

Antimicrobial resistance pattern of extended-spectrum β -lactamase-producing *Escherichia coli* isolated from fecal samples of piglets and pig farm workers of selected organized farms of India

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Abstract

Background and Aim: Extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* are gradually increasing worldwide and carry a serious public threat. This study aimed to determine the antimicrobial resistance pattern of ESBL-producing *E. coli* isolated from fecal samples of piglets and pig farm workers.

Materials and Methods: Fecal samples from <3-month-old piglets (n=156) and farm workers (n=21) were processed for the isolation of ESBL-producing *E. coli* in MacConkey agar added with 1 μ g/mL of cefotaxime. *E. coli* (piglets=124; farm workers=21) were tested for ESBL production by combined disk method and ESBL E-strip test. Each of the ESBL-positive isolate was subjected to antibiotic susceptibility testing. The ESBL-producing *E. coli* were further processed for genotypic confirmation to CTX-M gene.

Results: A total of 55 (44.4%, 55/124) and nine (42.9%, 9/21) ESBL-producing *E. coli* were isolated from piglets and farm workers, respectively. Antibiotic susceptibility testing of the ESBL-positive *E. coli* isolates from piglets and farm workers showed 100% resistance to ceftazidime, cefotaxime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, and cefpodoxime. A proportion of 100% (55/55) and 88.9% (8/9) ESBL-positive *E. coli* were multidrug resistance (MDR) in piglets and farm workers, respectively. On genotypic screening of the ESBL *E. coli* isolated from piglets (n=55), 15 were positive for the *bla*_{CTX-M} gene and of the nine ESBL *E. coli* from farm workers, none were positive for the *bla*_{CTX-M} gene.

Conclusion: Although there was no significant difference in isolation of ESBL-producing *E. coli* between piglets and farm workers, the ESBL-positive *E. coli* from piglets showed relatively higher MDR than farm workers.

Keywords: CTX-M gene, India, multidrug resistance, organized farm, piglets, workers.

Introduction

Treating infections associated with extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* are becoming difficult since they are capable of hydrolyzing penicillin, broad-spectrum cephalosporins, and monobactams and are often resistant to other antimicrobial classes such as fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole [1]. The general trend of antibiotic resistance around the world indicates that an overall decline in the total stock of antibiotic effectiveness [2].

In the livestock sector, antibiotics are not only used for the treatment of infections but also for disease

prevention and growth promotion [3]. India is one of the largest consumers of antibiotics in the world with 13 billion standard units in 2010 [2]. In India, human and animals live in close proximity which increases the risk of contamination and resistance between them and 95% of adults in India shows β -lactam antimicrobials resistance in Gram-negative bacteria [4,5]. Reports suggest the possibility of transferring ESBL-producing *E. coli* from farm workers to food animal and vice versa [6-10]. Multidrug-resistant *E. coli* are reported in food animals and pet animals of India [11-15]. Besides, food animals may act as a reservoir for ESBL-producing strains and foods as a vehicle for the transfer of β -lactam-producing bacteria [16].

This study was conducted to determine the antimicrobial resistance (AMR) pattern of ESBL-producing *E. coli* isolated from fecal samples of piglets and pig farm workers.

Materials and Methods

Ethical approval

Ethical approval was not required for this study.

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Sample collection

A cross-sectional study was conducted between August 2016 and May 2017 to sample five government organized pig farms covering three states, namely, Uttar Pradesh, Karnataka, and Tamil Nadu. The sample was collected from five different organized pig farms, namely, Aligarh (41), Bareilly (30), Chennai farm 1 (28), Chennai farm 2(21), and Hassan (36). The fecal samples were aseptically collected with the help of fecal swab (HiMedia, Mumbai, India) directly from piglet's rectum and in case of piglet farm workers, swabs were distributed to the farmers which were collected in the next morning (n=21) and transported to the laboratory under cold chain for the isolation of ESBL-producing *E. coli*.

Isolation and identification of ESBL-producing *E. coli*

Fecal swabs were pre-enriched in buffered peptone water and incubated at 37°C for 4-6 h. Initial screening was done using MacConkey agar supplemented with cefotaxime at 1 mg/L and plates were then incubated at 37°C for 12 h. The purified cultures of presumptive *E. coli* were stored in nutrient agar for identification by biochemical tests (IMViC pattern). For phenotypic identification of ESBL producers, the combination disk method by cefotaxime and ceftazidime with and without clavulanic acid was used [17] and the isolates were further confirmed by Triple ESBL detection Ezy minimum inhibitory concentration (MIC) Strip (HiMedia, India). The isolates were also tested for antibiotic susceptibility pattern with ceftazidime/clavulanic acid (20/10 µg), chloramphenicol (30 µg), cefpodoxime (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefixime (5 µg), cefoxitin (30 µg), piperacillin-tazobactam (100/10 µg), cefotaxime/clavulanic acid (30/10 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), amikacin (30 µg), and ciprofloxacin (5 µg) by disk diffusion method. The MIC of cefotaxime, cefepime, and ceftazidime was determined by E strips (HiMedia, India). Antimicrobial susceptibility results were interpreted by the following criteria established by the Clinical and Laboratory Standards Institute [18]. *E. coli* showing resistance to at least three classes of antibiotics were categorized as multi-drug-resistant strains.

Genotypic detection of *bla*_{CTX-M} gene

The genomic DNA was extracted from ESBL-positive isolates using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and the polymerase chain reaction (PCR) for *bla*_{CTX-M} (forward: CAATGTGCAGCACCAAGTAA; reverse: CGCGA TATCGTTGGTGGTG) was carried out in a final reaction volume of 25 µL [11]. The amplified PCR product was visualized by a gel documentation system (UVP, UK) after electrophoresis in 1.5% (w/v) agarose gel containing ethidium bromide (0.5 µg/mL, Loba Chemie, India). The positive PCR amplicons were sent to commercial sequencing services (Eurofins Ltd.,

Bangalore) for further purification and sequencing by Sanger method. The homology searches were made using the BLAST algorithm available at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>, and the representative sequences were submitted to GenBank for accession number.

Statistical analysis

The association of the isolation of ESBL-producing *E. coli* between piglets and farm workers was tested by Chi-square test/Fisher's exact (two-tailed) using SPSS version 20.0 statistical software (IBM Corp., Armonk, NY, USA).

Results

A total of 124 and 21 *E. coli* were isolated from piglets and human fecal samples, respectively, on MacConkey agar supplemented with cefotaxime. There was no significant difference in isolation of ESBL-producing *E. coli* between piglets and farm workers ($p>0.05$). Of the 124 isolates from piglets and 21 from farm workers, 55 (44.4%) and 9 (42.9%) isolates were identified as ESBL producers, respectively, by combined disk method. The ESBL-producing isolates from piglets and farm workers had the MIC of cefotaxime >128 µg/mL, cefepime > 32 µg/mL, and ceftazidime > 64 µg/mL. Antibiotic susceptibility testing of the ESBL-positive *E. coli* isolates from piglets (n=55) showed 100% resistance to ceftazidime, cefotaxime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, and cefpodoxime. In addition, they were also resistant to cefepime (53, 96.3%), piperacillin (52, 94.5%), amikacin (42, 76.4%) ciprofloxacin (16, 29.1%), chloramphenicol (18, 32.7%), tetracycline (34, 61.8%), and sulfamethoxazole/trimethoprim (28, 50.9%). The ESBL *E. coli* from farm workers showed 100% resistant to tetracycline, ceftazidime, cefotaxime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, and cefpodoxime. Further, *E. coli* from farm workers were also resistant to cefepime (3, 33.3%) and piperacillin (4, 44%), amikacin (8, 88.8%), chloramphenicol (5, 55%), sulfamethoxazole/trimethoprim (7, 77%), and ciprofloxacin (6, 66.6%) (Figure-1). A proportion of 100% (55/55) and 88.9% (8/9) ESBL-positive *E. coli* was resistant

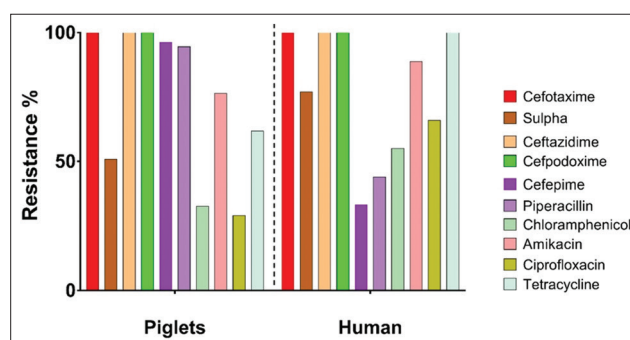


Figure-1: Antimicrobial resistance pattern of extended-spectrum β -lactamase-producing *Escherichia coli* isolates of piglets (n=55) and farm workers (n=9).

to more than three classes of antibiotics in piglets and farm workers, respectively, and classified as multi-drug resistance (MDR). On genotypic screening of ESBL *E. coli* (n=55), 15 isolates were positive for *bla*_{CTX-M} gene and of the nine ESBL *E. coli* from farm workers, none were positive for the *bla*_{CTX-M} gene. The representative *bla*_{CTX-M} gene sequences were submitted to GenBank which were assigned with the accession numbers (MF177899, MF 177900).

Discussion

The emergence and spread of ESBLs producing *E. coli* in food-producing animals is a major public health issue worldwide [11-15]. Piglets and farm workers harbored ESBL *E. coli*, the percentage of cefotaxime and ceftazidime resistance is higher among isolates as compared to ciprofloxacin and chloramphenicol indicates the abundant use of beta-lactam and cephalosporin antimicrobials in piglets and human [5,15]. A recent study documented that the occurrence of ESBL-positive *E. coli* in pigs was not related to total antimicrobial use, but associated with the presence or absence of cephalosporin use at the farm [10]. The current investigations showed that once the bacteria are on the farm or in the farm environment may be widely spread among animals (including insects and rodents) and can reach the environment through manure [19,20]. In this study, the ESBL-producing *E. coli* showed resistance to clinically important antibiotics such as cephalosporins, fluoroquinolones, and aminoglycosides and jeopardize the effective prevention and treatment of various bacterial infections [21]. The present study revealed that all the ESBL-producing *E. coli* were 100% resistant to cefotaxime and ceftazidime and were concordance with others [11-13]. This study reported that the entire ESBL-positive *E. coli* from piglets as MDR, similarly, in a study from China reported MDR phenotype in total ESBL-producing *E. coli* isolated from pigs [22]. AMR rate depends on the factors around the farm, human behavior, improper sanitation, and hygiene [23]; the improper antibiotic use with route of administration is important for multidrug-resistant bacteria. This study revealed lower resistance for ciprofloxacin, tetracycline, amikacin, and chloramphenicol compared with other antibiotics. The difference might be linked with the overall decline in the use of these antibiotics in India since 2000 [24]. It is also observed that ESBL producers also show resistance to non- β -lactam antimicrobials, such as fluoroquinolones, aminoglycosides, and sulfonamides [22]. The study noticed the CTX-M genotype in ESBL-positive isolates, similar findings were reported in ESBL-producing enterobacterials which might indicate rapid dissemination of *bla*_{CTX-M} genes [25-27]. In ESBL *E. coli*, the most commonly identified enzymes are CTX-M family [28].

Conclusion

The study highlights the AMR pattern of ESBL-producing *E. coli* isolated from fecal samples of

piglets and farm workers. The ESBL-positive *E. coli* from piglets showed relatively higher MDR than farm workers. Hence, necessary steps are needed to reduce the use of antimicrobials in pig farming to decrease the AMR.

Authors' Contributions

ORVK, BRS, and DKS conceptualized and designed this research. The research was carried out by ST and BSP. ORVK analyzed the data and result. ORVK, ST, SVS, RK, RR, DKS, and BRS drafted, revised, and finalized the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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