

Research Article

Effects of Prolonged Empirical Antibiotic Administration on Post-Surgical Intestinal Bacterial Flora of Local Dogs Undergoing Non-Laparoscopic Gastrectomy

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Abstract: Prolonged post-surgical antibiotic administration may be of less advantage in prevention of post-surgical infections. This study therefore, aimed at investigating the prolonged effect of empiric administration of three most-prescribed antibiotics (amoxicillin, cefotaxime and oxytetracycline) by veterinary practices in Southwest Nigeria on intestinal bacterial population of dogs undergoing partial, non-laparoscopic gastrectomy. Using conventional quantitative and qualitative microbial culture procedures, the total bacterial populations were mostly too numerous to count (TNTC) before gastrectomy but log₁₀3-10⁵ cfu/mL after, while control were log₁₀5-10⁷ cfu/mL after gastrectomy. On general-purpose, special, differential and selective culture media, total bacterial counts with increasing post-operative days were- amoxicillin (11 mg/kg) day 4: log₁₀5-10-9/TNTC cfu/mL vs. day 8: log₁₀3-10⁵ cfu/mL; cefotaxime (25 mg/kg) day 4: log₁₀3-10⁸/TNTC/cfu/mL vs. day 8: log₁₀2-10⁵ cfu/mL; oxytetracycline (10 mg/kg) day 4: log₁₀4-10⁹ TNTC cfu/mL vs. day 8: log₁₀2-10⁶ cfu/mL. Total bacterial counts of control animals were- day 4: log₁₀5-10⁸/TNTC cfu/mL vs. day 8: log₁₀5-10⁹. Total qualitative populations of predominant, easily-recoverable aerobic and anaerobic rectal canine bacteria, *Bacillus*, *Citrobacter aerogenes*, *Clostridium*, *E. coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Streptococcus*, *Staphylococcus* and *Lactobacilli* were significantly less after gastrectomy but reductions in post-operative bacterial populations were mostly more pronounced among the anaerobes (*Lactobacilli* and *Clostridium perfringens*). No post-operative infection was recorded among all the experimental animals, including the control animals. In conclusion, this study confirmed significant reduction effect of prolonged empiric antibiotic administration on rectal (intestinal) bacterial populations of experimental local dogs that had partial, non-laparoscopic gastrectomy.

Keywords: Canine bacterial populations, colonisation resistance, dogs, post-operative infection, veterinary gastrointestinal surgery, veterinary practices and public health, zoo noses

INTRODUCTION

Gastrointestinal surgeries are performed very commonly in small animals for biopsy, excision of foreign bodies, gastrointestinal bleeding, as well as resection of necrotic segments of the intestine and necrotic portion of the stomach (Monnet, 2009), while gastrectomy, as a surgical procedure is indicated in small animals presenting gastric neoplasms, bleeding gastric ulcer, perforation of the stomach wall and non-cancerous polyps. However, Surgical Site Infections (SSIs) are complications that result in increased expenses in the veterinary patients (Cockshutt, 2003), while the risk of infection in the surgical patients is based on the susceptibility of the surgical wound to microbial contaminations (Bowler *et al.*, 2001). Prevention of infection in the surgical patient without established infection at the time of surgery is therefore, essential for a good surgical outcome (Page *et al.*, 1993;

Howe and Boothe, 2006; Kang *et al.*, 2009; Monnet, 2009).

Gram-positive cocci and enteric Gram-negative bacilli are prevalent in the stomach and upper gastrointestinal tract, while the distal small intestine contain large numbers of aerobic and anaerobic microorganisms, such as enteric Gram-negative bacilli and enterococci but in the colon, facultative and strict anaerobic microbial loads increase markedly and typically greatly outnumber aerobic microorganisms (Cockshutt, 2003; Dunning, 2003). However, an important function of autochthonous intestinal micro biota in the gastrointestinal tract is to provide a natural defence against colonisation and translocation by exogenous, potentially pathogenic microorganisms or against the over-growth of indigenous opportunistic microbial flora. This feature, introduced by Van *et al.* (1972) and coined colonisation resistance, is considered to be a function of normal intestinal micro biota, which

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is related to the population of both aerobic and anaerobic non-pathogenic indigenous gut micro biota that normally reside in the gastrointestinal tract of humans and animals (Van *et al.*, 1972; Vollaard and Clasener, 1994; Donskey, 2006). It is a property of the indigenous intestinal micro flora that controls the growth and therewith, the chance of translocation of potentially pathogenic bacteria across the gut wall (Edlund and Nord, 2001; Williams, 2003).

The success of any surgical intervention, such as gastrectomy depends largely on the reduction in the degree or populations of opportunistic or pathogenic microorganisms that contaminate the wound. Meanwhile, it has been reported that pathogens are present during surgery regardless of how aseptic the surgery might appear; so, prophylactic antibiotics are generally administered both preoperatively and postoperatively (Burke, 1961; Kaiser, 1991). However, continued empiric administration of prophylactic antimicrobials after completion of surgery has therefore, been reported not to be likely beneficial (Howe and Boothe, 2006; Akinrinmade and Oke, 2012). Although it is known that several factors can affect gastrointestinal tract equilibrium (Mackie *et al.*, 1999), antibiotic usage normally affects or destroys the innate or already established colonisation resistance within the intestine (Nord and Heimdahl, 1986; Nord and Edlund, 1990; Levy, 1998; Sullivan *et al.*, 2001; Harmoinen, 2004; Donskey, 2006; Kang *et al.*, 2009) and this can lead to profound changes in the intestine. This study therefore, investigated the prolonged effect of empiric administration of three most-prescribed antibiotics (amoxicillin, cefotaxime and ox tetracycline) by veterinary practices in Southwest Nigeria on intestinal bacterial population of dogs undergoing partial, non-laparoscopic gastrectomy, using conventional quantitative and qualitative microbial culture procedures.

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 and University of Ibadan, Nigeria.

Experimental animals: Twelve local adult dogs, consisting of 6 males and 6 females with body weight ranging from 11-15 kg were used in this study. The dogs were randomised into 3 treatment groups, each consisting of 3 dogs, while an additional group served as control. All the experimental dogs were clinically examined prior to the experiments and declared healthy. They were housed separately for 10 weeks in clean

kennels at the Veterinary Surgery and Reproduction Unit experimental animal house but released to periodically socialise and maintained on a standard diet once daily with water ad lib. None of the experimental dogs received any form of antibiotic therapy at least five weeks before the commencement of the study, while coprophagy was avoided by ensuring regular disposition of faeces and cleaning of the animal cages.

The dogs were stabilised for 5 weeks during which complete physical examination were performed, followed by recording of vital parameters through appropriate laboratory work-up, such as faecal examination for parasitic ova, Complete Blood Counts (CBC), Biochemistry Profile (BF) and urinalysis. Animals with end parasites and ectoparasites were treated appropriately with anthelmintics, ivermectin dose rate of 0.2 mg/kg body weight and praziquantel 50 mg (Prazisam®) at dose rate of 5 mg/kg body weight, as well as multivitamin. Surgery commenced after all results were negative or within established reference ranges and all the animals were ascertained to be healthy and with no manifested disease conditions. No antibiotics were administered on any of the experimental dogs prior to surgery.

Pre-medication and pre-operative preparation: All the animals were weighed to determine appropriate anaesthesia with least possible dose and food was withheld from all dogs overnight, following thorough bathing and clipping of hairs at the surgical areas prior to surgery. Chlorhexadine and methylated spirit was used to aseptically prepare the area between the xiphoid and the pubic area after it has been thoroughly clipped. Each animal was pre-medicated with atropine sulphate (0.04 mg/kg, im) and xylozine (2 mg/kg, im). Anaesthesia was induced with thiopentone sodium (10 mg/kg, iv) and monitored with halothane and oxygen mixture. All dogs were administered a balance electrolyte solution (10 mL/kg/h, iv) throughout the surgery.

Surgical procedures: Laparotomy was performed on each of the experimental dogs after aseptic preparation according to the method of Nakazawa *et al.* (2011). Each anaesthetised dog was retained and placed on a dorsal recumbence with the limbs tied to the operating table, followed by administration of lactated Ringer's solution as intra-venous fluid throughout the surgical period. The abdomen was opened through a ventral midline incision, extending caudally from the xiphoid process to a point beyond the umbilicus. The stomach was exteriorised from the abdominal cavity with warm sterile saline moistened sponges, while the gastric fundus was grasped and the stomach was palpated to confirm the presence of any foreign body or

abnormality. The stomach was then retracted with stay suture to prevent gastric spillage and associated contamination and the wall of the stomach was grasped with a piece of gauze on each side at a time to elevate the stomach wall; thereby, exposing the least vascular area midway between the greater and lesser curvatures and approximately equidistant from its extremities. This portion was clamped with forceps at either end of the planned incision and large gauze sponges, moistened with warm sterile saline were used to pack the exposed sites from the rest of the visceral, after which an elliptical excision of 6 cm long and 3 cm wide tissue was removed from the fundus along the greater curvature of the section of the stomach.

The stomach was then closed using a 2-layer inverting (continuous Lembert) suture pattern with 2/0 absorbable suture and a 2nd row of continuous sutures is inserted in the serosa and placed in situ. The abdominal cavity was flushed with warm sterile saline to reduce contamination and also inspected before closure to ensure that all foreign materials and surgical equipment were removed. The abdomen was closed with 3-layer closure using simple interrupted suture pattern with 2/0 chromic catgut. Skin incision was finally closed with 2/0 nylon sutures using horizontal mattress suture pattern. Three intramuscular antibiotics, amoxicillin (11 mg/kg), cefotaxime (25 mg/kg) and ox tetracycline (10 mg/kg) Body Weights (BW) were administered on groups 1, 2 and 3 respectively, except the control experimental animals, which received equal volume of normal saline, immediately after the animals showed signs of recovery from anaesthesia and for 4 consecutive, post-operative days. Intravenous supportive therapy was continued until the 2nd day after surgery, when bland diet was gradually introduced. The animals however, tolerated semi-solid oral meals on the 3rd post-operative day.

Collection of dogs' rectal contents for microbiological analyses and identification of isolated bacterial flora: To determine the effect of administered antibiotics on the intestinal micro biota, rectal swabs from 10 adult dogs (extracted manually per dog rectum immediately before the operation and for eight days post-surgery) were analysed for bacterial culture, using different sterile swabs sticks, which were directly inoculated into sterile, unbuffered peptone water (Lab M, Basingstoke, England) in sterile McCartney bottles. The inoculated peptone water samples were transferred within one h after collections to the Department of Microbiology, Faculty of Science and University of Ibadan for microbiological analyses.

Inoculated unbuffered peptone water containing the rectal contents of the experimental animals were incubated at 32-35°C for 12 h, after which serial

dilutions were prepared from each of the broth culture and selected aliquots were placed on plate count agar (PCA), Blood agar (BA), Cystein Lactose Electrolyte Deficient (CLED) agar, Eosin Methylene Blue (EMB) agar, MacConkey (MCC) agar, de Mann, Rogosa and Sharpe (MRS) agar, Mannitol Salt Agar (MSA) and *Salmonella-Shigella* agar (SSA agar), all manufactured by Lab M. MRS broth and agar were modified to pH 5.5 prior to the selective isolation of lactic acid bacteria. The culture plates were then incubated aerobically and an aerobically to determine the quantitative aerobic and anaerobic populations of the rectal contents of the experimental animals, based on the colony forming units of the viable and culturable colonies on the different culture plates. Colonies on the primary plates were repeatedly streaked until assured purity and pure cultures were kept in duplicates on CLED agar slants as bench and stock cultures. Presumptive identification of the randomly selected pure bacterial isolates from rectal contents of healthy local experimental dogs undergoing gastrectomy was based on standard phenotypic taxonomic tools (Chessborough, 2000); while general keys for identification confirmation of phenotypic identities was according to Bergey's manual of systemic bacteriology (Buchanan and Gibbons, 1974).

RESULTS

Total numbers of predominant aerobic and anaerobic bacteria isolates, as cultured from rectal contents of the experimental dogs before and after gastrectomy were as presented in Table 1 and 2. Total colony forming units (cfu) without species differentiation on certain general purpose agar (plate count agar), special agar (De Man, Rogosa and Sharpe [MRS] agar) and differential agars (MacConkey agar, cystein lactose electrolyte deficient agar) were as shown in Table 1. Colonies obtained from the rectal swabs of the experimental dogs were Too Numerous To Count (TNTC) before the gastrectomic surgeries, while there were significant reductions with increasing post-surgery days after partial gastrectomy ($\log 10^3$ - 10^5 cfu/mL), except among the control animals in which bacterial loads were $\log 10^5$ - 10^8 cfu/mL after gastrectomy. Quantitative bacterial counts with increasing post-surgery days were-amoxicillin (11 mg/kg) day 4: $\log 10^5$ - 10^9 /TNTC cfu/mL vs. day 8: $\log 10^3$ - 10^5 cfu/mL; cefotaxime (25 mg/kg) day 4: $\log 10^3$ - 10^8 /TNTC cfu/mL vs. day 8: $\log 10^2$ - 10^5 cfu/mL; oxytetracycline (10 mg/kg) day 4: $\log 10^4$ - 10^9 /TNTC cfu/mL vs. day 8: $\log 10^2$ - 10^6 cfu/mL. Total bacterial counts of control animals were day 4: $\log 10^5$ - 10^8 /TNTC cfu/mL vs. day 8: $\log 10^5$ - 10^9 , while the reducing effects of administered antibiotics on bacterial loads were in the increasing order, amoxicillin (11 mg/kg), cefotaxime (25 mg/kg), ox tetracycline (10 mg/kg).

Table 1: Total plate counts of rectal contents of local experimental dogs on general purpose/differential media before and after surgery
Total plate counts (cfu/g)

Lab codes of dogs	Isolation period	Days	PCA	MCC	MCC	MRS agar*
Amoxicillin (11 mg/kg)						
3A	Before surgery	2	TNTC	TNTC	TNTC	1.05×10 ⁴
	After surgery	4	1.53×10 ⁵	2.16×10 ⁶	1.37×10 ⁵	1.48×10 ³
		8	1.43×10 ⁴	1.31×10 ⁴	1.59×10 ⁵	2.21×10 ³
3B	Before surgery	2	TNTC	TNTC	TNTC	2.05×10 ⁴
	After surgery	4	2.09×10 ⁶	2.08×10 ⁶	1.37×10 ⁶	1.37×10 ⁴
		8	1.52×10 ⁵	1.48×10 ⁴	1.13×10 ⁴	1.48×10 ³
4A	Before surgery	2	TNTC	TNTC	TNTC	2.31×10 ⁴
	After surgery	4	5.1×10 ⁵	3.7×10 ⁶	3.6×10 ⁶	3.2×10 ⁴
		8	4.8×10 ⁵	2.1×10 ⁴	2.8×10 ⁵	4.8×10 ⁴
4B	Before surgery	2	TNTC	TNTC	TNTC	3.5×10 ⁴
	After surgery	4	4.7×10 ⁷	1.65×10 ⁷	3.6×10 ⁷	3.2×10 ⁵
		8	2.11×10 ⁵	4.1×10 ⁵	1.2×10 ⁴	5.4×10 ⁴
5A	Before surgery	2	TNTC	TNTC	TNTC	2.11×10 ⁴
	After surgery	4	2.1×10 ⁶	TNTC	2.09×10 ⁵	2.9×10 ⁴
		8	1.14×10 ⁵	2.16×10 ⁵	2.15×10 ³	2.26×10 ³
5B	Before surgery	2	2.5×10 ⁸	1.13×10 ⁷	2.8×10 ⁵	2.6×10 ⁴
	After surgery	4	1.16×10 ⁹	TNTC	2.06×10 ⁶	2.5×10 ⁴
		8	1.56×10 ⁵	2.15×10 ⁵	1.48×10 ⁴	2.5×10 ³
Cefotaxime (25 mg/kg)						
6A	Before surgery	2	TNTC	TNTC	TNTC	1.26×10 ⁴
	After surgery	4	TNTC	1.95×10 ⁵	2.01×10 ⁷	2.19×10 ⁴
		8	1.39×10 ⁴	2.56×10 ⁵	1.50×10 ⁵	2.10×10 ³
6B	Before surgery	2	TNTC	TNTC	TNTC	2.03×10 ⁴
	After surgery	4	TNTC	2.05×10 ⁵	1.06×10 ⁸	1.64×10 ⁴
		8	1.62×10 ⁵	1.91×10 ⁴	2.12×10 ³	2.01×10 ³
7A	Before surgery	2	TNTC	TNTC	TNTC	1.27×10 ³
	After surgery	4	TNTC	2.36×10 ³	1.12×10 ⁴	1.59×10 ⁴
		8	2.01×10 ⁵	1.24×10 ⁴	1.12×10 ⁴	1.27×10 ³
7B	Before surgery	2	2.37×10 ⁷	TNTC	TNTC	1.59×10 ⁴
	After surgery	4	TNTC	2.14×10 ³	2.39×10 ⁶	1.41×10 ⁴
		8	2.11×10 ⁴	2.6×10 ³	2.61×10 ³	1.48×10 ³
8A	Before surgery	2	TNTC	1.67×10 ⁸	TNTC	2.13×10 ³
	After surgery	4	TNTC	2.17×10 ⁵	2.12×10 ⁵	2.64×10 ⁴
		8	1.72×10 ⁵	9.7×10 ⁴	1.18×10 ⁴	1.64×10 ²
8B	Before surgery	2	TNTC	TNTC	1.38×10 ⁶	1.25×10 ⁴
	After surgery	4	2.06×10 ⁷	TNTC	2.42×10 ⁵	2.73×10 ³
		8	1.69×10 ⁵	2.51×10 ⁴	2.58×10 ⁴	1.04×10 ³
Ox tetracycline (10mg /kg)						
9A	Before surgery	2	TNTC	TNTC	TNTC	2.7×10 ⁴
	After surgery	4	TNTC	2.49×10 ⁹	2.05×10 ⁸	1.12×10 ⁴
		8	1.42×10 ⁴	2.16×10 ⁴	2.48×10 ³	6.3×10 ³
9B	Before surgery	2	TNTC	TNTC	TNTC	TNTC
	After surgery	4	2.25×10 ⁶	TNTC	TNTC	1.24×10 ⁴
		8	2.37×10 ³	1.52×10 ⁴	1.24×10 ⁵	1.02×10 ⁴
10A	Before surgery	2	TNTC	TNTC	TNTC	1.58×10 ⁴
	After surgery	4	TNTC	1.13×10 ⁶	TNTC	2.41×10 ⁴
		8	2.25×10 ⁴	1.49×10 ⁵	1.24×10 ⁴	2.22×10 ³
10B	Before surgery	2	TNTC	TNTC	TNTC	1.12×10 ⁴
	After surgery	4	TNTC	2.51×10 ⁶	1.38×10 ⁵	1.35×10 ⁴
		8	1.31×10 ⁵	1.56×10 ⁶	2.54×10 ⁵	2.49×10 ³
11A	Before surgery	2	TNTC	7.3×10 ⁸	6.9×10 ⁹	1.58×10 ⁴
	After surgery	4	2.11×10 ⁴	TNTC	2.16×10 ⁴	1.01×10 ⁴
		8	1.81×10 ⁵	6.8×10 ⁵	5.9×10 ³	2.7×10 ²
11B	Before surgery	2	TNTC	TNTC	TNTC	4.8×10 ⁶
	After surgery	4	TNTC	TNTC	4.8×10 ⁶	4.8×10 ⁴
		8	1.46×10 ⁵	5.9×10 ⁵	2.5×10 ⁵	3.6×10 ³
Control [No Amoxicillin (11 mg/kg)]						
	Before surgery	2	TNTC	1.67×10 ⁵	TNTC	2.07×10 ⁵
	After surgery	4	TNTC	TNTC	1.26×10 ⁵	1.04×10 ⁵
		8	2.51×10 ⁷	2.09×10 ⁶	2.09×10 ⁶	1.04×10 ⁵

Table 1: (Continue)

Lab codes of dogs	Isolation period	Days	Total plate counts (cfu/g)			
			PCA	MCC	MCC	MRS agar*
Control [No Cefotaxime (25 mg/kg)]						
	Before surgery	2	TNTC	TNTC	TNTC	2.14×10 ⁴
	After surgery	4	1.64×10 ⁸	TNTC	1.39×10 ⁸	1.01×10 ⁵
		8	1.43×10 ⁷	1.2.0×10 ⁸	1.14×10 ⁷	1.81×10 ⁶
Control [No O×tetracycline (10 mg /kg)]						
	Before surgery	2	TNTC	TNTC	TNTC	1.65×10 ⁴
	After surgery	4	TNTC	TNTC	1.01×10 ⁸	2.04×10 ⁵
		8	1.36×10 ⁹	1.196×10 ⁸	1.51×10 ⁷	1.30×10 ⁵

Table 2: Differential and selective plate counts of the rectal contents of local experimental dogs before and after surgery

Dog	Isolation Days Period	Total plate counts (cfu/g)									
		EMB agar (<i>E.coli</i>)	EMB agar (<i>Klebsiella</i>)	PCA agar (<i>Clostridium</i>)	CLED agar (<i>Pseudomonas</i>)	MSA agar (<i>Staph.</i>)	BA (<i>Strept.</i>)	SSA agar (<i>Salmon.</i>)	PCA agar (<i>Bacillus</i>)	MRS*(LAB)	
Amoxicilline (11 mg/kg)											
3A	BS	2	2.8×10 ⁵	6.2×10 ⁵	6.2×10 ⁵	-	5.6×10 ³	4.1×10 ³	3.7×10 ³	5.2×10 ⁵	1.3×10 ³
	AS	8	9.7×10 ²	5.1×10 ³	-	-	2.1×10 ²	5.3×10 ¹	-	1.8×10 ³	-
3B	BS	2	1.3×10 ⁵	9.8×10 ⁵	1.4×10 ³	-	8.2×10 ⁴	1.3×10 ³	1.8×10 ⁴	1.9×10 ⁴	1.02×10 ⁴
	AS	8	7.1×10 ³	4.5×10 ³	-	-	6.3×10 ³	-	-	-	-
4A	BS	2	6.4×10 ⁵	4.9×10 ⁵	4.2×10 ⁴	2.2×10 ²	1.03×10 ⁴	3.2×10 ³	5.3×10 ³	2.7×10 ⁴	7.3×10 ⁴
	AS	8	9.3×10 ²	5.7×10 ⁴	-	-	6.9×10 ³	2.9×10 ¹	1.2×10 ¹	-	2.6×10 ²
4B	BS	2	1.9×10 ⁴	8.1×10 ⁵	5.1×10 ³	1.9×10 ³	6.9×10 ⁴	2.1×10 ³	8.5×10 ³	3.8×10 ⁴	2.11×10 ³
	AS	8	6.4×10 ²	4.3×10 ³	-	1.7×10 ¹	3.7×10 ³	-	4.8×10 ²	2.1×10 ²	5.8×10 ²
5A	BS	2	6.9×10 ⁵	9.1×10 ⁵	3.1×10 ⁴	-	1.32×10 ³	3.6×10 ³	1.1×10 ⁴	6.4×10 ⁴	1.25×10 ⁴
	AS	8	2.8×10 ³	6.4×10 ²	1.7×10 ²	-	6.1×10 ²	-	-	-	-
5B	BS	2	4.3×10 ⁵	3.7×10 ⁵	3.5×10 ³	2.8×10 ³	1.04×10 ³	6.3×10 ³	5.7×10 ³	9.0×10 ⁴	5.1×10 ³
	AS	8	5.1×10 ³	1.2×10 ²	2.1×10 ²	5.4×10 ²	7.3×10 ²	-	4.3×10 ¹	1.6×10 ²	-
Cefotaxime (25 mg/kg)											
6A	BS	2	7.3×10 ⁵	5.6×10 ³	1.3×10 ³	4.1×10 ²	2.3×10 ³	3.1×10 ³	3.8×10 ³	2.3×10 ³	1.02×10 ³
	AS	8	3.9×10 ⁴	3.1×10 ²	-	-	3.4×10 ²	-	5.1×10 ²	1.7×10 ³	-
6B	BS	2	4.1×10 ⁵	3.9×10 ⁴	3.9×10 ³	5.6×10 ³	2.5×10 ³	1.7×10 ³	1.2×10 ³	1.5×10 ⁴	3.1×10 ⁴
	AS	8	2.3×10 ³	2.5×10 ³	5.8×10 ⁴	-	2.8×10 ¹	-	-	7.0×10 ²	-
7A	BS	2	1.2×10 ⁵	8.5×10 ⁴	3.5×10 ²	-	9.3×10 ⁴	4.8×10 ³	2.4×10 ⁴	5.9×10 ³	7.6×10 ⁴
	AS	8	4.6×10 ⁴	4.1×10 ⁴	5.2×10 ⁴	-	4.1×10 ²	-	3.7×10 ³	4.3×10 ¹	1.2×10 ²
7B	BS	2	8.7×10 ⁵	6.4×10 ⁵	2.7×10 ¹	-	5.7×10 ³	4.2×10 ³	-	2.9×10 ⁴	3.1×10 ⁴
	AS	8	3.5×10 ³	8.3×10 ³	2.9×10 ⁴	2.7×10 ³	-	1.3×10 ¹	-	7.1×10 ³	1.2×10 ³
8A	BS	2	6.7×10 ⁵	4.5×10 ⁵	-	-	4.8×10 ⁴	1.3×10 ⁴	2.8×10 ⁴	2.1×10 ⁵	5.8×10 ⁴
	AS	8	6.3×10 ⁴	6.1×10 ⁴	6.1×10 ⁴	2.9×10 ⁴	2.1×10 ²	-	4.6×10 ²	1.4×10 ³	1.5×10 ²
8B	BS	2	7.9×10 ⁵	2.3×10 ⁵	2.3×10 ⁵	6.3×10 ³	5.8×10 ⁴	1.5×10 ³	5.7×10 ³	3.6×10 ⁵	1.6×10 ⁴
	AS	8	4.2×10 ⁴	4.8×10 ⁴	4.8×10 ⁴	-	3.3×10 ¹	-	6.1×10 ¹	2.4×10 ³	2.4×10 ³
O×tetracycline (10mg/kg)											
9A	BS	2	TNTC	5.9×10 ⁵	2.9×10 ³	9.1×10 ³	1.3×10 ⁴	3.8×10 ²	3.8×10 ²	3.4×10 ³	2.5×10 ⁴
	AS	8	6.3×10 ⁵	7.2×10 ³	-	5.7×10 ²	-	-	-	1.2×10 ³	-
9B	BS	2	7.9×10 ⁵	3.7×10 ⁵	3.1×10 ³	5.7×10 ²	1.04×10 ⁴	4.0×10 ³	2.1×10 ⁴	3.6×10 ⁴	1.02×10 ³
	AS	8	5.2×10 ⁴	7.0×10 ⁴	6.7×10 ²	-	2.1×10 ³	-	-	2.9×10 ³	4.1×10 ²
10A	BS	2	9.5×10 ⁴	2.1×10 ⁵	6.8×10 ³	-	4.7×10 ⁴	1.5×10 ⁴	-	7.3×10 ⁴	3.5×10 ³
	AS	8	4.7×10 ⁴	8.9×10 ³	7.3×10 ²	-	-	-	-	4.1×10 ²	2.1×10 ²
10B	BS	2	3.7×10 ⁴	6.3×10 ⁴	2.8×10 ³	-	5.2×10 ⁴	2.1×10 ³	1.2×10 ³	-	3.2×10 ⁴
	AS	8	-	4.0×10 ²	5.9×10 ²	-	6.0×10 ²	3.1×10 ²	2.9×10 ³	-	-
11A	BS	2	3.8×10 ⁴	7.1×10 ³	2.0×10 ³	1.7×10 ³	3.1×10 ⁴	-	-	2.8×10 ⁴	6.9×10 ⁴
	AS	8	2.6×10 ³	-	-	1.0×10 ²	5.3×10 ²	-	-	2.1×10 ³	1.2×10 ³
11B	BS	2	2.7×10 ⁴	5.2×10 ⁴	4.0×10 ³	1.5×10 ³	5.0×10 ³	-	-	1.8×10 ³	1.21×10 ³
	AS	8	8.0×10 ³	4.8×10 ⁴	-	-	1.9×10 ³	-	-	2.5×10 ³	2.7×10 ³
[No Amoxicilline (11 mg/kg)]											
Control	BS	2	3.9×10 ⁵	3.4×10 ⁶	5.0×10 ²	3.1×10 ³	3.6×10 ⁴	5.7×10 ⁴	1.2×10 ³	2.8×10 ⁵	7.1×10 ⁵
	AS	8	4.6×10 ³	2.1×10 ⁵	3.4×10 ²	1.5×10 ³	3.1×10 ⁴	2.2×10 ³	1.5×10 ³	2.5×10 ⁵	2.2×10 ⁵
[No Cefotaxime (25 mg/kg)]											
Control	BS	2	1.5×10 ⁴	1.8×10 ⁶	4.1×10 ³	1.7×10 ²	1.4×10 ⁶	3.1×10 ⁵	3.1×10 ²	1.2×10 ⁶	1.9×10 ⁵
	AS	8	1.0×10 ⁶	2.8×10 ⁸	1.1×10 ³	-	2.2×10 ⁵	1.9×10 ⁵	2.7×10 ⁵	1.1×10 ⁷	3.7×10 ⁴
[No O×tetracycline (10 mg/kg)]											
Control	BS	2	2.7×10 ⁶	1.7×10 ⁸	2.4×10 ³	2.3×10 ²	2.9×10 ³	1.4×10 ³	3.6×10 ³	1.3×10 ⁵	2.8×10 ⁴
	AS	8	1.9×10 ⁷	1.5×10 ⁷	1.7×10 ³	1.5×10 ²	2.5×10 ⁴	1.6×10 ³	2.0×10 ⁴	2.2×10 ⁵	1.4×10 ⁵

Keys: BS = before surgery, AS = after surgery, EMB agar = eosin methylene blue agar; CLED agar = cystein lactose electrolyte deficient agar; MSA agar = mannitol salt agar; BA = Blood agar; SSA agar = *Salmonella-Shigella* agar; PCA agar = plate count agar

Table 2 shows the cfu counts of the obtained colonies from the rectal swabs on some general purpose, differential and selective agars. Total quantitative and qualitative bacterial populations of predominant, easily-recoverable aerobic and anaerobic rectal bacteria before and after gastrectomy indicated that the bacterial loads were significantly less after partial gastrectomy. *Bacillus* [(amoxicilline 11 mg/kg): log 10⁴-10⁵ vs. log 10²-10³; (cefotaxime 25 mg/kg): log 10³-10⁵ vs. log 10¹-10³; (oxytetracycline 10 mg/kg): log 10³-10⁴ vs. log 10²-10³], *Clostridium perfringens* [(amoxicilline 11 mg/kg): log 10³-10⁴ vs. log 10²-10³; (cefotaxime 25 mg/kg): log 10³-10⁴ vs. log 10²-10³; (oxytetracycline 10 mg/kg): log 10³ vs. log 10²], lactobacilli [(amoxicilline 11 mg/kg): log 10³-10⁴ vs. log 10²-10³; (cefotaxime 25 mg/kg): log 10⁴ vs. log 10²-10³; (oxytetracycline 10 mg/kg): log 10³-10⁴ vs. log 10²-10³], *Streptococcus* [(amoxicilline 11 mg/kg): log 10³ vs. log 10¹; (cefotaxime 25 mg/kg): log 10³-10⁴ vs. log 10³; (oxytetracycline 10 mg/kg): log 10³-10⁴ vs. log 10²], *Staphylococcus aureus* [(amoxicilline 11 mg/kg): log 10³-10⁴ vs. log 10²-10³; (cefotaxime 25 mg/kg): log 10³-10⁴ vs. log 10¹-10²; (oxytetracycline 10 mg/kg): log 10³-10⁴ vs. log 10²-10³]. Bacterial loads of Gram-negative bacteria before and after partial gastrectomy were- *E. coli* [(amoxicilline 11 mg/kg): log 10⁴-10⁵ vs. log 10²-10³; (cefotaxime 25 mg/kg): log 10⁵ vs. log 10³-10⁴; (oxytetracycline 10 mg/kg): log 10⁴-10⁵/TNTC vs. log 10³-10⁴], *Klebsiella pneumoniae* [(amoxicilline 11 mg/kg): log 10⁵ vs. log 10²-10⁴; (cefotaxime 25 mg/kg): log 10³-10⁵ vs. log 10²-10⁴; (ox tetracycline 10 mg /kg): log 10³-10⁵ vs. log 10²-10⁴], *Pseudomonas aeruginosa* [(amoxicilline 11 mg/kg): log 10³ vs. log 10¹-10²; (cefotaxime 25 mg/kg): log 10²-10³ vs. log 10²; (ox tetracycline 10 mg /kg): log 10³ vs. 10²], *Salmonella* [(amoxicilline 11 mg/kg): log 10³-10⁴ vs. log 10¹-10²; (cefotaxime 25 mg/kg): log 10³-10⁴ vs. log 10¹-10³; (oxytetracycline 10 mg/kg): log 10²-10⁴ vs. log 10²].

Bacterial populations of the control animals before and after partial gastrectomy were *Bacillus* [log 10⁴-10⁵ vs. log 10⁵-10⁷]; *Clostridium perfringens* [log 10²-10³ vs. log 10²-10³]; lactobacilli log 10⁴-10⁵ vs. log 10⁴-10⁵]; *Streptococcus* log 10³-10⁵ vs. log 10³-10⁵]; *Staphylococcus aureus* [log 10³-10⁶ vs. log 10⁴-10⁵]; *E. coli* [log 10⁴-10⁶ vs. log 10³-10⁷]; *Klebsiella pneumoniae* [log 10⁵-10⁸ vs. log 10⁵-10⁸]; *Pseudomonas aeruginosa* [log 10²-10³ vs. log 10²-10³]; *Salmonella* [log 10²-10³ vs. log 10³-10⁶].

A total of 116 predominant bacterial strains were randomly obtained from rectal contents of the 12 local experimental and control dogs that underwent gastrectomy in this study. The isolates were phenotypically identified as *Bacillus cereus*, *Bacillus* sp.,

Clostridium perfringens, *Citrobacter aerogenes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* sp. and *Shigella dysenteriae*. Plate count agar was to determine total obtainable colonies, including *Bacillus*, *Clostridium*, *E. coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Streptococcus* and *Staphylococcus* species. No post-operative infection was recorded among the animals, including the control animals.

DISCUSSION

In the present study, the most recovered viable and easily culturable bacterial species from rectal contents of healthy local experimental dogs undergoing partial gastrectomy were the aerobic bacterial flora, *Bacillus*, *E. coli*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Salmonella*, *Staphylococcus*, *Streptococcus* species and the anaerobic flora, *Clostridium* and lactobacilli species. These species of bacteria have also been earlier reportedly isolated from GIT and rectal contents of animals (Schein and Wittmann, 1993; Howe and Boothe, 2006; Chow *et al.*, 2007; Monnet, 2009) but there is the likelihood of observable and significant changes in the microbial loads and diversity of animals receiving post-operative antibiotic therapy compared to healthy animals. It may not be entirely clear, which bacterial groups in the indigenous micro biota are involved in colonisation resistance (Edlund and Nord, 2001; Williams, 2003) but it has been reported that the most significant cause of decreased colonisation resistance is known to be administration of antibiotics (Nord *et al.*, 1984; Donskey, 2006). Although there is a dearth of information in Nigerian literatures on the residual effects of post-operative administration of antibiotics on the gut micro flora in small animals; however, present study corroborated by logarithmic counts of the colony forming units (cfu) confirmed that bacterial populations were generally less following prophylactic antibiotic administration in gastrectomised animals, although reductions in post-surgical bacterial populations were mostly more pronounced among the lactobacilli and *Clostridium perfringens*, which are anaerobes.

Antibiotic administration in gastrointestinal surgery is a common veterinary practice, while cefotaxime (cephalosporin), amoxicillin (penicillin) and ox tetracycline (tetracycline) were mostly among the drugs of choice by veterinary practitioners following intestinal surgeries (Dunning, 2003; Bratzler and Houck, 2004). However, it was discovered in this study that post-surgical bacterial loads of the rectal contents of experimental dogs, dosed with these antibiotics decreased significantly with time, from 4 days and

beyond. After 8 days of surgery, the microbial populations in the current study were on the average of \leq cfu 10^2 , the significant reduction in the bacterial counts could have therefore, been due to continued administration of antibiotics after surgery. Reduction rates in microbial loads of the rectal contents of the experimental animals in the current study obviously further lay credence to the controversies about surgical prophylactic antibiotic administration (Holmberg, 1990; Esposito *et al.*, 2002). Moreover, the intestinal flora is a rapidly changing ecosystem and although the effect of antibiotics may be transient, it can induce resistance to antibiotics and establishment of new microbial resistant strains, as well as transfer of resistant genes (Davison *et al.*, 2000; Smet *et al.*, 2010), which probably may be geographic dependent.

It is relatively common for veterinary surgeons to continue antimicrobial prophylaxis, even beyond 48 h after closure of surgical wounds but findings and recommendations on the use of antibiotics in surgery, both prophylactic ally and as therapy, suggested that adverse events associated with such mode of antibiotic administration remain a major cause of morbidity and mortality (Barie, 2000; Bratzler and Houck, 2004). According to Howe and Boothe (2006), each type of surgical procedure and each body system encountered has its own unique risks and potential pathogens that could result in Surgical Site Infections (SSIs). Therefore, antibiotic usage following surgery for prevention of increased morbidity and expenses associated with surgical infection is a well-accepted part of clinical practice (Holmberg, 1978; Ludwig *et al.*, 1993; Furukawa *et al.*, 2004; Bowater *et al.*, 2009); whereas, the bacterial populations of the control animals in this study were not reduced after partial gastrectomy, while also, there were no recorded post-operative infections in both the experimental animals and the control animals. Moreover, the study of Kang *et al.* (2009) also reported that there was no significant difference in the incidence of postoperative wound infections between patients who had received postoperative prophylactic antibiotic administration and those who had not.

If a post-operative wound infection occurs in spite of the effective prophylactic antibiotic administration before surgery, it could be concluded that the bacteria in the infected wound are not sensitive to the administered prophylactic antibiotics and since the clinicians may not be too sure of which microorganisms can contaminate surgical wounds/sites, in such cases, an appropriate antibacterial therapy should be determined by a culture test of the bacteria found in the infected region (Schein and Wittmann, 1993; Chow *et al.*, 2007), i.e., empiric therapy should be based on

intra-operative antibiotic findings. In addition, since the choice of antibiotics are mostly broad spectrum, beneficial bacterial flora are at a disadvantage; therefore, selection of antimicrobial agents for prophylactic and therapeutic use should be based on knowledge of expected flora, culture and susceptibility testing results, ability of the antimicrobial to reach the target tissue at appropriate concentrations, drug pharmacokinetics and pharmacodynamics, as well as bacterial resistance patterns, (Wilcke, 1990; Classen *et al.*, 1992; DiPiro *et al.*, 1996; McDonald *et al.*, 1998; Whittem *et al.*, 1999; Manian *et al.*, 2003; Nichols *et al.*, 2005; Akinrinmade and Oke, 2012). Consideration of these factors can reduce avoidable expenses, antimicrobial therapy failure and associated morbidity and mortality in surgical cases.

Unlike in most of the developed countries where extensive investigations had been carried out on antibiotic resistance among companion animals, this study, which is the first reported data on the post-surgical effects of prolonged antibiotic administration on animals undergoing partial gastrectomy in Nigeria, concluded that prolonged empiric (post-operative) antibiotic administration significantly decreased post-operative intestinal bacterial populations in Nigerian local dogs undergoing gastrectomy. In addition, since no post-operative infection was recorded among the control animals, there is therefore, the need to strongly consider the hazardous effects of prolonged antibiotic administration on the normal gut flora of the animals undergoing gastrointestinal surgeries, even in spite of likely reduction of surgical/post-surgical infections, especially with regards to the possibility of acquiring and transference of antibiotic resistance among the intestinal flora;. However, a limitation of this study was the impossibility of recovery of more fastidious and not-easily recoverable bacterial species due to lack of special selective culture media and automated identification kits.

ACKNOWLEDGMENT

The authors thank Dr. O. Eyarefe of Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Dr. Afusat Jagun-Jibril of Department of Veterinary Pathology, Faculty of Medicine University of Ibadan, as well as Celestina Obiekea and Roy Adeyemi of Department of Botany and Microbiology, Faculty of Science, University of Ibadan for laboratory assistance during the studies. J. Harmoinen of Department of Clinical Veterinary Sciences, Faculty of Veterinary Medicine and University of Helsinki, Finland is acknowledged for usage of some literature materials and references.

REFERENCES

- Akinrinmade, J.F. and B.O. Oke, 2012. Antibiotic prophylaxis in gastrointestinal surgery: An evaluation of current veterinary practices in Southwest Nigeria. *Int. J. Anim. Vet. Adv.* 4(4): 256-262.
- Barie, P.S., 2000. Modern surgical antibiotic prophylaxis and therapy-less is more. *Surg. Infect. (Larchmt)*, 1(1): 23-29.
- Bowater, R.J., S.A. Stirling and R.J. Lilford, 2009. Is antibiotic prophylaxis in surgery a generally effective intervention? Testing a generic hypothesis over a set of meta-analyses. *Ann. Surg.*, 249(4): 551-556.
- Bowler, P.G., B.I. Duerden and D.G. Armstrong, 2001. Wound microbiology and associated approaches to wound management. *Clin. Microbiol. Rev.*, 14(2): 244-269.
- Bratzler, D.W. and P.M. Houck, 2004. Antimicrobial prophylaxis for surgery: An advisory statement from the national surgical infection prevention project. *Clin. Infect. Dis.*, 38(12): 1706-1715.
- Buchanan, R.E. and N.E., Gibbons, 1974. *Bergey's Manual of Determinative Bacteriology*. 8th Edn., Williams and Wilkins Co., Baltimore, Md. USA. pp: 21202. xxvi + 1246.
- Burke, J.F., 1961. The effective period of preventative antibiotic action in experimental incisions and dermal lesions. *Surg.*, 50: 161-168.
- Classen, D.C., R.S. Evans, S.L. Pestotnik, S.D. Horn, R.L. Menlove and J.P. Burke, 1992. The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection. *N. Engl. J. Med.*, 326(5): 281-286.
- Chessborough, M., 2000. *District Laboratory Practice in Tropical Countries*. Part 2, Cambridge University Press. New York, USA.
- Chow, L.K., B. Singh, W.K. Chiu and N. Samman, 2007. Prevalence of postoperative complications after orthognathic surgery: A 15-year review. *J. Oral Maxillofac. Surg.*, 65: 984-992.
- Cockshutt, J., 2003. *Principles of Surgical Asepsis*. In: Slatter, D. (Ed.), *Textbook of Small Animal Surgery*. 3rd Edn., Elsevier Science, Philadelphia, pp: 149-155.
- Davison, H.C., J.C. Low and M.E.J. Woolhouse, 2000. What is antibiotic resistance and how can we measure it? *Trends Microbiol.*, 8: 554-559.
- DiPiro, J.T., C.E. Edmiston and J.M.A. Bohnen, 1996. Pharmacodynamics of antimicrobial therapy in surgery. *Am. J. Surg.* 171(6): 615-622.
- Donskey, C.J., 2006. Antibiotic regimens and intestinal colonization with antibiotic-resistant Gram-negative bacilli. *Clin. Infect. Dis.*, 43(Suppl. 2): S62-S69.
- Dunning, D., 2003. Surgical wound infection and the use of antimicrobials. In: Slatter, D., (Ed), *Textbook of Small Animal Surgery*. 3rd Edn. Elsevier Science, Philadelphia, pp: 113-122.
- Edlund, C. and C.E. Nord, 2001. The evaluation and prediction of the ecologic impact of antibiotics in human phase I and II trials. *Clin. Microbiol. Infect.*, 7: 37-241.
- Esposito, S., A. Novelli and F. de Lalla, 2002. Antibiotic prophylaxis in surgery: news and controversies. *Infez. Med.*, 10(3): 131-144.
- Furukawa, H., H. Imamura, J. Shimizu, S. Iijima, S. Sugihara, T. Tsujinaka, 2004. Can preoperative antibiotics take the place of postoperative long period antibiotics on the surgical site infection in Japan? – A phase II study by Osaka Gastrointestinal Cancer Chemotherapy Study Group (OGSG) in Japan. *ASCO Annual Meeting Proceedings (Post-Meeting Edition)*. *J. Clin. Oncol.*, 22 (14S): 4162.
- Harmoinen, J., 2004. A novel enzymic therapy, targeted recombinant β -lactamase, in the prevention of antibiotic-induced adverse effects on gut microbiota. Academic Dissertation, Division of Medicine, Department of Clinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, Finland.
- Holmberg, D.L., 1978. Prophylactic use of antibiotics in surgery. *Vet. Clin. North Am.*, 8(2): 219-227.
- Holmberg, D.L., 1990. Prophylactic antibiotics, friend or foe. *Vet. Comp. Orthop. Traumatol.*, 1: 18-19.
- Howe, L.M. and H.W. Boothe, 2006. Antimicrobial use in the surgical patient. *Vet. Clin. Small Anim.* 36: 1049-1060.
- Kaiser, A.B., 1991. Surgical-wound infection. *N. Engl. J. Med.*, 324: 123-124.
- Kang, S., J-H. Yoo and C-K. Yi, 2009. The efficacy of postoperative prophylactic antibiotics in orthognathic surgery: A prospective study in le fort iosteotomy and bilateral intraoral vertical ramus osteotomy. *Yonsei Med. J.*, 50(1): 55-59.
- Levy, S.B., 1998. The challenge of antibiotic resistance. *Sci. Am.* 278: 32-39.
- Ludwig, K.A., M.A. Carlson and R.E. Condon, 1993. Prophylactic antibiotics in surgery. *Ann. Rev. Med.*, 44: 385-393.
- Mackie, R.I., A. Sghir and H.R. Gaskis, 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am. J. Clin. Nutr.* 69: 1035S-1045S.
- Manian, F.A., P.L. Meyer, J. Setzer and D. Senkel, 2003. Surgical site infections associated with methicillin-resistant *Staphylococcus aureus*: Do postoperative factors play a role? *Clin. Infect. Dis.* 36(7): 863-868.

- McDonald, M., E. Grabsch, C. Marshall and A. Forbes, 1998. Single-versus multiple-dose antimicrobial prophylaxis for major surgery: a systematic review. *Aust. NZ. J. Surg.* 68(6): 388-396.
- McHugh, S.M., C.J., Collins, M.A., Corrigan, A.D.K. Hill and H. Humphreys, 2011. The role of topical antibiotics used as prophylaxis in surgical site infection prevention. *J. Antimicrob. Chemother.* 66(8):
- Monnet, E., 2009. Principles of GI surgery. Retrieved from: http://secure.aahanet.org/eweb/images/AAHANet/phoenix_proceedings/pdfs/01_scientific/098_PRINCIPLES%20OF%20GI%20SURGER.pdf Accessed on: July 26, 2011.
- Nakazawa, H., Y. Itoh, K. Tani, K. Itamoto, E. Tsuchihashi, T. Haraguchi, Y. Taura and M. Nakaichi, 2011. Partial gastro-duodenectomy and gastrojejunostomy in a dog with pyloric adenocarcinoma. *Japan J. Anest. Surg.*, 42 (2): 29-34.
- Nichols, R.L., R.E. Condon and P.S. Barie, 2005. Roundtable Discussion: Antibiotic Prophylaxis in Surgery-2005 and beyond. *Surg. Infect.*, 6(3): 349-361.
- Nord, C.E. and C. Enlund, 1990. Impact of antimicrobial agents on human intestinal microflora. *J. Chemother.*, 24: 218-237.
- Nord, C.E. and A. Heimdahl, 1986. Impact of orally administered antimicrobial agents on human oropharyngeal and colonic microflora. *J. Antimicrob. Chemother.*, 18: 159-164.
- Nord, C.E., L. Kager and A. Heimdahl 1984. Impact of antimicrobial agents on the gastrointestinal microflora and the risk of infections. *Am. J. Med.*, 76: 99-106.
- Page, C.P., J.M. Bohnen, J.R. Fletcher, A.T. McManus, J.S. Solomkin and D.H. Wittmann, 1993. Antimicrobial Prophylaxis for Surgical Wounds: Guidelines for Clinical Care. *Arch. Surg.*, 128(1): 79-88.
- Schein, M. and D.H. Wittmann, 1993. Antibiotics in abdominal surgery: The less the better. *Eur. J. Surg.*, 159: 451-453.
- Smet, A., A. Martel, D. Persoons, J. Dewulf, M. Heyndrickx, L. Herman, F. Haesebrouck, and P. Butaye, 2010. Broad-spectrum β -lactamases among Enterobacteriaceae of animal origin: Molecular aspects, mobility and impact on public health. *FEMS Microbio. Revs.*, 34 (3): 295-231.
- Sullivan, Å., C. Edlund and C.E. Nord, 2001. Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet.* 1: 101-114.
- Van D. W., D., J.M. Berghuis-de Vries and J.E.C.M. Lekkerkerk - van der Wees, 1972. Colonization resistance of the digestive tract and spread of bacteria to the lymphatic organs in mice. *J. Hyg.*, 70: 335-342.
- Vollaard, E.J. and H.A.L. Clasener, 1994. Colonization Resistance. *Antimicrob. Agents Chemother.*, 38: 409-414.
- Whittem, T.L., A.L. Johnson, C.W. Smith, D.J. Schaeffer, B.R. Coolman, S.M. Averill, T.K. Cooper and G.R. Merkin, 1999. Effect of perioperative prophylactic antimicrobial treatment in dogs undergoing elective orthopedic surgery. *J. Am. Vet. Med. Assoc.*, 215(2): 212-216.
- Wilcke, J.R., 1990. Use of antimicrobial drugs to prevent infections in veterinary patients. *Probl. Vet. Med.*, 2(2): 298-311.
- Williams, B., 2003. GIT microflora and host health. 4th International Advanced Course, Ecophysiology of the gastrointestinal tract. Wageningen, The Netherlands.