SHORT COMMUNICATION

Occurrence of antibiotic resistant Salmonella isolated from dogs in Klang Valley, Malaysia

Mustapha Goni Abatcha¹, Zakaria Zunita¹*, Dhalwal Kaur Gurmeet², and Kwai Lin Thong³

¹Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
²Department of Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
³Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
Email: zunita@vet.upm.edu.my

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Aims: Salmonellosis continues to be a major public health concerned globally. The objective of the study was to determine the occurrence and antimicrobial resistance pattern in Salmonella isolated from non-diarrheic stray and pet dogs in Klang Valley, Malaysia.

Methodology and results: A total of 162 dogs were sampled, 15 (9.3%) were positive for Salmonella (stray dogs, n=12; pet dogs, n=3). All the isolates were identified as Salmonella using conventional culture methods and confirmed by PCR-targeting the invA gene. Four different Salmonella serovars were identified upon serotyping including Salmonella Corvalis (53.3%), S. Typhimurium (13.3%), S. Mbandaka (20%), and S. Agona (6.7%). Salmonella isolates were resistant to tetracycline (86.7%), sulphamethoxazole-trimethoprim (40%), ampicillin (40%), chloramphenicol (33.3%), streptomycin (33.3%), and enrofloxacin (26.7%). None of the isolates was resistant to gentamycin, cephalaxin and amoxicillin-clavulanic acid. Eight isolates (53.3%) were multiple drugs resistant.

Conclusion, significance and impact study: High number of canine Salmonella isolates developed resistance and this may likely be public health concern.

Keywords: Dogs, Salmonella, invA genes, serotyping, antibiotic resistance.

INTRODUCTION

Salmonellosis is one of the most important zoonotic diseases with global distribution and importance (Sanchez et al., 2002). Salmonella is a common inhabitant of the intestinal tracts of a broad range of animal hosts, including mammals, reptiles, birds and even insects. Dogs have been reported to be a carrier of Salmonella spp. worldwide which has the potential to serve as sources of exposure or infection for humans (Filip et al., 2004). Many findings have indicated that Salmonella is uncommonly present in healthy dogs. The prevalence of Salmonella in household pet ranges between 0-2.9% and in stray dogs was 6.3-23.5% respectively (Hackett and Lappin 2003; Lefebvre et al., 2006; Bagcigil et al., 2007). The incidence of salmonellosis and intestinal carriage of Salmonella in pets and stray dogs is of important public health concern worldwide. There have been many reports on Salmonella shedding and transmission to humans (Sato et al., 2000; Cherry et al., 2004; Wright et al., 2005).

The increasing incidence of antibiotic resistant bacteria is also an important heath concern internationally, and animals are potential reservoirs for many resistant bacteria (Boerlin and Reid-Smith, 2008). Recent studies showed that close contact between humans and animals can lead to the exchange of pathogenic bacteria, including those carrying antibiotic resistant genes (Johnson et al., 2006). Multiple drug resistant strains of Salmonella serovars have being isolated from dogs (Guardabassi et al., 2004; Umber and Bender, 2009). These Salmonella serovars include Albany, Anatum, Havana, London and Typhimurium (Van et al., 2007). Resistance towards the first line of antibiotics such as ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole are of concern because of the potential transfer to humans, limiting the options for chemotherapy in invasive salmonellosis (Crump and Mintz, 2010). The aim of the study was to determine the occurrence of antibiotic resistance in Salmonella in dogs in Klang Valley, Malaysia.

*Corresponding author
MATERIALS AND METHODS

Source of samples

A total of 162 rectal swab samples from dogs of different age, sex, and breed were taken. Rectal swabs were collected from 75 pet dogs at a Veterinary Hospital, Universiti Putra Malaysia located in Selangor city and for 85 stray dogs were collected from a municipal animal shelter located Kuala Lumpur, Malaysia. The swabs were transported in Cary Blair transport medium to the laboratory within 24 h of collection.

Isolation and identification of Salmonella

The isolation of Salmonella from rectal was performed after selective enrichment in Rappaport-Vassiliadis-Soy peptone (Oxoid, UK) broth and incubated at 37 °C for 24 h. A loopful of enriched broth was streaked on Xylose-Lysine deoxycholate (XLD) and Brilliant green agar (BGA) (Oxoid, UK) agar plates and incubated at 37 °C for 24 h. All presumptive Salmonella colonies were subcultured onto nutrient agar (Oxoid, UK) at 37 °C for 24 h, and further confirmed by biochemical tests as recommended by the guidelines of the ISO 6579 (2002). These biochemical tests included the Triple Sugar Iron (TSI), Sulfide Indole Motility (SIM), Simmons citrate, and Urease test reactions. The Slide agglutination test as done on presumptive Salmonella isolates using a Salmonella polyvalent O antisera (Gp A-S) test (Remel Europe, UK) to identify the organism as Salmonella spp. The Salmonella serotypes were determined using the Kauffmann-White classification scheme using a battery of somatic and flagellar antisera (OIE Terrestrial Manual, 2008). The serotyping was done at the Salmonella Reference Centre at Veterinary Research Institute (VRI) Ipoh, Malaysia.

Polymerase chain reaction for Salmonella confirmation

DNA template for PCR was prepared by direct crude boiled cell lysate. The Salmonella genus specific primers, invA gene were employed to confirm the identity of the isolates (Rahn et al., 1992). The sequences for the primers are; Forward (5’-3’): GTG AAA TTA TCG CCA CGT TCG GGC AA and Reversed (5’-3’): TCA TCG CAC CGT CAA AGG AAC C. Amplification for the PCR was performed in 50 µL reaction volumes containing template (DNA) 5 µL; Top taq master mix 25 µL (Qiagen); 1x coral load 5 µL (Qiagen); invA primer forward and reverse 1 µL each and RNAse free water 13 µL (Qiagen). The reaction was conducted in Thermal cycler (Eppendorf) under the following cycling condition: An initial incubation 94 °C for 60 s, followed by 35 cycles of denaturation at 94 °C for 60 sec. annealing at 55 °C for 30 sec and elongation at 72 °C for 45 sec, followed by 7 min final extension period. The amplified DNA products were electrophoresed on 1.5% agarose gel for 45 min at 100 voltages. Salmonella Typhimurium ATCC 14028 use as the positive control while deionised water was used as the negative control. Then the gels stained with ethidium bromide and visualized by UV illumination. The DNA ladder used is 100 bp as a marker for products of the PCR.

Antibiotic susceptibility test

Susceptibility to antimicrobial agents was tested using the Kirby-bauer disk diffusion method on Muller-Hinton agar with commercial antibiotic disks (Oxoid Ltd, Basingstoke, UK) as recommended by Clinical and laboratory Standard Institute (CLSI, 2009). A total of 12 antimicrobials used included tetracycline (30 µL), streptomycin (25 µg), amoxicillin-clavulanic acid (30 µg), kanamycin (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), sulphamethoxazole/trimethoprim (25 µg), gentamicin (10 µg), neomycin (10 µg), cephalaxin (30 µg), cephalothin (30 µg), enrofloxacin (5 µg).

RESULTS AND DISCUSSION

Fifteen dogs (9.3%) were tested positive for the presence of Salmonella. All presumptive Salmonella isolates contained invA gene by producing the 284 bp amplicon (Figure 1) confirming the identity of isolates as Salmonella. Of these Salmonella-positive dogs, 12 (14/87, 13.8%) were from stray dogs and 3 (5/75, 4.0%) were from pet household dogs. Upon serotyping, 4 serovars were identified including Salmonella Corvallis (53.3%), Salmonella Typhimurium (13.3%), Salmonella Mbandka (20%), and Salmonella Agona (6.7%). The remaining 6.7% of the isolates were untypable using the available antisera and regarded as Salmonella spp. (Table 1). Out of 15 Salmonella isolates, one was susceptible to all 12 antimicrobials tested, while the other 14 (66.7%) were resistant to at least one antimicrobial. Higher resistance rates were observed for tetracycline (86.7%) followed by sulphamethoxazole-trimethoprim (40%), ampicillin (40%), chloramphenicol (33.3%), streptomycin (33.3%), and enrofloxacin (26.7%). None of the isolates was resistant to gentamicin, cephalaxin and amoxicillin-clavulanic acid (Table 2). Eight isolates exhibited multiple drug resistance (resistant to more than 3 classes of antibiotics) (Table 2).

The Multiple antibiotic resistant (MAR) Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was tested (Krumperman, 1985). The MAR indexes of the isolates were calculated and noted in Table 2.

Salmonellosis constitutes a major public health burden and represents a significant cost to society in many countries. In this study, the overall incidence of Salmonella detected in dogs was 9.3%. The rate of Salmonella isolation is less from that reported in Thailand (12.4%) (Arunee et al., 2012), USA (20.8%) (Frye and Fedorka-Cray, 2007) but higher than that of Taiwan
Figure 1: PCR amplification of invA (284 bp) genes. Lane MC, Molecular ladder 100 bp, Lane PC, Positive control Salmonella Typhimurium ATCC 14028, Lane SD3, SD4, SD6, SD37, SD47, SD48, SD72, D38, D54 samples and lastly lane NC: Negative control.

Table 1: Antimicrobial resistance of Salmonella isolates from dogs.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (AMP) 10 μg</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Chloramphenicol (C) 30 μg</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Gentamycin (CN) 120 μg</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Kanamycin (K) 30 μg</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>Streptomycin (S) 25 μg</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Neomycin (N) 10 μg</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>Cephalaxin (CL) 30 μg</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cephalexin (KF) 30 μg</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>Enrofloxacin (ENR) 5 μg</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Sulphamethazole-TriM (SxT) 25 μg</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Tetracycline (TE) 30 μg</td>
<td>13 (86.7)</td>
</tr>
<tr>
<td>Amoxicillin-Clavulanic acid (AMC) 30 μg</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 2: Antibiotic profile of different Salmonella serovars isolated from stray and pet dogs.

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Serovars</th>
<th>Antimicrobial resistance pattern</th>
<th>MAR index</th>
</tr>
</thead>
<tbody>
<tr>
<td>*D54</td>
<td>Agona</td>
<td>C, K, N, ENR, S, AMP, SXT</td>
<td>0.6</td>
</tr>
<tr>
<td>*SD6</td>
<td>Corvallis</td>
<td>C, KF, AMP, SXT, TE</td>
<td>0.4</td>
</tr>
<tr>
<td>*SD47</td>
<td>Corvallis</td>
<td>C, KF, AMP, SXT, TE</td>
<td>0.4</td>
</tr>
<tr>
<td>*SD61</td>
<td>Typhimurium</td>
<td>C, KF, AMP, SXT, TE</td>
<td>0.4</td>
</tr>
<tr>
<td>*SD68</td>
<td>Salmonella enterica</td>
<td>C, K, N, AMP, SXT, TE</td>
<td>0.4</td>
</tr>
<tr>
<td>*SD63</td>
<td>Mbandaka</td>
<td>AMP, SXT, TE</td>
<td>0.3</td>
</tr>
<tr>
<td>*SD 37</td>
<td>Corvallis</td>
<td>ENR, S, TE</td>
<td>0.2</td>
</tr>
<tr>
<td>*SD72</td>
<td>Corvallis</td>
<td>ENR, S, TE</td>
<td>0.2</td>
</tr>
<tr>
<td>SD3</td>
<td>Corvallis</td>
<td>S, TE</td>
<td>-</td>
</tr>
<tr>
<td>D38</td>
<td>Mbandaka</td>
<td>S, TE</td>
<td>-</td>
</tr>
<tr>
<td>SD4</td>
<td>Corvallis</td>
<td>TE</td>
<td>-</td>
</tr>
<tr>
<td>SD64</td>
<td>Corvallis</td>
<td>TE</td>
<td>-</td>
</tr>
<tr>
<td>SD48</td>
<td>Corvallis</td>
<td>TE</td>
<td>-</td>
</tr>
<tr>
<td>SD57</td>
<td>Typhimurium</td>
<td>TE</td>
<td>-</td>
</tr>
</tbody>
</table>

*MDR SD, Stray dog; D, Pet household dog
(4.3%) (Tsai et al., 2007), and Trinidad (3.6%) (Seepearsadsingh et al., 2004).

The prevalence of Salmonella varied among different countries and this might be attributed to the sample size of dogs, sample origin, type of faecal sample, and isolation methods used in the different countries. Our study shows that stray dogs had higher Salmonella positive rate than household dogs. The diversity of diet source, free movement and environment of stray dogs apparently determine the number and occurrence of Salmonella serovars (Finley et al., 2007). All the serovars identified in this study have previously been found in humans, animals and food products (Ammari et al., 2009; Le Bouquin et al., 2010; Thong and Moderni, 2011).

The serovars were S. Corvallis, S. Mbandaka, S. Typhimurium, S. Agona and S. enterica. This shows that Salmonella found in dogs may be related to human Salmonellosis. In Malaysia, S. Typhimurium, S. Corvallis and S. Agona are frequently incriminated in human illness (MOH, 2005), and Salmonella Mbandaka has been isolated in humans in Denmark (Torpdahl et al., 2009).

In this study, the invasion gene, invA was detected in all Salmonella isolated. The invA gene of Salmonella contains sequence unique to this genus and confirms the usefulness of this target for PCR confirmation of Salmonella (Rahn et al., 1992; Jamshidi et al., 2008). This finding closely agrees with other studies which reported the detection of this gene in almost all Salmonella isolates (Shanmugasamy et al., 2011; Tafida et al., 2013).

In the past decade, the emergence of antimicrobial-resistant Salmonella has become a major public health problem. The present study demonstrated that 66.7% of the Salmonella isolates exhibited resistance to at least more than one antibiotic. Also a higher number of multidrug resistances were found among the Salmonella, being resistance to 3 or more groups of antimicrobial agents. Higher resistances rates are in tetracycline (86.7%) were found among the Salmonella. This finding was higher than those from Thailand 43.5% and Taiwan 38.8% of the Salmonella strains in dogs are resistant to tetracycline (Chang et al., 2011; Arunee et al., 2012). This probably due to drug being widely use in human and veterinary medicine and in animal feed as a growth promoter and additive supplement (Cardoso et al., 2006). Notably high levels of resistance to sulphonamethazole-trimetrophrom (40%), ampicillin (40%), chloramphenicol (33.3%) and streptomycin (33.3%) were observed. These findings concurred with previous reports that Salmonella strains from dogs were resistant to multiple antimicrobials, including tetracycline, sulphonamides and streptomycine (Leonard et al., 2012).

The most commonly observed MDR Salmonella serovars were S. Agona (D54), S. Corvallis (SD6, SD47), S. Typhimurium (SD61), S. enterica (SD68) and S. Mbandaka (63) (Table 2). However, continuous monitoring revealed the isolation frequency of MDR Salmonella in general sources is on increased worldwide (Frye and Fedorka-Cray, 2007). The MAR Index analysis reveals that 6 isolates had a very high MAR index value (> 0.2). Bacteria having MAR Index > 0.2 originate from an environment where several antibiotics are used (Tambekar et al., 2006). The broader range of MAR index observed from the Salmonella isolated from dogs might be due to antimicrobial use in veterinary treatment or feeds additive. Likewise, none of Salmonella in this study is resistant to gentamycin, cepahlexin, and amoxicillin-clavulanic acid. Finally, findings of the present study ascertain that Salmonella serovars in dogs have developed resistance for routinely prescribed antimicrobial drugs and pose considerable health risk to the public.

CONCLUSION

In conclusion, this study confirmed that the stray and pet dogs might act as reservoirs for Salmonella thereby serving as a source of human infection and a potential threat to public health. The occurrence of multiple antibiotic resistances in the Salmonella is worrisome as it could pose a risk to animal and human health. This finding provides a starting point for investigating the impact of antimicrobial resistance in Malaysian dogs.

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