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ASSESSMENT OF THE ANTIBIOTIC RESISTANCE OF BACTERIAL ISOLATES RECOVERED FROM DOG FEED SOLD IN ADO-EKITI METROPOLIS

ATERE Ayowole Victor

Address (es):
Department of Microbiology, Federal University of Technology, Akure, P.M.B 740 Akure, Nigeria.

*Corresponding author: victor_efosa001@yahoo.com

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ABSTRACT

Animal feed has been incriminated in many animal infectious diseases. This study was carried out to investigate and document the bacteriological safety of dog feed sold in Ado-Ekiti Metropolis. Five feed samples were analyzed, the bacterial load, coliform count, total Salmonella count as well as test for campylobacter was carried out on the feed samples. Pure culture of the isolates were subjected to antibiotic susceptibility test using disc diffusion method. The total bacterial count ranged between 4.41 log_{10}CFU/g and 6.36 log_{10}CFU/g. All feed samples had coliform ranging between 2.09 log_{10}CFU/g and 3.93 log_{10}CFU/g. Salmonella was only recovered from feed sample DED. Only sample DDL harbours *Campylobacter* sp. Other bacteria isolated from the feed were *Escherichia coli*, *Bacillus* sp, *Enterobacter* sp, *Klebsiella* sp, *Staphylococcus* sp and *Lactobacillus* sp. The antibiogram showed that nitofuratoin and ciproflocacin had the best activity where 93.10 % (54 out of 58) were susceptible to the two antibiotic. Ampicilin was most resisted with only 36.21 % (21) susceptibility. Thirty one (31) of the isolates showed resistance to 3 or more antibiotic constituting 53.44 % of the entire bacterial isolate. Antibiotic resistance pattern mostly encountered were AMP, AMO, TLY and AMP, AMO, FUR with thirteen (13) isolates each. These results showed that dog feed may not be totally safe for the pets. Based on the type of bacteria isolated and the antibiotic resistance, good manufacturing practices should be ensured by manufactures in other to reduce the rate of contamination.

Keywords: Dogs, *Campylobacter*, *Salmonella*, Antibiotic, Resistance

INTRODUCTION

Animal feeds are so important to the overall productivity of the animal. In general, the composition of animal feed includes mixture of different ingredients that constitute the raw material. The ingredients may include cereals, fat supplements, vitamins, minerals, antioxidants as well as meat meal **(Cabarkapa et al., 2009; Atere et al., 2015a)**.
When feed is contaminated, the quality of the feed is reduced, this contamination often emanates from the ingredients of both plant and animal origin. The quality and quantity of biological contamination is often dependent on the temperature and humidity of the feed and storage environment.
Researchers have showed that the major source of microbial infection in animal is often through the feed consumed, at times, these feeds are contaminated by additives **(Atere, 2016)**. Several animal diseases and syndrome has been traced to the feed, such as diarrhoea, bacillary dysentery, salmonellosis, staphylococcosis, colibacillosis, erysipelas, listeriosis **(Healing and Greenwood, 1991, Xin, 2013)**.
Generally, animal feed has been reported as a source of infection in poultry **(Cabarkapa et al., 2009)**. The toxigenic content of the dog feeds are reported **(Boermans and Leung, 2007)**. A potentially risky factor is the presence of microbial toxins of bacterial and fungal origin in the feed which could lead to food poisoning **(Hussein and Brasel, 2001)**. However, little or no comprehensive information has been reported about the bacteriological contamination and antibiotic susceptibility of the bacteria isolated from dog feed. The bacteriology of the feed can give a clue to the risk feed may constitute, it can also create a public health issue since this can be a source of infection in humans. It is therefore imperative to investigate the bacteriological quality of dog feed sold around Ado-Ekiti metropolis.

AIM AND OBJECTIVE

This research was design to investigate the bacteriological quality of dog feeds by the way of determining the microbial load, the type of bacteria associated with each feed as well as the antibiotic susceptibility of the bacterial isolates.

MATERIALS AND METHOD

The materials used for this research was gotten from pet shops in Ado Ekiti. Triplicate samples of five different feed samples were collected and transported to the microbiology laboratory within two hours of collection.

Determination of moisture content

Five grams of the feed was weighed and dry to a constant weight in a dry oven (DHG-9101-1US Royalcare England). The change in weight divided by the initial weight multiply by 100 was recorded as the moisture content **(AOAC, 2005)**.

Determination of the Bacteriology of the feed

The feed samples were aseptically weighed, 5 gram of each feed sample was homogenized in 45ml of sterile buffer peptone water. A serial dilution of the stock was then carried out using sterile buffered peptone water. The pour plate technique was used where 1 ml of the diluted sample was plated on Nutrient agar, MacConkey and Salmonella-Shigella Agar. All plates were allowed to gel, and inverted then incubated at 37^{o}C for 24h in an incubator (DNP-9022A Royalcare England). The colonies on each of the plates were counted using colony counter. The colonies on the Nutrient agar was recorded as the total bacterial count, the coliforms were determined on MacConkey agar, the total salmonella was determined on Salmonella-Shigella Agar. Preston selective agar was used in testing for the presence of *Campylobacter* sp **(Weese et al., 2005)**.

Identification of bacteria isolates

Pure culture of each isolates were made on nutrient agar. From the pure culture, the cultural characteristics of the colonies were determined. The gram reaction and biochemical testes were also carried out on each of the pure isolates according to the standard as described by **Atere *et al*. (2015b)**. The biochemical characteristics observed were citrate, methyl red voges-proskauer, nitrate, urease, oxidase, coagulase, and catalase.

Antibiotic susceptibility

Muller-Hinton agar was used for the antibiotic susceptibility. The organisms were standardized going by the McFarland standard. While the disk diffusion method was adopted, isolates were inoculated on the agar and the antibiotics disks were introduced. The plate was inverted and incubated in the incubator at a temperature of 37^{o}C for 24 h. The susceptibility and resistance of the bacteria isolates was determined based on the diameter of the zone of inhibition which was compare with the internationally acceptable standard.

RESULTS

The total bacterial count of the feed analyzed ranged between 4.28 log_{10}CFU/g and 6.36 log_{10}CFU/g in feed samples of PED and ERY respectively (Table 1). The highest coliform count was recorded in feed sample DDL with a value of 3.93 log_{10}CFU/g which is significantly higher than all other samples. *Salmonella* sp was not isolated in four of the feeds, the salmonella count of 1.34 log_{10}CFU/g was recorded for feed DED. The moisture content of the feed ranged from 9.16 % to 19.20 %.

Table 1 The moisture content, total bacteria, coliform and *Salmonella* count of dog feed samples

Feed	Total bacterial count (log_{10}CFU/g)	Total coliform count (log_{10}CFU/g)	Total salmonella (log_{10}CFU/g)	Moisture content (%)
DDL	5.68±0.35^{c}	3.93± 0.20^{d}	0	19.2±2.50^{c}
DCO	4.77±0.40^{ab}	3.09±0.40^{c}	0	9.16±0.83^{a}
ERY	6.36±0.50^{d}	2.09±0.30^{a}	0	17.50±2.50^{bc}
PED	4.28±0.10^{a}	2.33±0.15^{ab}	0	11.87±1.49^{ab}
DED	4.41±0.16^{ab}	3.15±0.10^{a}	1.34±0.02	10.00±1.70^{a}

Table 2 showed the distribution of the isolated bacteria in the feed samples. A total of fifty eight (58) bacterial isolates were recovered from all the feeds samples with 8 different bacteria species. *Bacillus* sp was recovered from DDL, DCO, and ERY while *E. coli* was present in all the feed sample analyzed. *Staphylococcus* sp was found in ERY, PED and DED. Only DCO had *Enterobacter* sp. *Campylobacter* sp, was found only in DDL.

Table 2 Number of Bacterial isolates recovered from dog feed samples

Feeds	*E. coli*	*Campylobacter* sp	*Bacillus* sp	*Enterobacter* sp	*Klebsiella* sp	*Lactobacillus* sp	*Staphylococcus* sp	*Salmonella* sp	Total
DDL	6	3	4	-	-	-	-	-	13
DCO	4	-	4	2	2	-	-	-	12
ERY	3	-	2	-	-	6	3	-	14
PED	2	-	-	-	1	-	2	-	5
DED	5	-	-	-	-	-	3	6	14
Total	20	3	10	2	2	6	8	6	58

Table 3 showed the susceptibility of the bacteria isolate to the antibiotic used in this research. The bacterial isolates are most susceptible to nitrofurantoin and ciprofloxacin with 54 of the bacterial isolates being susceptible constituting 93.10% of the total isolates. The least active antibiotic was ampicillin with only 36.21 % (21) susceptible.

Table 3 Antibiotic susceptibility profile of bacterial isolates recovered from dog feed samples

		CEF	CEZ	AMO	OFL	TLY	CIP	ENR	NIT	FUR	GEN	AMP
DDL	*Bacillus* sp n=4	4	3	3	4	1	4	2	4	1	3	1
	Campylobacter sp n=3	2	2	1	2	2	2	1	2	1	3	2
	E. coli n=6	4	5	1	5	5	5	4	6	4	6	2
DCO	*Enterobacter* sp n=2	2	2	2	2	1	2	2	2	2	2	1
	E. coli n=4	3	4	2	4	2	4	2	4	1	3	1
	Bacillus sp n=4	4	4	3	4	2	4	4	4	3	4	3
ERY	*Klebsiella* sp n=2	2	2	2	2	1	2	2	2	2	2	1
	E. coli n=3	2	1	1	2	1	3	1	2	1	2	0
	Lactobacillus sp n=6	6	6	5	6	5	6	5	6	4	6	3
	Bacillus sp n=2	2	1	0	2	2	2	1	2	2	2	1
	Staphylococcus sp n=3	1	3	3	3	1	3	3	3	3	3	2
PED	*Klebsiella* sp n=1	1	1	0	0	0	1	1	1	0	1	0
	Staphylococcus sp n=2	2	2	0	2	0	2	2	2	0	0	0
	E. coli n=2	2	2	2	2	2	2	2	2	0	2	0
	Salmonella sp n=6	5	4	3	5	4	6	2	5	3	5	2
DED	*E. coli* n=5	4	4	2	5	3	4	3	4	2	4	1
	Staphylococcus sp n=3	2	3	1	3	1	2	1	3	3	2	1
Total	58	48	49	31	53	33	54	38	54	32	50	21

Key: Ceftazidime (CEZ) and Cefuroxime (CEF) Amoxicillin(AMO), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CIP), Enrofloxacin (ENR), Nitrofurantoin (NIT), Furasol (FUR), Gentamicin (GEN), Ampicillin (AMP).

The antibiotic resistance pattern AMP, AMO, TLY and AMP, AMO, FUR were the most encountered resistance pattern with 13 bacterial isolates each displaying such resistance pattern. NIT, GEN, AMP and CIP AMP TLY resistance patterns were least encountered with one isolate each displaying this antibiotic resistance pattern (table 4). Table 5 showed the distribution of bacterial isolates showing resistance to 3 or more antibiotics. A total of 31 out of 58 bacterial isolates recovered from dog feeds showed resistance to 3 or more antibiotics.

Table 4 Antibiotic resistance pattern of Bacterial isolates recovered from dog feed samples

	DDL			DCO				ERY				PED			DED			
Resistance pattern	*Bacillus* sp	*Campylobacter* sp	*E.coli*	*Enterobacter* sp	*E. coli*	*Bacillus* sp	*Klebsiella* sp	*E. coli*	*Lactobacillus* sp	*Bacillus* sp	*Staphylococcus* sp	*Klebsiella* sp	*Staphylococcus* sp	*E. coli*	*Salmonella* sp	*E. coli*	*Staphylococcus* sp	Total
AMP, AMO, TLY	1	1	1	0	1	1	0	1	0	0	0	1	2	0	2	1	1	13
AMP, AMO, FUR	1	1	0	0	1	1	0	1	1	0	0	1	2	0	2	2	0	13
CEF, CEZ, OFL	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	3
NIT, GEN, AMP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
ENR, AMP, AMO, FUR	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	3
CIP, AMP, TLY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

Key: Ceftazidime (CEZ) and Cefuroxime (CEF) Amoxicillin(AMO), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CIP), Enrofloxacin (ENR), Nitrofurantoin (NIT), Furasol (FUR), Gentamicin (GEN), Ampicillin (AMP).

Table 5 Bacterial isolates recovered from dog feeds showing resistance to 3 or more antibiotics

Feed	*E. coli*	*Campylobacter* sp	*Bacillus* sp	*Enterobacter* sp	*Klebsiella* sp	*Lactobacillus* sp	*Staphylococcus* sp	*Salmonella* sp	Total
DDL	**3**	**2**	**3**	**-**	**-**	**-**	**-**	**-**	**8**
DCO	**3**	**-**	**0**	**0**	**0**	**-**	**-**	**-**	**3**
ERY	**3**	**-**	**1**	**-**	**-**	**1**	**1**	**-**	**6**
PED	**0**	**-**	**-**	**-**	**1**	**-**	**2**	**-**	**3**
DED	**4**	**-**	**-**	**-**	**-**	**-**	**3**	**4**	**11**
Total	**13**	**2**	**4**	**0**	**1**	**1**	**6**	**4**	**31**

DISCUSSION

Foods have been recognized as the major health determinant, what you eat determined your wellbeing. Animal feed has been reported as a source of microbes in farmed animals and poultry **(Weese et al., 2005; Atere et al., 2015a)**. This may also be true of pets. The bacterial load and the type of bacteria present in the feed samples analyzed indicated that the feeds might not be totally safe for the animals. Isolating *Campylobacter* sp in one of the feed sample is an indication that such a food is so unfit. The bacteria recovered is an indication of potential hazard to the animals. This study revealed that eight (8) bacterial species were isolated from these feeds. The occurrence of these bacteria in the feed and direct consumption of bacterial contaminated feed or their toxins by such animal may constitute a public health issue **(Fraizer and Westhoff, 1978)**. Animal feeds are rich in nutrients, this encourages the proliferation of microorganism and when the environmental/storage conditions such as moisture increased, the growth of these bacteria are pronounced **(Atere et al., 2015)**.

In a similar investigation carried out by **Weese *et al.* (2005)**, *E. coli, Salmonella* sp *Staphylococcus* sp and *Clostridium* sp were isolated. Meanwhile there was no *Clostridium* sp isolated in this research, a report of coliform present in all the samples analyzed is also similar to what is observed in this research where *E.coli* was isolated in all the feed samples **(Weese et al., 2005)**. **Nemser et al. (2014)** reported that *Salmonella*, *Listeria* and *E. coli* are often isolated in pet food, the bacteria load and the type of bacteria isolated from feed can tell more about the safety of the feed.

In a previous research of **Atere et al. (2015a)**, it was reported that the presence of coliforms in poultry feed may have resulted from feacal or environmental contamination. This might also be true of what is observed in this study where the coliform level of three of the feeds are higher than what is recommended by Canadian food inspection agency where the maximum level of coliform should be less or equal to 1000 CFU/g **(Fraizer and Westhoff, 1978)**. Coliform count is always seen as index of sanitation. The increase above this level in this feed may suggest that good manufacturing practices are not being stocked to, or may have resulted from improper handling.

Isolating *Salmonella* sp in one of the feed is of concern. This is because salmonella is a pathogen of many farm animals including pets like dog and cats. There are indications that there could be zoonosis through direct contact or through environmental contamination within the house hold **(Atere et al., 2015b)**. The presence of *Campylobacter* sp in one of five feeds is of concern, this is because it has been responsible for food infection in humans **(Brieseman, 1990)**. Meanwhile *Campylobacter* sp is one of the recognized enteropathogen of dogs and cats, where contact with these pets has been reported as the means of transmission of *Campylobacter* sp to human population **(Brieseman, 1990)**.

The presence of *Staphylococcus* sp in three of the feed samples may have resulted from human source, possibly during dispensing, since these are normal flora of human body. It should be recalled that *Staphylococcus aureus* and *Salmonella* sp are capable of producing acute and chronic infection in all or most type of animal **(Mallinson, 1984)**. Therefore, the effect these bacteria could have on dogs should not be underestimated.

The antibiotic susceptibility of the bacterial isolate can also be of great public health concern. Though the isolates are well susceptible to the antibiotic used in this research when compared with the susceptibility of bacteria isolates from poultry feed **(Atere et al., 2015a)**. In research of **Atere et al. (2018)**, it was reported that the bacterial isolates from dog are less resistant to antibiotic when compare to isolates recovered from poultry. The reason for the increased susceptibility of bacterial isolates recovered from the dog feed when compared with that of poultry may be related to what **Atere et al. (2018)** earlier reported as the sub-therapeutic addition of antibiotic to poultry feeds. Nevertheless, this study showed that some of the bacteria showed multiple resistance, it is of great importance to acknowledge that some of the bacteria multiple resistance strain can find human population, through a trend of being infectious in the pets that feeds on them and through human contact with the pets finds its way into human population.

CONCLUSION

There are potential risks attached to the pet feeds, the type of bacteria found in the feed, the load of the bacteria and the antibiotic resistance of these organisms are of public health concern. The storage condition and the moisture content can also encourage the growth of bacteria. Chemical amendment, heat treatment, careful sourcing for raw materials and good manufacturing practices can go a very long way in reducing bacterial infestation of the feed thereby, improving the safety of the feed.

REFERENCES

AOAC (2005). Official methods of Analysis international. 18th edition. Association of official Analytical chemists. USA.

Atere, V. A, Bamikole, A. M. and Ajurojo, O. A. (2015a). Antibiotic Susceptibility of Bacteria Isolated from Poultry Feeds Sold in Ado Ekiti, Nigeria. *Journal of Advancement in Medical and Life Sciences* V3I2. https://doi:10.15297/JALS.V3I2.06 .

Atere, V. A., Bamikole, A. M., Ajurojo, O. A. and Alo, O. S. (2015b). Antimicrobial Resistance Pattern of Pathogenic *Escherichia coli* Isolated from Chicken Liver and Trachea. *Journal of Advancement in Medical and Life Sciences.* V3I3. https://doi:10.15297/JALS.V3I3.02

Atere A. V. (2016). Multidrug resistant Salmonella sp isolated from chicken. *International Journal of biological research* 4(1): 64-66. https://doi:10.14419/ijbr.v4il.6030

Atere, A.V., Alo, O. S. and Daniel, F. (2018). Comparative study of antibiotic resistance in bacteria isolated from dog and chicken. *International Journal of scientific world* 6 (1): 34-37. https://doi10.14419/ijsw.v6il.8744

Boermans, J. H. and Leung, M. K. C. (2007). Mycotoxins and the pet food industry: Toxicological evidences and risk assessment. *International Journal of food Microbiology*, 119: 95-102. https://doi:10.1016/j.ijfoodmicro.2007.07.063

Brieseman, M. A. (1990). A further study of the Epidemiology of *Campylobacter jejuni* infection. *N Z Med J*. 103: 207-209.

Cabarkapa, I., Kokic, B., Plavsic, D., Ivanov, D. and Levic, J. (2009). Microbiological safety of animal feed. *Biotechnology in Animal Husbandry*, 25: 1155-1162.

Fraizer, W. C and Westhoff, D. C. (1978). Food Microbiology. 3rd Edition Tata McGraw Hill Publishing Company Limited, New Delhi.

Healing, T. O and Greenwood, M. H. (1991). Frequency of Isolation of Campylobacter spp, *Yersinia* spp and *Salmonella* spp from small mammals. *Southern Britain International Journal of Environmental Health Research*. 1: 54 – 62.

Hussein, S. H. and Brasel, M. J. (2001). Toxicity, metabolism and impact of mycotoxins on humans and animals. *Toxicology*, 167:101-134. https://doi:10.1016/s0300483x(01)00471-1

Mallinson E. T. (1984). Infectious diseases. In Animal Health. Jack Hayes (ed) Bureau of animal Industry (publisher), Maryland USA.

Nemser, S, M., Doran, T., Grabenstein, M., McConnell, T., McGrath, T., Pamboukia, R., Smith, A. C., Achen, M., Danzeizen, G., Kim, S., Liu, Y., Robeson, S., Rosario, S., Wilson, M. K. and Reimschuessel, R. (2014). Investigation of *Listeria, Salmonella* and Toxigenic *Escherichia coli* in various pet foods. Foodborne pathogen and disease 11(9): 706-709. http://doi:10.1089/fpd.2014.1748

Weese, J. S., Rousseau, J. and Arroyo, L. (2005). Bacteriological evaluation of commercial canine and feline raw diets. *Canadian Veterinary Journal* 46: 513-516.

Xin, L. (2013).Pet Food Safety. U S Food and Drug Administration Protecting and Promoting Public Health. FASS 2013 Symposium Washington DC. www.fda.gov/FASS2013Symposium