The Journal of Animal & Plant Sciences, 26(1): 2016, Page: 13-16
ISSN: 1018-7081

OCCURRENCE OF ANTIMICROBIAL RESISTANT BACTERIA IN DOGS SUFFERING FROM ENTERITIS

[1]I. Habib, [1]A. A. Anjum, [2]M. Rabbani, [3]M.U.D. Ahmad, [1]M. A. Ali, [1]M. Nawaz,[1] M. Kamran and [4]H. M. Khan

[1]Department of Microbiology, [2]University Diagnostic Laboratory, [3]Department of Epidemiology and Public Health, [4]Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore
Corresponding Author: aftab.anjum@uvas.edu.pk

ABSTRACT

Aim of the present study was to isolate bacteria from feacal material of dogs suffering from enteritis and determine their antibiotic resistance pattern. Fecal samples (n=100) were collected from dogs presented at Pet Clinic of University of Veterinary and Animal Sciences, Lahore with symptoms of enteritis.. A total of 210 different bacterial isolates were selected and identified as *E. coli* (127; 60.47%), *Salmonella spp.* (50; 23.81%), *Proteus vulgaris* (12; 5.71%), *Citrobacter spp.* (17; 8.09%) and *Pseudomonas spp.* (04; 1.90%). *E. coli* were moderately resistant to ampicillin (59.65%) followed by tetracycline (54.33%), kanamycin (52.75%), gentamycin (49.60%), vibramycin (46.45%), ceftriaxone (44.88%), norfloxacin (30.70%) and ciprofloxacin (25.98%). *Salmonellae* were also moderately resistant to ampicillin (42%), followed by gentamycin (38%), kanamycin (30%), tetracycline (28%), cepharadine (20%), ceftriaxone (16%), vibramycin (14%), ciprofloxacin (12%) and norfloxacin (8%). All the isolates were sensitive to amikacin. The isolates found resistant to more than two antibiotics were declared as multiple drug resistant (MDR) bacteria. Out of 127 *E. coli* isolates, 52 (40.94%) were multiple drug resistant bacteria, followed by *Salmonella enterica* isolates 17(34.00%), *Citrobacter diversus* 06 (35.29%), *Proteus vulgaris* 06 (50%). It is concluded that multiple drug resistance is present in gut pathogens of dogs which may be alarming for public health as well.

Key words: Dogs, enteritis, *Salmonella*, *E. coli* and multiple drug resistance.

INTRODUCTION

Pet animals are reservoirs of antibiotic resistant bacteria which may disseminate antibiotic resistance to other microbes (Guardabassi *et al.*, 2004). Antibiotic resistance has been a problem since the discovery of first antibiotic (Davies and Davies, 2010). The problem has been compounded in recent times because of overuse and misuse of antibiotics in veterinary and human clinical set ups. Use of antibiotics as growth promoters in poultry and turkey rearing have also contributed to the problem (Castanon, 2007). Antibiotic resistant gut (Guardabassi *et al.*, 2004) normal flora and pathogens can transfer resistance determinants to other bacteria (Huddleston, 2014). Resistant gut pathogens increase the treatment cost and cause mortality and morbidity. Due to the close contact of pets with human, pets are alarming reservoir of antibiotic resistance. Antibiotic resistant bacteria of zoonotic importance pose substantial threat to public health as well (Damborg *et al.*, 2015).

Antibiotic resistance can be natural or acquired. Natural or intrinsic antibiotic resistance is because of internal structural or physiological nature of microbes. It is generally chromosome encoded and non-transferable. Acquired antibiotic resistance is generally acquired from the environment. It is plasmid or chromosome encoded and transferable to other bacteria (Davies and Davies, 2010). Mechanisms of development of antibiotic resistance are generally decreased permeability, lesser affinity with the target, efflux pumps, target protection and modifications and antibiotic degrading enzymes (Blair *et al.*, 2015).

Pet animals including dogs harbor complex diversity of microbes (more than 10 bacterial phyla) in their gut including *E. coli*, Clostridia, *Helicobacter spp.*, fimicutes and bacteroidetes (Deng and Swanson, 2015; Suchodolski, 2011) . Microbes such as *E. coli*, *S. aureus*, *Salmonella*, *Shigella* cause different gut problems including enteritis in dogs (Puno-Sarmiento *et al.*, 2013, Faires *et al.*, 2010). Enteritis with multiple drug resistant microbes is cause of sever and chronic enteritis which may lead to morbidity and mortality (Pedersen *et al.*, 2007). It is of great importance to isolate etiological agent of dog enteritis and their antibiotic resistance pattern. Dogs carry drug resistant *E. coli* in their feces which may readily contaminate the environment (Johnson *et al.*, 2006, Sidjabat *et al.*, 2006). Commensals flora of dogs carry antibiotic resistance and possible antibiotic resistance reservoirs (Murphy *et al.*, 2009).

Although there are many studies reporting antibiotic resistance in bacteria causing enteritis in dogs and role of pet animals in transmission of antibiotic resistance, none is from Pakistan (Minton *et al.*, 1983, Gronvold *et al.*, 2010, Trott *et al.*, 2004, Barton *et al.*, 2003, Warren *et al.*, 2001). Lack of data on antibiotic resistance in dogs insinuates for the isolation of bacteria

causing enteritis in dogs and resistance of isolates to commonly used antibiotics. Study will not only help in improving the efficacy of empirical treatment, it will also help to quantify the problem of antibiotic resistance in Pakistan. Aim of the current study was to highlight the bacterial etiology of dog enteritis and determination of resistance pattern of isolated bacteria.

MATERIALS AND METHODS

Study Animals: A total of 100 dogs were enrolled in study. Dogs were presented at pet clinic of University of veterinary and Animal Sciences, Lahore with general symptoms of enteritis. Enteritis was diagnosed from apparent signs (diarrhea), any change in dog feed. All the pet dogs were vaccinated for various diseases including for viral enteritis but none was vaccinated for bacterial enteritis.

Sample collection: Rectal swab samples (n=100) were collected from dogs diagnosed with enteritis. Samples were immediately transported to bacteriology laboratory of Department of Microbiology, University of Veterinary and Animal Sciences, Lahore and processed for isolation of etiological bacteria.

Bacteriological Study: Samples were cultured on MacConkey's agar plate. Post incubation, colonies with different morphology were selected and purified by three way streaking method. Isolates were identified by their microscopic, cultural and biochemical characteristics following the Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). Pathogenicity test was performed using Congo Red Medium to differentiate between invasive and non-invasive *E. coli* isolates. *E. coli* cultures were streaked on Congo Red Medium and results were observed after 24-72hrs of incubation.

Antibiotic sensitivity: Antibiotic sensitivity of all isolates to different antibiotics including ampicillin, gentamycin, kanamycin, tetracycline, ceftriaxone, vibramycin, ciprofloxacin, norfloxacin and cepharadin was determined by Kirby-baud method. Briefly, fresh growth of isolates was adjusted to I McFarland and a lawn was prepared on Muller Hinton agar. Antibiotic disks were placed on appropriate distance and incubated at 37 ^{o}C for 24 hours. Post incubation, diameters of zone of inhibitions was measured. Isolates were declared as resistant or sensitive on the basis of microbiological break points adopted from clinical laboratory institute.

RESULTS

From 100 samples, a total of 210 bacterial isolates were recovered. Out of 210 bacterial isolates, *E. coli* were most prevalent (127, 60.47%) followed by *Salmonella spp.* (50, 23.81%), *Proteus vulgaris* (12, 5.17%), *Citrobacter diversus* (17, 8.09%) and *Pseudomonas spp*. (4, 1.90%). Out of 127 *E. coli*, 46 (36.50%) were invasive. *E. coli* were moderately resistant to ampicillin (59.65%) followed by tetracycline (54.33%), kanamycin (52.75%), gentamycin (49.60%), vibramycin (46.45%), ceftriaxone (44.88%), norfloxacin (30.70%) and ciprofloxacin (25.98%). *Salmonellae* were also moderately resistant to ampicillin (42%), followed by gentamycin (38%), kanamycin (30%), tetracycline (28%), cepharadine (20%), ceftriaxone (16%), vibramycin (14%), ciprofloxacin (12%) and norfloxacin (8%). The isolates found resistant to more than two antibiotics were declared as multiple drug resistant (MDR) bacteria. All the isolates were sensitive to amikacin. Out of 127 *E. coli* isolates, 52 (40.94%) were multiple drug resistant, followed by *Salmonellae* 17(34.00%), *Citrobacter diversus* 06 (35.29%), *Proteus vulgaris* 06 (50%).

Table 1: Antimicrobial resistance pattern of bacteria isolated from faecal material of dogs

Antibiotics	Resistant Bacterial Isolates n^{a} (%)					
	E. coli **(n=127)**	***S. enterica*** **(n=50)**	***Proteus spp.*** **(n=12)**	***Citrobactor spp.*** **(n=17)**	***Pseudomonas spp.*** **(n=04)**	**Total** (n=210)
Ampicillin	75 (59.05)	21(42)	09(75)	07(41.1)	02(50)	114(54.28)
Gentamycin	63 (49.60)	19(38)	05(41.66)	06(35.29)	0(0)	93(44.28)
Amikacin	00(00)	0(0)	0 (0)	0(0)	0(0)	0(0)
Kanamycin	67(52.75)	15(30)	08(66.66)	0(0)	0(0)	9(42.85)
Vibramycin	59(46.45)	07(14)	07(58.33)	0(0)	0(0)	73(34.76)
Tetracycline	69(54.33)	14(28)	09(75)	03(17.6)	0(0)	93(44.28)
Norfloxacin	39(30.70)	04(08)	05(41)	03(17.6)	0(0)	51(24.28)
Ciprofloxacin	33(25.98)	06(12)	04(33.33)	01(5.80)	0(0)	44(20.95)
Cepharadin	23(18.11)	10(20)	06(50)	01(5.80)	02(50)	42(20)
Ceftriaxone	57(44.88)	09(18)	05(41.66)	0(0)	0(0)	71(33.80)
MDR	52(40.94)	17(34)	06(50)	06(35)	0(0)	81(38.57)

n^{a}: Number of antibiotic resistant isolates, MDR: Multiple drug resistance.

DISCUSSION

Present study report the isolation of *E. coli, Salmonellae, Proteus spp., Citrobacter spp.* and *Pseudomonas spp.* from fecal samples of dogs suffering from enteritis. *E. coli* were most common isolates from dog enteritis followed by *Salmonellae* and *Citrobacter*, *Proteus* and *Pseudomonas.* Dogs enteritis is caused by many viruses, bacteria and protozoans (Bodewes *et al.,* 2014, Decaro *et al.,* 2014, Okanishi *et al.,* 2013, Sasaki *et al.,* 1999). Among bacteria *E. coli, Salmonellae, Campylobacter*, *Clostridia* are more common cause of dog enteritis (Sasaki *et al.,* 1999, Adesiyun *et al.,* 1997, Schlegel *et al.,* 2012, Giacomelli *et al.,* 2015). Finding of the present study that *E. coli* is most common isolate from the fecal samples of enteritis dogs is in accordance with the findings of DebRoy and Maddox (2001); Starcic *et al.* (2002) but in contrast to the findings of Marks and Kather (2003).

E. coli isolates had high to moderate resistance to ampicillin (56.65%), tetracycline (54.33%), kanamycin (52.75%), gentamycin (49.60%), vibramycin (46.45%), ceftriaxone (44.88%), norfloxacin (30.70%) and ciprofloxacin (25.98%). Similar resistance pattern of *E. coli* have also been reported previously (Minton *et al.,* 1983). Leonard *et al.,* (2012) reported that most of *E. coli* and *Salmonella* (80.4%) isolated from stray dogs in Australia were pan-sensitive. Monaghan *et al.* (1981) reported moderate to high level of antibiotic resistance to different antibiotics in *E. coli*. Pedersen *et al.* (2007) also reported high level of ampicillin, sulphonamides, tetracycline and streptomycin resistance in *E. coli*.

Multiple drug resistant *E. coli* and *Salmonella* were also reported in present study. Multiple drug resistance has been reported in *E. coli* and *Salmonella* of different origins throughout the world (Gronvold *et al.,* 2010; Barton *et al.,* 2003; Warren *et al.,* 2001; Bodewes *et al.,* 2014; Okanishi *et al.,* 2013). Multiple drug resistance in pathogens is a constant threat for public health (Guardabassi *et al.,* 2004). Antibiotic resistance is either intrinsic or acquired. Intrinsic or natural antibiotic resistance such as macrolides resistance in *E. coli* and *Salmonella* is chromosomal encoded and non-transferable to other microbes. Acquired antibiotic resistance is of great importance as it generally is acquired from the environment. Determinants of acquired antibiotic resistance are located on plasmid or chromosome and can be transferred to other bacteria by conjugation, transformation or transduction (Davies and Davies, 2010). Antibiotic resistance in *E. coli* and *Salmonella* reported in the present study is acquired and may be transferred to commensals in gastrointestinal tract of dogs (Huddleston, 2014). People associated with dogs may get infections from antibiotic resistant *E. coli* and *Salmonella* which can act as reservoir of antibiotic resistance (Guardabassi *et al.,* 2004; Johnson *et al.,* 2006).

It is concluded that enteritis in dogs is mostly caused by *E. coli* and Salmonella. Data on occurrence of antimicrobial resistance in *E. coli* and *Salmonella* may provide guidelines for small animal practitioners. MDR *E. coli* and *Salmonella* in dogs are potential hazard for people associated with dogs. It is insinuated that antibiotics should be prescribed carefully and regulatory authorities may initiate epidemidemiological survey of antibiotic resistance at national level.

REFERENCES

Adesiyun, A.A., M. Campbell and J.S. Kaminjolo (1997). Prevalence of bacterial enteropathogens in pet dogs in Trinidad. Zentralbl. Veterinarmed. B., 44: 19-27.

Barton, M.D., R. Pratt and W.S. Hart (2003). Antibiotic resistance in animals. Commun Dis Intell Q Rep. 27 Suppl: S121-126.

Blair, J.M., M.A. Webber, A.J. Baylay, D.O. Ogbolu and L.J. Piddock (2015). Molecular mechanisms of antibiotic resistance. Nat. Rev. Microbiol., 13: 42-51.

Bodewes, R., S. Lapp, K. Hahn, A. Habierski, C. Forster, M. Konig, P. Wohlsein, A.D. Osterhaus and W. Baumgartner (2014). Novel canine bocavirus strain associated with severe enteritis in a dog litter. Vet Microbiol. 174: 1-8.

Castanon, J.I.R. (2007). history of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci., 86: 2466-2471.

Damborg, P., E.M. Broens, B.B. Chomel, S. Guenther, F. Pasmans, J.A. Wagenaar, J.S. Weese, L.H. Wieler, U. Windahl, D. Vanrompay and L. Guardabassi (2015). Bacterial zoonoses transmitted by household pets: state-of-the-art and future perspectives for targeted research and policy actions. J. Comp. Pathol., DOI:10.1016/J.JCPA.2015.03.004.

Davies, J. and D. Davies (2010). Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev., 74: 417-433.

DebRoy, C. and C.W. Maddox (2001). Identification of virulence attributes of gastrointestinal *Escherichia coli* isolates of veterinary significance. Anim. Health Res. Rev., 2: 129-140.

Decaro, N., V. Martella, C. Desario, G. Lanave, E. Circella, A. Cavalli, G. Elia, M. Camero and C. Buonavoglia (2014). Genomic characterization of a circovirus associated with fatal hemorrhagic enteritis in dog, Italy. PLoS One. 9: e105909.

Deng, P. and K.S. Swanson (2015). Gut microbiota of humans, dogs and cats: current knowledge and future opportunities and challenges. Br. J. Nutr., 113(Suppl): S6-17.

Faires, M.C., M. Traverse, K.C. Tater, D.L. Pearl and J.S. Weese (2010). Methicillin-resistant and -susceptible *Staphylococcus aureus* infections in dogs. Emerg. Infect. Dis., 16: 69-75.

Giacomelli, M., N. Follador, L.M. Coppola, M. Martini and A. Piccirillo (2015). Survey of *Campylobacter spp*. in owned and unowned dogs and cats in Northern Italy. Vet. J., 204: 333-337.

Gronvold, A.M., T.M. L'Abee-Lund, H. Sorum, E. Skancke, A.C. Yannarell and R.I. Mackie (2010). Changes in fecal microbiota of healthy dogs administered amoxicillin. FEMS Microbiol. Ecol. 71: 313-326.

Guardabassi, L., S. Schwarz and D.H. Lloyd (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria: Review. J. Antimicrob. Chemother., 54: 321-332.Huddleston, J.R. (2014). Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infect. Drug Resist., 7: 167-176.

Holt, J.G., N.R. Krieg, P.H. Sneath, J.T. Staley and S.T. Williams (1994). Bergeys manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, London, UK.

Johnson, J.K., E.N. Perencevich, D.P. Lincalis and R.A. Venezia (2006). Dog bite transmission of antibiotic-resistant bacteria to a human. Infect. Control Hosp. Epidemiol., 27: 762-763.

Leonard, E.K., D.L. Pearl, R.L. Finley, N. Janecko, R.J. Reid-Smith, A.S. Peregrine and J.S. Weese (2012). Comparison of antimicrobial resistance patterns of *Salmonella spp*. and *Escherichia coli* recovered from pet dogs from volunteer households in Ontario (2005-06). J. Antimicrob. Chemother., 67: 174-181.

Marks, S.L. and E.J. Kather (2003). Antimicrobial susceptibilities of canine *Clostridium difficile* and *Clostridium perfringens* isolates to commonly utilized antimicrobial drugs. Vet. Microbiol., 94: 39-45.

Minton, N.P., J. Marsh and T. Atkinson (1983). The R-factors of multiple antibiotic resistant faecal coliforms isolated from a domestic dog. J Appl Bacteriol. 55: 445-452.

Monaghan, C., U. Tierney and E. Colleran (1981). Antibiotic resistance and R-factors in the fecal coliform flora of urban and rural dogs. Antimicrob. Agents Chemother., 19: 266-270

Murphy, C., R.J. Reid-Smith, J.F. Prescott, B.N. Bonnett, C. Poppe, P. Boerlin, J.S. Weese, N. Janecko and S.A. McEwen (2009). Occurrence of antimicrobial resistant bacteria in healthy dogs and cats presented to private veterinary hospitals in southern Ontario: A preliminary study. Can. Vet. J., 50: 1047-1053.

Okanishi, H., J. Matsumoto, S. Nogami, Y. Kagawa and T. Watari (2013). *Echinostoma hortense* infection with enteritis diagnosed by upper gastrointestinal endoscopy in a dog. J. Vet. Med. Sci., 75: 991-994.

Pedersen, K., H. Jensen, K. Finster, V.F. Jensen and O.E. Heuer (2007). Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. J. Antimicrob. Chemother., 60: 775-781.

Puno-Sarmiento, J., L. Medeiros, C. Chiconi, F. Martins, J. Pelayo, S. Rocha, J. Blanco, M. Blanco, M. Zanutto, R. Kobayashi and G. Nakazato (2013). Detection of diarrheagenic Escherichia coli strains isolated from dogs and cats in Brazil. Vet. Microbiol., 166: 676-680.

Sasaki, J., M. Goryo, M. Asahina, M. Makara, S. Shishido and K. Okada (1999). Hemorrhagic enteritis associated with *Clostridium perfringens* type A in a dog. J. Vet. Med. Sci., 61: 175-177.

Schlegel, B.J., T. Van Dreumel, D. Slavic and J.F. Prescott (2012). *Clostridium perfringens* type A fatal acute hemorrhagic gastroenteritis in a dog. Can. Vet. J., 53: 555-557.

Sidjabat, H.E., K.M. Townsend, N.D. Hanson, J.M. Bell, H.W. Stokes, K.S. Gobius, S.M. Moss and D.J. Trott (2006). Identification of bla(CMY-7) and associated plasmid-mediated resistance genes in multidrug-resistant *Escherichia coli* isolated from dogs at a veterinary teaching hospital in Australia. J. Antimicrob. Chemother., 57: 840-848.

Starcic, M., J.R. Johnson, A.L. Stell, J. Van Der Goot, H.G. Hendriks, C. Van Vorstenbosch, L. Van Dijk and W. Gaastra (2002). Haemolytic *Escherichia coli* isolated from dogs with diarrhea have characteristics of both uropathogenic and necrotoxigenic strains. Vet. Microbiol., 85: 361-377.

Suchodolski, J.S. (2011). Intestinal microbiota of dogs and cats: a bigger world than we thought. Vet. Clin. North. Am. Small Anim. Pract., 41: 261-272.

Trott, D.J., L.J. Filippich, J.C. Bensink, M.T. Downs, S.E. McKenzie, K.M. Townsend, S.M. Moss and J.J. Chin (2004). Canine model for investigating the impact of oral enrofloxacin on commensal coliforms and colonization with multidrug-resistant Escherichia coli. J Med Microbiol. 53: 439-443.

Warren, A., K. Townsend, T. King, S. Moss, D. O'Boyle, R. Yates and D.J. Trott (2001). Multi-drug resistant escherichia coli with extended-spectrum beta-lactamase activity and fluoroquinolone resistance isolated from clinical infections in dogs. Aust Vet J. 79: 621-623.