OCCURRENCE OF ANTIMICROBIAL RESISTANT BACTERIA IN DOGS SUFFERING FROM ENTERITIS

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

1Department of Microbiology, 2University Diagnostic Laboratory, 3Department of Epidemiology and Public Health, 4Department 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 fecal 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%), cephradine (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. Antimicrobial resistant bacteria of zoonotic importance pose substantial threat to public health as well (Damborg et al., 2015).

Antimicrobial 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 °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 (36.5%) 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%). Salmonella were also moderately resistant to ampicillin (42%), followed by gentamycin (38%), kanamycin (30%), tetracycline (28%), cepharadine (20%), ceptriaxone (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 Salmonella 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**

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

n*: Number of antibiotic resistant isolates, MDR: Multiple drug resistance.


**DISCUSSION**

Present study report the isolation of **E. coli**, **Salmonella**, **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 **Salmonella** 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**, **Salmonella**, **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 epidemiological survey of antibiotic resistance at national level.

**REFERENCES**


