0)	(19.0-23.0)	(10.0-16.0)	(20.0-23.0)			(12.0-21.0)	(12.0-37.0)	(17.0-32.0)	(11.0-35.0)		
Proteus mirabilis [10]	100	50.0%	90.0%	80.0%	30.0%	50.0%	70.0%	20.0%	37.5-100%	37.5-100% **[2]	10.0%	40.0%	30.0%	20.0%	50.0-75.0%	25.0-75.0% *[2]
	-	(13.0-22.0)	(13.0)	(20.0-22.0)	(12.0-21.0)	(13.0-30.0)	(10.0-11.0)	(17.0-35.0)			(14.0-32.0)	(21.0-37.0)	(13.0-40.0)	(13.0-31.0)		

Ps. aeruginosa [11]	100	53.6%	100%	90.9%	81.8%	0.91%	18.2%	-	50.0-75.0%	50.0-75.0%	9. 1%	72.7%	53.6%	-	50.0-75.0%	25.0-75.0% *[2]
	-	(13.0-15.0)	-	(12.0)	(18.0-20.0)	(15.0-28.0)	(10.0-12.0)	(11.0-32.0)			(11.0-28.0)	(15.0-31.0)	(17.0-26.0)	(12.0-32.0)		
Salmonella sp. [14]	100	57.1%	100%	92.9%	35.7%	42.9%	35.7%	42.9%	37.5-87. 5%	37.5-87. 5%	21.4%	42.9%	57.1%	21.4%	50.0-75.0%	25.0-75.0% *[3]
	-	(11.0-23.0)	-	(27.0)	(11.0-22.0)	(15.0-30.0)	(10.0-13.0)	(16.0-30.0)			(11.0-32.0)	(14.0-35.0)	(13.0-37.00	(12.0-35.0)		
Shigella dysentariae [4]	100	25.0%	100%	100%	100%	25.0%	25.0%	25.0%	50.0-100%	50.0-100% **[1]	25.0%	100%	100%	50.0%	50.0-100%	50.0-100% **[1]
	-	(13.0-16.0)	-	-	-	(14.0-16.0)	(10.0-13.0)	(21.0-28.0)			(11.0-24.0)	-	-	(12.0-37.0)		

Keys: AUG = augmentin; TET = tetracycline; AMX = amoxicillin; COT = cotrimoxazole; NIT = nitrofurantoin; NAL = nalidixic acid; GEN = gentamicin; OFL = ofloxacillin; AMXT = amoxil; ORT = oritaxim; NXT = nitaxim; OXYT = oxytetracycline; %MAR = percentage multiple antibiotic resistance; % ARP = percentage antibiotic resistance profiles; [* = no resistance / 0.0%; ** = 100.0% MAR]

Table 2: Overall *in vitro* antibiotic resistance profiles of Gram-negative bacterial species isolated from rectal contents of healthy local dogs (antibiotic discs)

		Antibiotic profiles									
		Antibiotic discs (µg-1/ mg l-1)									
No. of profiles	Gram-negative bacterial species per profile	AUG	TET	AMX	COT	NIT	NAL	GEN	OFL	%MAR	No. of strains per profile
1	Citrobacter aerogenes [1], Kleb. pneumoniae [1]	AUG		AMX						25.0%	[2]
2	Proteus mirabilis [1]	AUG		AMX			NAL			37.5%	[1]
3	Ent. aerogenes [1], Kleb. pneumoniae [1]p, Proteus mirabilis [1], Salmonella sp. [1]	AUG		AMX	COT					37.5%	[4]
4	Citrobacter aerogenes [1]	AUG			COT		NAL	GEN		50.0%	[1]
5	Kleb. pneumoniae [1], E. coli [1], Pr. mirabilis [1]	AUG		AMX	COT		NAL			50.0%	[3]
6	Citrobacter aerogenes [1]p, Salmonella sp. [1], Sh. dysenteriae [3], Ps. aeruginosa [2]	AUG		AMX	COT	NIT				50.0%	[7]
7	Ps. aeruginosa [1]p	AUG	TET	AMX		NIT				50.0%	[1]
8	Pr. mirabilis [1]	AUG	TET	AMX				GEN		50.0%	[1]
9	E. coli [1], Salmonella sp. [1], Pr. mirabilis [1], Ps. aeruginosa [2]	AUG	TET	AMX	COT					50.0%	[5]
10	Salmonella sp. [1]p	AUG		AMX	COT			GEN		50.0%	[1]
11	Ps. aeruginosa [1]	AUG		AMX	COT	NIT		GEN		50.0%	[1]
12	Salmonella sp. [1], Ps. aeruginosa [2]p	AUG	TET	AMX	COT	NIT				62.5%	[3]
13	Citrobacter aerogenes [1], Pr. mirabilis [1]p, Ps. aeruginosa [1]	AUG		AMX	COT	NIT		GEN		62.5%	[3]
14	Pr. mirabilis [1]	AUG		AMX	COT		NAL	GEN		62.5%	[1]
15	Salmonella sp. [2]p	AUG	TET	AMX	COT				OFL	62.5%	[2]
16	Salmonella [1]	AUG		AMX			NAL	GEN	OFL	62.5%	[1]

17	Pr. mirabilis [1], Salmonella sp. [1], Ps. aeruginosa [1]	AUG	TET	AMX	COT			GEN		62.5%	[3]
18	Citrobacter aerogenes [1]p	AUG	TET	AMX	COT	NIT				62.5%	[1]
19	Salmonella sp. [1]p, Citrobacter aerogenes [1]	AUG		AMX	COT		NAL	GEN	OFL	75.0%	[2]
20	Salmonella sp. [1]	AUG		AMX	COT	NIT	NAL	GEN		75.0%	[1]
21	Kleb. pneumoniae [1]	AUG	TET		COT	NIT	NAL		OFL	75.0%	[1]
22	Salmonella sp. [1]	AUG	TET	AMX	COT		NAL		OFL	75.0%	[1]
23	Citrobacter aerogenes [1]	AUG	TET	AMX	COT	NIT	NAL			75.0%	[1]
24	Salmonella sp. [1]	AUG	TET	AMX	COT	NIT	NAL			75.0%	[1]
25	Ps. aeruginosa [1]p	AUG	TET	AMX	COT	NIT	NAL			75.0%	[1]
26	Kleb. pneumoniae [1]	AUG	TET	AMX	COT		NAL		OFL	75.0%	[1]
27	Salmonella sp. [1]	AUG	TET	AMX	COT		NAL	GEN	OFL	87.5%	[1]
28	Citrobacter aerogenes [1], Pr. mirabilis [2]p, Sh. dysenteriae [1]	AUG	TET	AMX	COT	NIT	NAL	GEN	OFL	100%	[4]
Overall % resistance		100%	49.1%	96.4%	89.1%	49.1%	38.2%	32.7%	23.6%		

Keys: AUG = augmentin; TET = tetracycline; AMX = amoxicillin; COT = cotrimoxazole; NIT = nitrofurantoin; NAL = nalidixic acid; GEN = gentamicin; OFL = ofloxacillin; p = species including post-antibiotic administered strains.

Table 3: Overall *in vitro* antibiotic resistance profiles of Gram-negative bacterial species isolated from the rectal contents of healthy local dogs undergoing gastrectomy (intramuscular antibiotic drugs)

		Antibiotic profiles					
		Intramuscular antibiotic drugs (µg-1/ mg l-1)					
No. of profiles	Gram-negative bacterial species per profile	AMX	ORT	NTX	OXYT	%MAR	No. of Strains per profile
1	E. coli [1], Kleb. pneumoniae [2]p, Pr. mirabilis [4]p, Ps. aeruginosa [1], Salmonella sp. [1].					0.0%	[16]
2	Citrobacter aerogenes [1], Kleb. pneumoniae [1], Pr. mirabilis [1], Ps. aeruginosa [1]		ORT			25.0%	[4]
3	Kleb. pneumoniae [1]p, Salmonella sp. [1]	AMX				25.0%	[2]
4	Ps. aeruginosa [1], Salmonella sp. [2]			NTX		25.0%	[3]
5	Proteus mirabilis [1], Salmonella sp. [2]p				OXYT	25.0%	[3]
6	Citrobacter aerogenes [1]p, Ps. aeruginosa [1]	AMX	ORT			50.0%	[2]
7	Kleb. pneumoniae [1]	AMX			OXYT	50.0%	[1]
8	Citrobacter aerogenes [3], Salmonella sp. [4]p, Sh. dysenteriae [2]p, Pr. mirabilis [1], Ps. aeruginosa [6]p		ORT	NTX		50.0%	[16]
9	Citrobacter aerogenes [1], Pr. mirabilis [1], Salmonella sp. [1]	AMX	ORT	NTX		75.0%	[3]
10	Enterobacter aerogenes [1p]	AMX	ORT		OXYT	75.0%	[1]
11	Salmonella sp.[1]	AMX		NTX	OXYT	75.0%	[1]
12	Pr. mirabilis [1]p, Sh. dysenteriae [1]		ORT	NTX	OXYT	75.0%	[2]
13	Shigella dysenteriae [1]	AMX	ORT	NTX	OXYT	100%	[1]
Overall % resistance		20.0%	52.7%	47.3%	18.2%		

Keys: AMX = amoxicillin; ORT = oritaxim; NTX = nitaxim; OXYT = oxytetracycline; %MAR = % multiple antibiotic resistance; ^{p} = species including post-antibiotic administered strains.

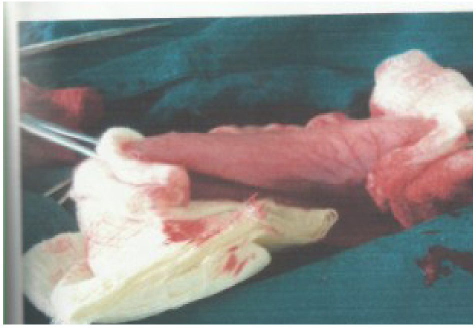

Fig. 1: Normal stomach (fundus) retracted with forceps to show branches of the left gastroepiploic vessels along the greater curvature

Fig. 2: Removal of an elliptical portion of the tissue of the stomach to expose its contents

Fig. 3: Placement of the stomach in situ

Fig. 4: Closure of the stomach with a 2-layered inverting (Connell) of 3.0 chromic catgut

Figs. 5a & 5b: Placement of interrupted horizontal matters sutures in the upper flap to create an overlap

Discussion

Dogs are the commonest pets in Nigeria; however, most of the surgical interventions required in dog health in the country are gastrectomic-related (Figs. 1-5), during which antibiotics are usually administered to prevent post-surgical infections. It has been suggested that prophylactic antimicrobials with anaerobic and enteric Gram-negative coverage should be considered in surgery of the large intestine or other areas containing anaerobic organisms (Harmoinen, 2004). Whereas, use of antibiotic prophylaxis in gastrointestinal surgery can cause disruption of microflora, which leads to colonisation resistance; thereby, encouraging development of antibiotic resistance. Since few reported local studies in the country had confirmed inappropriate antibiotic use and prolonged antibiotic administration periods in clinical/surgical veterinary conditions (Akinrinmade and Oke, 2012), simulating gastrectomic conditions to investigate current surgically-related clinical outcomes of antibiotic administration, and determine a baseline antibiotic administration standards for the country is of significant importance.

Antimicrobial resistance has been increasing among certain Gram-negative bacteria that cause infections in pets (Guardabassi *et al*., 2004; Smet *et al*., 2010) due to selection pressure associated with the use of antimicrobial agents both in veterinary and human medicine (Prescott *et al*., 2002). This thereby, suggests that such cases might reflect abundant use of broad-spectrum antimicrobials. But studies on veterinary antibiotic resistance are still rarely conducted or reported in developing countries, including Nigeria, in spite of commonly reported mobility of pathogenic microorganisms of zoonotic importance, even across continents. In the current study, it was recorded in tables 2 & 3 that significant antibiotic resistance were exhibited by the Gram-negative bacteria to commonly available test antibiotic discs. None of the canine-borne Gram-negative bacteria was totally susceptible to the eight antibiotics (discs), while 87.3% exhibited ≥50.0% multiple antibiotic resistance profiles. This can lead to the inference that antibiotic resistance among pet animals is also likely to be very common in Nigeria.

Similarly, just 29.1% of the 55 Gram-negative bacterial species isolated from the local dogs that had partial gastrectomy were totally susceptible to the four antibiotic drugs (amoxicillin, nitaxim, oritaxim and oxytetracycline), while 49.1% of the bacteria exhibited as high as ≥50.0% multiple antibiotic resistance profiles. In Nigeria, local dogs are mostly free-range animals and do not receive as much clinical attention as the hybrid exotic dogs, and as such are not exposed to antibiotic treatments like foreign dogs. Therefore, the recorded significant antibiotic resistance against the four most-commonly administered antibiotics for clinical and surgical prophylaxis in veterinary practices in Nigeria further showed the alarming trend that had been earlier reported in most developed countries. Whereas, once resistance emerges, the continued selection pressure of antimicrobial drugs will maintain resistant bacteria in the populations (McGowan, 1996). Even if the selection pressure exerted by antimicrobial drug use is removed, once resistance has emerged, its prevalence will not necessarily decline (Prescott *et al.*, 2002).

Although, the intramuscular antibiotic drugs tested in the current study were reported to have an extended spectrum of activity with effective actions against a wider range of Gram-positive and Gram-negative bacteria (Esposito *et al.*, 2002; eMed, 2007); still, high percentage resistance and multiple antibiotic resistance were exhibited by the canine bacterial flora against the drugs, except among few *E. coli* and *Klebsiella* strains in which slightly lower resistance were recorded. In addition, there has been increasing evidence of transfer of antibiotic resistant bacteria between pet owners or veterinary staff and the pets which they readily come in contact with (Wegener *et al.*, 1999; Manian, *2003*). In spite of quite low overall resistance rates being recorded for oxytetracycline (18.2%) and amoxicillin (20.0%) drugs in this study, the strong possibility of transmission of antibiotic resistance from pet animals to humans laid credence to the cautions raised in extended use of antibiotic administration in clinical / surgical veterinary procedures. To buttress this point, a total of 70.9% of the Gram-negative bacterial strains in this study exhibited antibiotic resistance, while 49.1% exhibited multiple antibiotic resistance. The clinical veterinary implication being that in cases of such multiple antibiotic resistance, there would be likely treatment failure, even with combined therapy in dogs under antibiotic treatments.

It was documented that length of therapy in veterinary cases depended at least on the infection being treated and the toxicity of the selected antimicrobials (Howe *et al.*, 2006); whereas, as highlighted in tables 2 & 3, there were no significant variations in the phenotypic antibiotic resistance patterns and profiles of the Gram-negative bacteria isolated from rectal swabs of the experimental dogs before and after partial gastrectomy, i.e., four-day post-operative antibiotic administration (Akinrinmade *et al.*, 2013). However, the case may be worse *in vivo*, since four-day post-operative antibiotic administration may be considered as not being too prolonged for some of such bacterial strains to have probably acquired antibiotic resistant genes. This study, which attempted to highlight the world-known but not well-addressed serious issue of antibiotic resistant bacteria in pet animals, reported significant phenotypic antibiotic resistance and multiple antibiotic resistance. It is thus, necessary that administration of antibiotics in veterinary surgical cases be for as short duration as possible.

Veterinarians were quick to attribute an unsuccessful antimicrobial treatment to failure in microbial culture and susceptibility tests (Papich, 2013b). But as earlier suggested by Stinner *et al.* (1998), pre-clinical testing of therapeutic agents in complex clinically-modeled randomised trials should be mandatory to avoid possible hazards of misconducted clinical trials. Moreover, it has been reported that there are very few veterinary clinical studies to support recommended use and dose for treating resistant bacterial infections in small animals (Papich, 2013a). As an example, except for a strain of *Enterobacter aerogenes*, table 1 showed recorded resistance rates towards amoxicillin discs and drugs (60.0-100% vs. 9.1-100%) and tetracycline / oxytetracycline antibiotic discs and drugs (25.0-57.1% vs. 20.0-50.0%). Tables 2 & 3 showed recorded overall resistance profiles towards amoxicillin discs and drugs as 96.4% vs. 20.0% and tetracycline / oxytetracycline antibiotic discs and drugs as 49.1% vs. 18.2%, which indicated varied phenotypic resistance rates among corresponding antibiotic discs and drugs. Since antibiotic prescriptions and administrations are usually based on antibiotic susceptibility test results using antibiotic discs; interpreting the obtained results in the current study inferred that amoxicillin and tetracycline (discs) were generally highly or moderately resisted and would likely not be prescribed. Whereas, obtained results for amoxicillin and oxytetracycline drugs inferred that these particular antibiotics could be prescribed for clinical treatments, based on their low resisted rates. These conflicting findings suggested therefore, that such disparity in pre-clinical testing of therapeutic agents were not accurately predictive for clinical applications.

No study had been carried out in Nigeria on comparative potentials of antibiotics discs and intramuscular antibiotic drugs. Therefore, observed and reported significant differences in the obtainable results between antibiotic susceptibility profiles using antibiotic discs and corresponding antibiotic drugs is a point of interest as a major limitation and implication in antibiotic therapy for veterinary practices. Such findings can be addressed in future veterinary microbiology and antibiotic therapy research by adopting the effective strategy employed in the current study, whereby antibiotic drugs to be administered are directly screened using antibiotic susceptibility testing methods. Such developed criterion can significantly combat the problem of antibiotic resistance, in order to prevent endemic situations among companion animals.

There are many reasons why antimicrobial treatments fail (Papich, 2013b) but based on the findings of the current study, mode of evaluation of potency of antibiotic agents can be considered as one of the many factors that contribute to antibiotic treatment failures. In this study, the inhibitory potencies of the test antibiotics as denoted by zones of inhibition were mostly within low and moderate profiles. This can be interpreted that the antibiotics can only be minimally or moderately inhibitory in related clinical/treatment cases. So, routine determination of phenotypic antibiotic susceptibility profiles of isolated bacterial pathogens, using veterinary drugs that are commonly likely to be prescribed/administered in veterinary surgical conditions, as well as general clinical veterinary conditions must be strongly considered. Antibiotic resistance from companion animals may also be introduced into food chain, since local dogs are food delicacies in some parts of Nigeria. Therefore, as earlier suggested by Ghidini *et al*. (2011), a diagnostic protocol based on bacterial identification to species level and proper antibiotic susceptibility testing must be carried out prior to all prescriptions of antibiotics.

Conclusion

This study presented preliminary baseline data on antibiotic susceptibility and resistance profiles of indigenous Gram-negative bacterial flora of canine origin, which are presently lacking in the country. Prevalence of multiple antibiotic resistance to most-commonly administered antibiotics for surgical prophylaxis in veterinary practices in Nigeria by easily culturable Gram-negative bacterial species isolated from local dogs, are currently under-recognised / under-reported and not adequately documented. As reflected in this study, recorded significant antibiotic resistance against the four most-commonly administered antibiotic drugs for clinical and surgical prophylaxis in veterinary practices in the country, further showed the alarming trend that had been earlier reported in most developed countries. There is therefore, urgent need for appropriate guidance on antibiotic prophylaxis in veterinary surgery with regards to timing, duration, choice of antibiotics, etc.

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