Indian Journal of Animal Sciences **89**(7): 732–734, July 2019/Short communication
https://doi.org/10.56093/ijans.v89i7.92016

Characterisation of extended spectrum β-lactamase among *Escherichia coli* and *Klebsiella pneumoniae* associated with skin and urogenital tract infections in dog

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Received: 8 August 2018; Accepted: 29 November 2018

Key words: bla_{TEM}, bla_{CTX-M}, bla_{SHV}, *Escherichia coli,* Extended spectrum beta-lactamase, *Klebsiella pneumoniae*

Treatment of infections caused by Gram-negative bacilli is becoming increasingly difficult because of antibiotic resistance. Various mechanisms produce drug resistance. One such mechanism is the production of extended spectrum beta-lactamase (ESBL) enzymes by these bacteria. Extended spectrum beta-lactamases are plasmid mediated beta-lactamases that provide resistance to extended spectrum cephalosporins and monobactams as well as to older penicillins and cephalosporins, and production of ESBL is one of the significant features of *Escherichia coli* and *Klebsiella pneumoniae*. The number of pet animals are increasing in clinical practice and companion animals especially dogs, pose a potential source of ESBL (Zogg *et al*. 2018), either through transmission of resistant pathogens from animals to humans, or via transmission of resistance genes to sensitive bacterial species. The most common ESBL encoding genes isolated from clinical specimens are TEM, SHV and CTX-M (Paterson and Bonomo 2005, Bora *et al*. 2017). The occurrence of ESBL producing strains has emerged as a major challenge in hospitalized as well as community based patients. Since ESBL confers resistance to all third generation cephalosporins which are the most predominantly used antibiotics in several hospitals, such resistance can often lead to treatment failures. Hence the detection of these strains is of great significance in selecting an adequate regime for therapy. In addition, it could create an awareness to reduce the indiscriminate use of antibiotics and further a comprehensive date for surveillance studies.

The present research was envisaged to analyze the antibiogram of *E. coli* and *K. pneumoniae* isolated from dermatological and urogenital tract infections in dogs followed by phenotypic confirmation of the isolates for ESBL production and subsequent characterisation of ESBL encoding genes. This will give an overview of the current situation regarding the presence of ESBL in *E. coli* and *K. pneumoniae* associated with major clinical infections in dogs thereby facilitating an effective treatment schedule for the management of such infection.

Samples collected were urine, swabs from lesional skin and anterior vagina, from 75 dogs brought to the hospitals of College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. Brain heart infusion agar (BHIA), MacConkey agar (MAC) and eosin methylene blue agar were used for the isolation of bacteria from clinical samples. The isolates were identified based on cultural, morphological and biochemical characterisation (Quinn *et al*. 1994).

Kirby-Bauer disk diffusion method was performed on Mueller-Hinton agar (MHA) plates to determine the susceptibilities of different beta-lactam and non-beta-lactam antibiotics. Animicrobial discs used were amikacin (30 µg), amoxycillin-clavulanic acid (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), co-trimoxazole (30 µg), ceftazidime (30 µg), ciprofloxacin (30 µg), cefepime (30 µg), cefoxitin (30 µg), gentamicin (30 µg), piperacillin/tazobactam (30 µg).

As per CLSI (2011), isolates showing inhibition zone size of ≤ 25 mm with ceftriaxone, ≤27 mm with cefotaxime, ≤22 mm with ceftazidime, ≤17 mm with cefpodoxime and ≤25 mm with cefepime were considered to be potential ESBL producers.

For confirmation of ESBL producers, combination disc diffusion method was used which employed the use of a third-generation cephalosporin antibiotic disc alone, and in combination with clavulanic acid. In this study, disc of ceftazidime (30 µg) and ceftazidime + clavulanic acid (30 µg/10 µg) and disc of cefotaxime (30 µg) and cefotaxime (30 µg) + clavulanic acid were used (CLSI 2011).

All the isolates found positive in the initial screening

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test for ESBL production were examined for the presence of bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ genes by polymerase chain reaction (PCR) assay with specific primers (Table 1). The colonies of the isolates were dispensed in 2 ml of sterile PBS (pH 7.2) and incubated at 37°C for 6 h. Deoxyribonucleic acid was extracted using the Genomic DNA Purification Kit of Origin (Kerala, India) as per manufacturer's instructions. The PCR conditions were optimized by using different concentrations of $MgCl_2$ (1.5 mM, 1.8 mM and 2.0 mM) and by setting different time temperature combinations for annealing and extension. The combination that gave the best result for amplification was selected for carrying out further PCR. A master mix was prepared just before setting up the PCR assay by combining 10× PCR buffer containing $MgCl_2$, dNTP mix, primer pairs, Taq DNA polymerase and sterile nuclease free water in a total volume of 12.5 µl in 200 µl reaction tube. To each reaction tube, 10 µl of master mix and 2.5 µl of template DNA were added. The PCR amplification for individual genes was carried out in a thermal cycler (Eppendorf).

Extended spectrum beta lactamases have become a widespread problem and these enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a great potential for dissemination and animals acts as a major source in this regard. The presence of ESBL in bacteria compromises the activity of antibiotics and creates severe therapeutic difficulties, and is also posing diagnostic challenges to the clinical microbiology laboratories.

In the present study, out of 75 samples collected from dogs, 20 were positive for *E. coli* and 5 for *K. pneumoniae*, based on colony characters, morphology on Gram staining and biochemical characters. The other organisms isolated were *Staphylococcus* spp. (37) and *Pseudomonas* spp. (10) respectively. Similar results were obtained by Bassessar *et al.* (2013), Shakya *et al.* (2017) and Shetty (2017) from skin lesions, urinary tract infections and pyometra, respectively.

Multidrug resistance was observed in all the *E. coli* and *K. pneumoniae* isolates against amikacin, cefotaxime, ceftazidime, ceftriaxone and co-trimoxazole, and all were found sensitive to piperacillin-tazobactam combination.

Table 1. Sequences of the primers used for polymerase chain reaction

Primer	Primer sequence (5'-3' direction)	Product size	References
bla_{SHV} F	GGGTTATTCTTA TTTGTCGCT	929 bp	Bora *et al.* (2017)
bla_{SHV} R	TAGCGTTGCCAGTGCTCG		
bla_{CTX} F	TTTGCGATGTGC AGTACCAGTAA	544 bp	
bla_{CTX} R	CGATATCGTTGGT GGTGCCATA		
bla_{TEM} F	AAAATTCTTGAAGACG	1080 bp	
bla_{TEM} R	TTACCAATGCTTAATCA		

Resistance pattern shown by the isolates were similar as reported by Nisha *et al.* (2015), where a higher degree of resistance was observed among Gram negative bacteria when compared with that of Gram positive organisms. This might be attributed to the presence of plasmids harbouring antibiotic resistance genes, which might have conferred the resistance.

On screening test for ESBL, 19 *E. coli* isolates and 5 *K. pneumoniae* isolates showed resistance to third generation cephalosporins like ceftriaxone, ceftazidime, cefpodoxime and cefotaxime. On conducting combination disc diffusion method for extended spectrum beta lactamase production, 18 *E. coli* and all the 5 *K. pneumoniae* isolates showed positive results.

For characterizing the genes encoding for ESBL among the isolates obtained, the extracted DNA was subjected to PCR targeting the $bla_{TEM,}$ $bla_{CTX\text{-}M}$ and bla_{SHV} genes. In this study, out of 20 *E. coli*, 66% were positive for bla_{TEM}, 38.8% for $bla_{CTX\text{-}M}$ and 33.3% for bla_{SHV} genes whereas, among *K. pneumoniae*, 60% were positive for bla_{TEM}, 80% for $bla_{CTX\text{-}M}$ and 60% for bla_{SHV}. Only 3 out of the total 25 isolates were devoid of these 3 genes. All the isolates that were detected positive in phenotypic confirmation test showed positive results for the presence of SHV, CTX-M or TEM genes in PCR. The PCR positive amlpicons of TEM, CTX-M and SHV genes of *E. coli* and *K. pneumoniae* were confirmed by nucleotide sequencing. The sequences obtained from the current study showed similarity with reference sequences available in GenBank. The nucleotide sequences obtained from the present study were submitted to GenBank and the accession numbers obtained were MH684528, MH684529, MH684530, MH684531, MH684532 and MH684533.

Results from the SENTRY Asia-Pacific Surveillance Program of 9 countries reported 5.9% *E. coli* and 17.2% *K. pneumoniae* as the ESBL producers (Bell *et al*. 2007). A recent survey from 31 countries reported the prevalence of ESBL producers among *E. coli* and *K. pneumoniae* as low as 1.5% in Germany and as high as 39–47% in Russia, Poland and Turkey (Goosens 2001). Studies from India reported varied prevalence of ESBL producers ranging from 6 to 87% (Agrawal *et al.* 2008). A study conducted in a rural medical college hospital in North Kerala, documented the prevalence of ESBL as 68% among *E. coli* and 20% in *K. pneumoniae* isolates (Ahmed *et al*. 2014).

Current therapy for strains of Enterobacteriacece that express ESBL, is limited to carbapenem (Chaudhary and Aggrawal 2004). Carbapenems are expensive drugs, requires prolonged intravenous administration and have potential side effects. Again, the circumstances have changed and growing carbapenem resistance is a major clinical concern.

Thus, ESBL producing organisms pose a major problem for clinical therapeutics. Because of the close contact between dogs and man, and aimless utilization of antibiotics especially cephalosporins in veterinary sector, these animals have turned into potential source of ESBL producing

bacteria, thereby prompting the transfer of multidrug resistant strains in human. So far no studies have been conducted regarding the prevalence of ESBL among animals in Kerala. Because of the alarming rise in the number of these resistant strains, there is a urgent need for regular area based monitoring of the antibiogram profile of the various bacterial organisms associated with clinical infections in animals which could fluctuate throughout the years and from site to site. This could provide adequate data prompting the authorities to formulate strict antibiotic usage guidelines in veterinary and human sector.

REFERENCES

Agrawal P, Ghosh A N, Kumar S, Basu B and Kapila K. 2008. Prevalence of ESBL among *E. coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital. *Indian Journal of Pathology and Microbiology* **51**(1): 139–42.

Ahmed S M, Rajeevan S, Jasmin P T and Shakir V P. 2014. Detection of ESBL among the Gram negative uropathogens and their antibiotic resistance pattern in a rural medical college hospital North Kerala, India. *International Journal of Current Microbiology and Applied Sciences* **3**: 561–67.

Bassessar V, Verma Y and Swami M. 2013. Antibiogram of bacterial species isolated from canine pyometra. *Veterinary World* **6**: 546–49.

Bell J M, Chitsaz M, Turnidge J D, Barton M, Walters L J and Jones R N. 2007. Prevalence and significance of a negative ESBL confirmation test result after a positive ESBL screening test result for isolates of *E. coli* and *Klebsiella pneumoniae*: Results from the SENTRY Asia-Pacific Surveillance program. *Journal of Clinical Microbiology* **45**(5): 1478–82.

Bora A, Hazarika K N, Shukla K S, Prasad N K, Sarma B J and Ahmed G. 2017. Prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. *Indian Journal of Pathology Microbiol*ogy **57**: 249–59.

Chaudhary U and Aggrawal R. 2004. Extended spectrum lactamases (ESBL)—An emerging threat to clinical therapeutics. *Indian Journal of Medical Microbiology* **22**(2): 75–80.

Clinical and Laboratory Standard Institute. 2011. *Performance Standards for Antimicrobial Susceptibility Testing*. 21st International Supplement M100-S21. CLSI, Wayne, PA, USA.

Goosens H. 2001. Summary of European data from 1997 to 2000. *Diagnostic Microbiology Infectious Diseases* **41**: 183–89.

Nisha V K, Shenoy D R, Shetty V A, Shinoy M V and Shetty K A. 2015. Trends in antimicrobial resistance among uropathogens with special reference to *Escherichia coli* in community acquired pediatric urinary tract infections from Kerala. *Journal of Evolution of Medical and Dental Sciences* **4**: 9313–20.

Paterson L D and Bonomo A R. 2005. Extended-spectrum beta-lactamases: a clinical update. *Clinical Microbiology Reviews* **18**: 657–86.

Shakya P, Shrestha D, Maharjan E, Sharma V K and Paudyal R. 2017. ESBL production among *E. coli* and *Klebsiella* spp. causing urinary tract infection: A hospital base study. *Open Microbiology Journal* **11**: 23–30.

Shetty J. 2017. Skin and soft tissue infections associated with methicillin resistant *Staphylococcus aureus*, ESBL, Amp C and metallo β-Lactamase producing bacilli in a tertiary care hospital. *Journal of Dental and Medical Sciences* **16**: 08–14.

Zogg L A, Simmen S, Zurfluh K, Stephan R, Schmitt N S and Neusch-inderbinen M. 2018. High prevalence of extended-spectrum β-lactamase producing Enterobacteriaceae among clinical isolates from cats and dogs admitted to a veterinary hospital in Switzerland. *Frontiers in Veterinary Science* **5**: 62–68.