PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL SPECIES ISOLATED FROM DIARRHEIC DOGS IN JOS, PLATEAU STATE, NIGERIA

***[1]OGBU K. I., [2]CHUKWUDI I. C., [1]PETER Z. N., [3]SULE H., [1]MAGAJI A. D., [4]TION M. T., [5]OKPARA J. O.**

[1]Department of Animal Health, Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom, Plateau State, Nigeria
[2]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria
[3]Department of Animal Production, Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom, Plateau State, Nigeria
[4]Department of Veterinary Medicine, College of Veterinary Medicine, Federal University of Agriculture Makurdi, Benue State, Nigeria
[5]Department of Science Laboratory Technology, Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom, Plateau State, Nigeria

***Corresponding author:** OGBU K. I. drken2016@gmail.com; ogbu.kenneth@fcahptvom.edu.ng; +2348030852357; ORCID: 0000-0003-0924-1604

ABSTRACT

Diarrhea caused by bacterial species is an important and common problem in companion animal, especially dogs. Bacteria have the potential to develop resistance to antibiotic drugs at any time. Antibiotic susceptibility testing therefore provides information that allows clinicians to select the most appropriate antimicrobial drugs. Although, over the years, the antimicrobial resistance patterns associated with diarrhea in dogs have been changing. The research was carried out to determine the prevalence and antibiotics susceptibility pattern of bacterial specie(s) in diarrheic dogs attended in veterinary practice at Teaching Veterinary Hospital Polo, ECWA Veterinary Hospital Bukuru and NVRI Veterinary Hospital in Jos Plateau State. A total number of 60 fecal swabs were collected from diarrheic dogs in the study area and analyzed in the Central Diagnostic Microbiology Laboratory NVRI. The culture and sensitivity pattern of the bacteria species isolated were conducted against a panel of 10 commonly selected antibiotics using disc diffusion method. Out of the 60 diarrheic swab samples *Escherichia coli* 49 (81.67%) was significantly (P<0.01) higher than *Proteusmirabilis* 5(8.33%) and *Klebsiellapneumoniae* 4(6.67%). Total bacteria species isolated was significantly (P<0.01) higher in young 52(86.67%) than in adult 6(10%). Mongrel

24(40.00%) was significantly (P<0.01) higher followed by Caucasian 22(36.67%). Further (85%) of the isolates were observed to be multidrug resistant, limiting treatment of diarrheic infections with routinely used antibiotics. Hence, there is need for constant monitoring of antibiotic resistance for better management of patients on antibiotic treatment. In addition, the collected data could be use in determination of trends in antimicrobial susceptibility patterns and therefore assisting in policy formulation on the currently used antibiotics for management of diarrheic infections.

Keywords: Prevalence, Antimicrobial Susceptibility Pattern, Bacterial Species, Diarrheic Dogs, Jos, Plateau State, Nigeria

INTRODUCTION

Diarrhea caused by bacterial species is an important and common problem in companion animal, especially dogs. Bacteria have the potential to develop resistance to antibiotic drugs at any time. This means that antibiotics once used to kill or inhibit their growth may no longer be effective. Susceptibility testing is used to determine which antimicrobials will inhibit the growth of the bacteria causing a specific infection. Diarrhea is one of the most common clinical signs seen in dogs with gastrointestinal disease, and can result in electrolyte and fluid disturbance (McMichael, 2005), as well as disruption of intestinal function and the microbiota. Diarrhea is also associated with the highest incidence of mortality in infants and young farm animals (Buddington and Weiher, 1999). Potential causes of chronic diarrhea in dogs include food allergy, bacterial or parasitic infections, and inflammatory and neoplastic conditions (Weese *et al.,* 2001). *Clostridium difficile* and *Clostridium perfringens*are commonly associated with canine diarrhea, particularly with acute large intestinal diarrhea. However, their possible role as causative agents is confounded by their presence in the normal intestinal microbiota, as demonstrated by the fact that detection of these bacteria has been shown to be of low value in the diagnosis of diarrhea (Cave *et al*., 2002). Some studies however, have shown significant associations between the detection of their toxins or toxin gene and diarrhea (Weese *et al*., 2001; Marks *et al*., 2002). An abnormal gastrointestinal microbiota has been associated with Crohn's disease and ulcerative colitis in humans (Schaffer *et al* 2015; Sokol *et al*., 2006), irritable bowel syndrome in humans (Nobaek *et al.,* 2000) and canine inflammatory bowel disease (IBD) (Xenoulis *et al*., 2008).

Diarrhea can be caused by a wide variety of bacterial, viral, and protozoan pathogens. The most important of these are *Salmonella spp, Shigellaspp, Vibrio cholera, Entamoebahistolytica*, Norovirus, *Giardia lamblia*, *Escherichia coli, Campylobacter jejuni, Cryptosporidium spp,* and Rotavirus (Akinnibosun and Nwafor, 2015).

Members of the famiy Enterobacteriaceae include many species such as *Escherichia coli, Klebsiellaspp, Enterobacterspp and Salmonella spp.* Many organisms belonging to these species are commensals of the gastrointestinal tract. Increasing antimicrobial resistance among Enterobacteriaceae is emerging as a significant public health concern in human medicine (Pitout and Laupand, 2008).

Salmonellosis, the disease caused by *Salmonella*spp, is one of the most frequently occurring foodborne diseases worldwide. Salmonella is acquired by contact with contaminated domestic animals and their food products, farm animals or animals in petting zoo, and exotic pets such as turtles, hedgehogs, and iguanas. This can then be transmitted from person to person via the oral fecal route (Humphries and Linscott, 2015).

Antimicrobials contribute a vital role in treatment and prophylaxis. Even though different factors aggravate antimicrobial resistance, overuse, and misuse of antimicrobials in other sectors play a significant role (WHO, 2017). Beyene *et al.* (2011) reported that there is an increase in antimicrobial resistant in pathogenic bacteria causing diarrheal disease because of easy affordability of antibiotics in pharmacies and misuse. The spectrum of causative agents and their antimicrobial resistance pattern has been dynamic worldwide. There is pressure resulting from intensive and indiscriminate use of antibiotics in treatment leading to rapid spread of antimicrobial agent resistance gene (Annapurna and Lakshmi, 2013). There is need to carry out this research work, since there has been a little work done to evaluate the prevailing bacterial species mostly affecting dogs, commonly causing diarrhea. The study aimed at determining the prevalence and antimicrobial susceptibility pattern of bacterial specie(s) isolated from diarrheic dogs in Jos Plateau State, Nigeria. The results from this study will help a health care practitioner or Veterinarians to determine which drugs are likely to be most effective in treating an infection in a patient and to sensitize the society on the issue of drug resistance and it public health importance.

Research method

Study Location

The study was conducted in Jos Metropolis. Jos is a city in the middle belt of Nigeria. It is located on 9056'N8053'E.

Sample Size Determination

The required sample size will be determined using the formula as stated by Thrusfield (2005).

$n = z^2pq / d^2$

n= Desired sample size

z = 1.96 (normal distribution) from table

p = prevalence rate from the average of previous studies = 5.5% (Akwuobu*et al.*, 2018)

d = Desired absolute precision of + 5% with 95% confidence intervals.

$q = 1 - p$

$n = z^2 p (1 - p) / d^2$

$n = (1.96)^2 \times 5.5\% \times (1 - 5.5\%) / 0.05^{\circ}$

$n = 3.8416 \times 0.055 \times (1 - 0.055) / 0.0025$

$n = 79.866864$ $\qquad$ $n = 80$

Study Design

The study was conducted in Central Diagnostic Laboratory, National Veterinary Research Institute (NVRI) Vom, Plateau State. A total of 120 fecal swabs were collected from diarrheic dogs brought to some selected Veterinary hospitals (Veterinary hospital FCAH&PT Vom, ECWA Veterinary hospital Bukuru, and University of Jos Veterinary Teaching hospital, JosPlateau State).

Sample Collection

Diarrheic fecal swabs were collected from 120 dogs in the study area. The fecal swabs was collected using swab stick and immediately well-labeled. The samples was stored in a flask for preservation and transported to the Central Diagnostic Laboratory, National Veterinary Research Institute (NVRI) Vom for analysis.

Bacterial Isolation and Identification

The fresh fecal samples collected in the swab stick collected were inoculated into 9ml of sterile peptone water and incubated overnight at 37 C. A loop full of peptone water broth was streaked on Eosin methylene blue agar (EMB) using quadrant streak method. The plate was incubated aerobically at 37C for 24hours.The plates were examined for gross colony morphology, pigmentation such as greenish metallic sheen between 24 and 48 hours. Presumptive colonies of isolated bacteria species were selected and purified on Nutrient agar, and purified isolates were subjected to biochemical tests and growth was inspected to identify the bacteria (Mustapha *et al*., 2021) and antibiotic susceptibility tests according to Clinical Microbiology Procedures Handbook, (2016).

Antimicrobial Susceptibility Test

The antibiotic susceptibility test of the bacteria specie(s) isolated was conducted against a panel of 10 commonly selected antimicrobials, using Kirby- Bauer disc diffusion, according to the standard of Clinical and Laboratory Standards Institute (CLSI, 2015).

The diameter of the zones of each antibiotic inhibition, which was determined by the break points of antimicrobial Susceptibility discs, was measured with a ruler after 24 hours of incubation at 37^{o}c. The antimicrobial Susceptibility discs that was used for susceptibility testing was Pefloxacin (PEF, 10 µg), Ampicillin (AMP, 30 µg), Cephalexin (CEP, 10 µg), Ciprofloxacin (CPX, 10 µg), Streptomycin (S, 30 µg), Gentamicin (GEN, 10 µg), Amoxicillin (AU, 30 µg), Nalidixic Acid (NA, 30 ug), and Trimethoprim (SXT, 30 µg).

Data Analysis

The data obtained was analyzed using Chi-square and percentage (%) prevalence, P values less than 0.05 ($P<0.05$) was considered statistically significant. Antibiotic susceptibility profile of bacterial species isolated from the positive samples was analyzed using inhibition zones in diameter (mm). The result was presented in tables.

RESULTS

A total of 60 fecal swabs were collected from diarrheic dogs brought to some selected veterinary hospitals (Veterinary hospital FCAH&PT Vom, ECWA Veterinary hospital Bukuru, and University of Jos Veterinary Teaching hospital Plateau State) and were subjected to bacterial examination. A total of 116 samples were positive with *Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae* isolate and also determined their susceptibility pattern using some common selected antibiotics.

Table 1: Distribution of Bacteria species isolated from dogs with diarrhea in the study

Bacterial species	Number positive n (120)	Prevalence (%)
Escherichia coli	98	81.67^{a}
Klebsiellapneumonia	8	6.67^{b}
Proteus mirabilis	10	8.33^{b}
Total	116	96.67
Standard Deviation		26.938
Chi-square		114.454
P-value		0.000*

$^{a,\,b}$ Means in the same column with different superscripts are significantly different (P<0.01)

* = Significant

Shows the prevalence of the bacterial species isolated from diarrheic dogs. *Escherichia coli* was 98 (81.67), *Klebsiella pneumoniae* was 8(6.67) and *Proteus mirabilis* was 10(8.33) isolated from a total of 120 samples collected. *Escherichia coli* was significantly (P<0.01) higher than *Klebsiella pneumonia* and *Proteus mirabilis.*

Table 2: Distribution of Bacterial species isolated from dogs with diarrhea based on age

Bacteria species	Number positive (%) Young (0-6 months) n = 104	 Adult (>12 months) n = 12
Escherichia coli	90 (83.33^{a})	8(66.67^{b})
Klebsiella pneumonia	4(3.70)	4(33.33)
Proteus mirabilis	10(9.26)	-
Total	104(86.67^{a})	12(10.00^{b})
Standard Deviation	0.607	0.473
Chi-square	122.313*	11.560*
P- value	0.000	0.001

$^{a, b}$ Means in the same column with different superscripts are significantly different (P<0.01)

* = Significant

Shows the prevalence of bacterial species isolated from diarrheic dogs with respect to age. Young (age 0-6 months) was 104 (86.67) positive samples and in adult dogs (age >12 months) was 12(10.00) positive samples. *Escherichia coli* was 90(83.33) in young while in adult 8(66.67), *Klebsiella pneumoniae* was 4 (3.70) while in adult 4(33.33) and *Proteus mirabilis* was 10(9.26) in young while in adult was negative. Bacterial species isolated in young dogs was significantly (P<0.01) higher than in adult dogs. Likewise, *Escherichia coli* was significantly (P<0.01) higher than *Klebsiella pneumonia* and *Proteus mirabilis.*

Table 3: Distribution of Bacterial species isolated from dogs with diarrhea based on sex

Bacteria species	**Number positive (%)**	
	Male **n = 52**	**Female** **n = 64**
Escherichia coli	42(80.77)	56(87.50)
Klebsiella pneumonia	4(7.69)	4(6.25)
Proteus mirabilis	6(11.54)	4(6.25
Total	52(43.33)	64(53.33)
Standard Deviation	0.677	0.520
Chi-square	100.059*	134.480*
P- value	0.000	0.001

There was no significantly different (P<0.01) in respect to sex.

* = Significant

Shows the prevalence of bacteria species isolated from diarrheic dogs based on sex. Male was 26(43.33) positive samples and female was 32 (53.33) positive samples. *Escherichia coli* was 21 (80.77) in male while in female 28(87.50), *Klebsiella pneumoniae* (2.7.69) in male while in female 2(6.25), *Proteus mirabilis* 3(11.54) while in female 2(6.25). There was no significant difference (P<0.01) based on sex.

Table 4: Distribution of Bacteria species isolated from dogs with diarrhea based on breed

	Number positive (%)				
Bacteria species	**Mongrel** **n = 24**	**Caucasian** **n=22**	**German shepherd** **n=6**	**Mastiff** **n = 4**	**Rottweiler** **n=2**
Escherichia coli	23(95.83^{a})	17 (77.27 ^{a})	5(83.32^{b})	2(50.00^{b})	2(10.00^{b})
Klebsiella Pneumonia	-	2(9.09)	1(16.67)	1(25.00)	-
Proteus mirabilis	1(4.17)	3(13.64)	-	1 (25.00)	-
Total	24(40.00 ^{a})	22(36.67^{a})	6(10.00 ^{b})	4(6.67 ^{b})	2(3.33 ^{b})
Standard Deviation	0.384	0.720	0.378	0.833	
Chi-square	84.640*	86.180*	43.560*	12.500*	
P- value	0.000	0.000	0.000	0.002	

$^{a, b}$ Means in the same column with different superscripts are significantly different (P<0.01)

* = Significant

Shows the prevalence of bacteria species isolated from diarrheic dogs based on breed. Mongrel 24(40.00) positive samples, were *Escherichia coli* was 23(95.83), and *Proteus mirabilis* 1(4.17), while Caucasian 22(36.67) positive samples, were *Escherichia coli* was 17(77.27), *Klebsiella pneumoniae* was 2(9.09) and *Proteus mirabilis* (3(13.64), while German shepherd 6(10.00) positive samples, were *Escherichia coli* was 5(83.33), and *Klebsiella pneumoniae* was 1(16.67), while Mastiff 4(6.67) positive samples, were *Escherichia coli* was 2(50.00), *Klebsiella pneumoniae* 1(25.00) and *Proteus mirabilis* 1(25.00), lastly in Rottweiler 2(3.33) positive samples, were *Escherichia coli* was 2(100.00).Mongrel was significant (P<0.01) higher followed by Caucasian compared to other breed of dogs.

Table 5: Sensitivity pattern of *Escherichia coli* isolated from dogs with diarrhea

	Susceptibility Pattern n=10		
Antibiotics (mcg)	**Sensitive (%)**	**Intermediate (%)**	**Resistant (%)**
Ofloxacin (10)	5(50)	1(10)	4(40)
Pefloxacin (10)	1(10)	4(40)	5(50)
Ciprofloxacin (10)	0(0)	5(50)	5(50)
Amoxicillin (30)	0(0)	0(0)	10(100)
Gentamycin (10)	7(70)	2(20)	1(10)
Streptomycin (30)	8(80)	1(10)	1(10)
Cephalexin (10)	0(0)	1(10)	9(0)
Nalidixic Acid (30)	0(0)	0(0)	10(100)
Trimethoprim (30)	0(0)	2(20)	8(80)
Amplicin (30)	0(0)	0(0)	10(100)

Shows the sensitivity pattern of 10 *Escherichia coli* isolate to different antibiotics. Most isolates were resistance to between 2 to 8 agents, although ofloxacin, gentamycin and streptomycin were invariably sensitive to 5, 7 and 8 isolates respectively. Streptomycin showed the highest potency among the antibiotics used.

Table 6: Sensitivity pattern of *Proteus mirabilis* isolated from dogs with diarrhea

	Susceptibility Pattern n=5		
Antibiotics (mcg)	**Sensitive (%)**	**Intermediate (%)**	**Resistant (%)**
Ofloxacin (10)	2(40)	0(0)	3(60)
Pefloxacin (10)	0(0)	1(20)	4(80)
Ciprofloxacin (10)	0(0)	2(40)	3(60)
Amoxicillin (30)	0(0)	0(0)	5(100)
Gentamycin (10)	3(60)	0(0)	2(40)
Streptomycin (30)	3(60)	0(0)	2(40)
Cephalexin (10)	0(0)	0(0)	5(100)
Nalidixic Acid (30)	0(0)	0(0)	5(100)
Trimethoprim (30)	0(0)	0(0)	5(100)
Amplicin (30)	0(0)	0(0)	5(100)

Shows the sensitivity pattern of 5 *Proteus mirabilis* isolate to different antibiotics. Most isolates were resistance to all the agents, although ofloxacin, gentamycin and streptomycin were invariably sensitive to 2, 3 and 3 isolates respectively. Gentamycin and streptomycin showed the highest potency among the antibiotics used.

Table 7: Sensitivity pattern of *Klebsiella pneumoniae* isolated from dogs with diarrhea

	Susceptibility Pattern n=4		
Antibiotics (mcg)	**Sensitive (%)**	**Intermediate (%)**	**Resistant (%)**
Ofloxacin (10)	1(25)	0(0)	3(75)
Pefloxacin (10)	0(0)	0(0)	4(100)
Ciprofloxacin (10)	0(0)	1(25)	3(75)
Amoxicillin (30)	0(0)	0(0)	4(100)
Gentamycin (10)	0(0)	0(0)	4(100)
Streptomycin (30)	3(75)	1(25)	0(0)
Cephalexin (10)	0(0)	0(0)	4(100)
Nalidixic Acid (30)	0(0)	0(0)	4(100)
Trimethoprim (30)	0(0)	0(0)	4(100)
Amplicin (30)	0(0)	0(0)	4(100)

Shows the sensitivity pattern of 4 *Klebsiell apneumoniae* isolates to different antibiotics. Most isolates were resistance to almost all the agents, with the exception of streptomycin which invariably showed the highest potency among the antibiotics used.

Table 8: Standard antimicrobial inhibition zones according to Clinical Laboratory Standards Institute

Antibiotics (ug)	Resistant	Intermediate	Sensitive
Ofloxacin (10)	≤18mm	14-17mm	≥13mm
Pefloxacin (10)	≤17mm	13-14mm	≥12mm
Ciprofloxacin (10)	≤26mm	22-25mm	≥21mm
Amoxicillin (30)	≤18mm	14-17mm	≥13mm
Gentamycin (10)	≤12mm	13-14mm	≥15mm
Streptomycin (30)	≤15mm	12-14mm	≥11mm
Cephalexin (10)	≤14mm	11-15mm	≥18mm
Nalidixic Acid (30)	≤13mm	14-18mm	≥19mm
Trimethoprim (30)	≤10mm	11-15mm	≥16mm
Amplicillin (30)	≤13mm	14-16mm	≥16mm

DISCUSSION

The study reported Escherichia coli was the most predominant bacteria species which agrees with previous reports by Han *et al*., 2016; Moon *et al*., 2012 and DeGraef *et al.* (2004) from Korea and Belgium, which reported that among all isolate E. coli has higher prevalence as compared to other bacterial isolated. This may be due to the abundant of E. coli in the gastrointestinal tract at the time of infection and most opportunistic bacteria in both human and dogs (Chen *et al*., 2003; Hagman *et al.,* 2002; Johnson *et al.,* 2003). *Escherichia coli* are part of the normal intestinal microflora, but can be associated with gastroenteritis in the presence of bacterial virulence factors and impaired local or systemic immunity Hammermueller *et al*. (1995). Furthermore, studies from the American Animal Hospital Association Norris *et al*. (2000) and College of Veterinary Medicine, North Carolina State University. Santajit and Indrawattan. (2016), reported that *E.coli, Proteus spp., Klebsiella spp., Pseudomonas spp*., and *Enterococcus spp.* were the five most prevalent bacterial species in the urine of dogs with urinary tract infections (UTIs).These findings are consistent with that of Moon *et al.* (2022).

The results obtained in this study showed higher prevalence of bacteria species with in young dogs which agreed with the finding of Buddington and Weiher (1999), which noted that diarrhea is associated with highest incidence of mortality in infants and young farm animal. This may be attributed to compromised immunity, abnormal gastrointestinal microbiotamore with younger dogs, change in diet, seasonal variation with higher incidence during the summer months (Lekcharoensuk *et al.,* 2021).

The result in this study reported no significant association in respect to sex, although there was no previous study on diarrheic infections for comparison. Unlike findings in a previous study with urinary tract infections where mixed infections were more common in female dogs. This suggested that anatomy is the most likely culprit. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, 2021) in United States, female are more likely to develop urinary tract infections (UTIs) because they have a shorter urethra than male. Bacteria don't have to travel very far to reach and infect a female's bladder. Plus, the opening to the urethra is closer to the rectum, where the bacteria that can cause these infections mostly reside.

The study recorded higher rates of resistance to trimethoprim and ampicilin resistance *Escherichia coli* were detected which agreed with Ramos *et al*. (2022), recorded high rates to trimethoprim and ampicilin. Which suggest that feeding raw meat-based diets (RMBD) can change the *Escherichia coli* compostion in the canine gut microbiome, altering the frequency of certain phylogroups and increasing the shedding of diarreagenic pathotype (Ramos *et al.* (2020). This could also be due resistance as a result of prolonged exposure to antibacterial agents, particularly when applied in sub-therapeutic doses, and by acquiring antibacterial resistance genes from other commensals or transient pathogens that colonize the body or the environment (Tajbakhsh *et al.,* 2016; Shaheen *et al.,* 2011 and Torkan *et al.* (2016).However, contradict the findings with Aslani *et al*. (2008), who reported that only a few isolates were resistant to ciprofloxacin, and the results of Grave *et al.* (1999), where the high sensitivity was shown only to ciprofloxacin among the used antimicrobials. This may be attributed to newer drugs that are relatively expensive, and therefore less available for use and potential abuse in the study areas.

The study recorded resistance of almost half of *Proteus mirabilis* isolated to gentamycin and streptomycin which agreed with Han *et al.*(2020), recorded half of their isolates were resistance to all aminoglycosides evaluated (gentamycin, kanamycin, streptomycin, amikacin, tobramycin). Perhaps high resistance to aminoglycosides was due to presence of the rmtB gene Fritche *et al.* (2008), failure to follow the treatment regimen, prophylactic use of antibiotics, and the use of antibiotics as growth promoters. However, in this study gentamycin and streptomycin were the most potent drugs having invariably high sensitivity.

CONCLUSION

The prevalence study showed that *Escherichia coli* were more predominant bacteria species in diarrheic dogs than other species in the study. Prevalence of bacterial species in diarrheic dogs was more in young dogs than in adult dogs. Mongrel has the highest prevalence followed by Caucasian than in other breed of dogs. However, there was no prevalence different in respect to sex.The result indicates Ofloxacin (10mcg) and Streptomycin (30mcg) as drugs suitable for the treatment of diarrheic infection with Canine *Escherichia coli*. Ofloxacin (10mcg), Gentamycin (10mcg) and Stretomycin (30mcg) as drug of choice for the treatment of diarrheic infection with Canine *Proteus mirabilis*and Stretomycin (30mcg) as drug of choice for the treatment of diarrheic infection with Canine *Klebsiella pneumonia.*This study ascertained the prevalence and drug resistance profiles of *Escherichia coli, Klebsiella pneumoniae*and *Proteus mirabilis* isolated from diarrheic dogs, in order to provide update information and to sensitize the society on the issue of drug resistance and it public health importance.

RECOMMENDATIONS

It is recommended to avoid the use of irrational drugs, misuse of drugs and dogs should be fed with cooked meals or conventional feeds if possible. T the use of antibiotics as therapeutic and prophylaxis for animals especially pets should be carefully evaluated and monitored under the supervision of a Veterinarian to reduce acquisition of antibiotic resistant strains of bacterial species which post a serious health implication in domestic animals in general. Implementation of rules and regulations guiding the usage of antibiotic drugs and good hygiene practice are also recommended. Proper diagnosis is required in every ill-health for proper use of drugs and further studies are recommended for better in-depth of knowledge in the field of study.

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