RESEARCH ARTICLE

Isolation and Molecular Characterization of Extended Spectrum Beta-lactamase (ESBL) Producing Escherichia Coli from Dogs

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ABSTRACT

The present study was aimed to isolate and characterize the Extended Spectrum Beta-lactamase (ESBL) producing Escherichia coli from faecal samples of dogs in Puducherry, India. In this study, 100 E. coli isolates were obtained from 50 healthy dogs and 50 diarrheic dogs, out of which 33 (33%) isolates were confirmed as ESBL producers by the combination disc method. All the E. coli isolates were genotypically confirmed for the presence of genes responsible for ESBL production. Out of 100 E. coli isolates, fourteen (14%), seven (7%) and three (3%) isolates were found to be positive for blaTEM, SHV and CTX-M genes, respectively. The present study highlighted ESBL-producing E. coli in dogs in Puducherry region.

Keywords: Antimicrobial resistance, E. coli, ESBL, Dogs, Genotypic confirmation Public health.

INTRODUCTION

Antibiotics are the ‘wonder drugs’ to combat various infectious diseases caused by microbes. For decades, multiple varieties of antibiotics have not only been used for therapeutic purposes but practiced prophylactically across industries like agriculture and animal husbandry. Uncertainty has arisen, as microbes have become resistant to common antibiotics while the host remains unaware that antibiotic resistance has emerged (Zaman et al., 2017).

The resistance mechanism developed by several bacteria, especially Enterobacteriaceae, in E. coli is the production of extended spectrum beta-lactamase (ESBLs) enzyme, which could hydrolyse beta-lactam group of antibiotics such as penicillin, cephalosporin, cefotaxime, ceftazidime, aztreonam and related oxyimino beta lactam (Bush et al., 1995). The production of CTX-M mediates this resistance, TEM and SHV β-lactamases encoded by blaCTX-M, blaSHV, and blaTEM genes, respectively. These β-lactamase (bla) gene can be plasmid-mediated or expressed chromosomally (Bush and Jacoby 2010). ESBL-producing Enterobacteriaceae in dogs was first reported in Western Europe in the mid-1980s. (Knothe et al., 1983) Since then, their incidence has been increasing steadily and has become a worldwide problem (Giraud et al., 2003).

Reduced bacterial susceptibility to advanced antimicrobials may further enhance the uncontrolled spread of these resistant pathogens at the animal–environment–human interface. (Prestinaci et al., 2015) One of the driving forces behind increased beta lactam resistance among E. coli is the use of third and fourth-generation cephalosporins.

Furthermore, the conjugative nature of E. coli facilitates the transfer of beta-lactamase genes (blaCTX-M, blaSHV, and blaTEM genes) from resistant donors to susceptible bacteria (Eiamphungporn et al., 2018). The ESBL-producing E. coli isolates from companion animals is increasing and leading to alarming that the cross species spread of these
resistant bacteria could be of great public health significance (Albrechtova et al., 2012).

Although there are reports of phenotypic and genotypic detection of ESBL-producing E. coli in farm animals (Tewari et al., 2019) and poultry (Samanta et al., 2015) from India, the study on prevalence of ESBL producing E. coli in dogs is scanty in India. Considering which, the present study was undertaken with the objective of isolation and molecular characterization of ESBL producing E. coli from faecal samples of healthy dogs as well as diarrheic dogs samples.

**Materials and Methods**

**Collection and Processing of Samples**

A total of number 100 faecal samples (50 healthy and 50 diarrheic) were collected at the Veterinary Clinical Complex, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry. The samples were collected in sterile cotton swab individually packed in polypropylene tube (M/s Hi-Media, Mumbai) and were transported to Department of Veterinary Microbiology Laboratory, RIVER, Puducherry within 2 hours and immediately processed for culture and isolation.

**Isolation and Identification of E. coli**

Each fecal sample was inoculated into Luria broth individually in sterile test tubes and incubated for 18 h at 37°C. A loopful of the enriched culture was streaked onto Mac Conkey’s agar and incubated at 37°C for 24 h. Upon incubation, the pink lactose fermenting colonies were subjected to Gram’s staining. The isolated gram-negative bacteria were identified upto species level as E. coli based on the morphology, cultural characters and biochemical reactions described in Bergey’s Manual of Systematic Bacteriology (1984).

The E. coli isolates were further subjected to a polymerase chain reaction (PCR) using the primers targeting alr gene for genotypic confirmation (Yokoigawa et al., 1999). The PCR amplification was carried out in an automated thermal cycler (Eppendorf Mastercycler, Germany) with primers targeting blaTEM and blaSHV according to the following programme, amplification of blaCTX-M was carried out using the following primers and programmes:

- Initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min and final extension at 72°C for 5 min as per Bhattacharjee et al., 2007. The PCR amplification was carried out in an automated thermal cycler (Eppendorf Mastercycler, Germany) with primers targeting blaTEM and blaSHV according to the following programme, Initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min and final extension at 72°C for 5 min as per Bhattacharjee et al., 2007. The PCR amplification of blaCTX-M was carried out using the following programmes:
  - Initial denaturation at 95°C for 5 min followed by 5 cycles of denaturation at 95°C for 30 s,
  - annealing at 65°C for 30 s, extension at 72°C for 30 s, 10 cycles of denaturation at 95°C for 30 s,

<table>
<thead>
<tr>
<th>S. No</th>
<th>Primer name</th>
<th>Sequences</th>
<th>Amplicon size (bp)</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>alr gene</td>
<td>FS’CTGGAAGGCTAGCCTGGACGAG3’</td>
<td>366</td>
<td>(Yokoigawa et al., 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS’AAAATCGGCCAGGTTGAGACGT3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TEM</td>
<td>FS’ ATGAGTATTCAACATTTCCG 3’</td>
<td>867</td>
<td>(Bhattacharjee et al., 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS’CTGACAGTACCAATGCTTA 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>SHV</td>
<td>FS’AGGATTGACTGGCTTTTGG 3’</td>
<td>393</td>
<td>(Bhattacharjee et al., 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS’ATTTGCTGTATTTCGCTG 3’</td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>CTX-M</td>
<td>FS’CAATGGTGACGACAAAGTAA 3’</td>
<td>540</td>
<td>(Dutta et al., 2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS’CGCCGATATCGTGGTG 3’</td>
<td></td>
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</tbody>
</table>

References

- Albrechtova et al., 2012
- Yokoigawa et al., 1999
- Zhang et al., 2015
- CLSI, 2019
- Bhattacharjee et al., 2007
- Dutta et al., 2013
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**Results and Discussion**

In the present study, among 100 canine faecal swabs (50 healthy and 50 diarrheic) examined, a total of 100 *E. coli* isolates were obtained, in using cultural and biochemical characterization followed by genotypic confirmation by PCR using alr gene specific for *E. coli* (366 bp) (Fig. 1).

In this study, a total of 33 (33%) *E. coli* isolates were confirmed as ESBL producers by combination disc method (Fig. 2). Of the 33 isolates, 16% (8/50) of the isolates were from the healthy dogs and 50% (25/50) isolates from the diarrheic dogs.

A similar frequency of occurrence of ESBL producing *E. coli* in dogs ranging from 1 to 33.3%, has been reported previously from various countries such as United States (O’Keefe et al., 2010), Europe (Ewers et al., 2010), Korea (So et al., 2012), Netherland (Hordijk et al., 2013), Switzerland (Huber et al., 2013), Denmark (Dierikx et al., 2013), Germany (Schmiedel et al., 2014) Similarly, Tamang et al. (2012), Mandakini et al. (2015) and Gundran et al. (2019) have also reported ESBL production in *E. coli* isolates from dogs ranging from 15.75 to 44.23 % from Korea, India and the Philippines.

The genotypic method helps confirm genes’ presence in ESBL production in *E. coli* isolates. The isolates were screened for the presence of TEM, SHV, CTX-M or other bla genes by PCR amplification (Falagas and Karageorgopoulos, 2009). By Polymerase chain reaction, out of 100 *E. coli* isolates, fourteen (14%), seven (7%) and three (3%) isolates were found positive for bla TEM, SHV and CTX-M genes, respectively, by PCR (Fig. 3 to Fig. 5). Of the 14 TEM positive isolates, 16% (8/50) were from the healthy dogs and 12% (6/50) from the dogs with diarrhea.

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**Fig 1: Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 366 bp for the alr gene of *E. coli* isolates.**

Lane 1: Negative control; Lane 2,3,4,5&6: Field isolates and positive control for alr gene of *E. coli* respectively; Lane 7: 100 bp ladder.

**Fig 2: Phenotypic confirmation of ESBL production of *E. coli* isolate**

- annealing at 62°C for 30 s, extension at 72°C for 30 s, 15 cycles of denaturation at 95°C for 30 s,
- annealing at 60°C for 30 s, extension at 72°C for 30 s, 15 cycles of denaturation at 95°C for 30 s,
- annealing at 58°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 7 min as per Dutta et al., 2013 and the primers details were described in Table 1. The PCR products were analyzed by agarose gel electrophoresis.
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Similarly, of the 3 CTX-M positive isolates, 2% (1/50) were from healthy dogs and 4% (2/50) from dogs with diarrhea and all the 7 (14%) SHV positive isolates were from healthy dogs. In this study, the most common ESBL genotype among E. coli isolates was found to be SHV and TEM.

Whereas the presence of TEM, SHV and CTX-M genes in ESBL-positive E. coli isolates at different ranges has been reported by several studies from different countries such as O’Keefe et al. (2010) detected one (9.09%) and 10 (90.09%) of 11 ESBL positive isolates harbored SHV and CTX-M respectively in isolates from dogs and cats associated with urinary tract infection in the United States. Similarly, Mandakini et al. (2015) detected TEM (6.47%) and CTX-M (2.94%) genes in isolates from faecal samples of diarrhoeic piglets in India.

Tamang et al. (2012) reported 1.91% (12/628) of the isolates confirmed phenotypically as ESBL producer harbored CTX-M gene in stray dogs in South Korea. Das et al. (2017) reported 36% and 12% isolates harbored blaCTX-M and blaTEM genes, respectively and none of the isolates carried blaSHV genes from subclinical mastitis in cattle in West Bengal. However,
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Resistance might probably result from indiscriminate use of third generation cephalosporins in canine practice, which may lead to therapeutic failures in treating the infections caused by E. coli.

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Reference

Rawat et al. (2018) reported a much higher prevalence of the TEM and SHV of 20% and 31.6% of the E. coli isolates from faecal samples of dairy cattle from Chhattisgarh, India.

In the present study, higher rates of positivity of ESBL production in E. coli confirmed by phenotypic methods in comparison with PCR might be because other resistance genes of ESBL production were not targeted in the present study. IMP, VIM, OXA, NDM, KPC, CMY, etc.

The difference in prevalence and occurrence of different genes between different studies can have several explanations. These include geographic differences, selection of animals, methods used for sampling, antibiotic usage and bacterial isolation (such as enrichment procedures and the media used).

This is the first study to describe the carriage of ESBL-producing E. coli in fecal samples of dogs in Puducherry. Hence such dogs harboring ESBL-producing E. coli may pose a threat to humans.

Conclusions
It can be concluded that a high prevalence 33% of ESBL-producing E. coli isolates in faecal samples of dogs in Puducherry. The most common ESBL genotype among E. coli isolates was SHV and TEM. The incidence of beta-lactamase resistance might probably result from indiscriminate use of third generation cephalosporins in canine practice, which may lead to therapeutic failures in treating the infections caused by E. coli.
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