



Detection of carbapenem resistant *Escherichia coli* in cattle and pigs

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Abstract

The aim of this study was to detect the presence of carbapenem resistant *Escherichia coli* (*E. coli*) in cattle and pigs. Altogether, 100 samples were collected, 50 each from cattle and pigs including mastitic milk and faecal samples from healthy as well as diarrhoeic cattle and faecal samples and slaughter specimens from pigs. Bacteria were isolated from the samples and antibiotic sensitivity test was carried out. Eight isolates of *E. coli* were obtained from cattle and 15 isolates from pigs. The phenotypic resistance patterns against carbapenems (ertapenem, imipenem and meropenem) were analysed. In cattle, the four isolates from milk were observed to be sensitive to all the three carbapenems whereas, all the isolates obtained from faecal samples were resistant to all the three carbapenems. The four faecal and three intestinal isolates of *E. coli* from pigs showed sensitivity to all the three carbapenems. From a total of 10 faecal samples from pigs, four, three and one isolates showed resistance to ertapenem, imipenem and meropenem respectively. Among five isolates from intestine, one isolate showed resistance to ertapenem and imipenem and no isolate showed resistance to meropenem. Polymerase chain reaction (PCR) was carried out to find the presence of carbapenem resistance genes (*bla_{SHV}*, *bla_{CTX}*, *bla_{TEM}* and *bla_{NDM}*) in all the 23 isolates and the results revealed that seven (58.3 per cent) *E. coli* isolates carried *bla_{SHV}* gene and four (33.3 per cent) contained *bla_{CTX}* gene which encodes carbapenem resistant enzymes. Antibigram using 14 commonly used antimicrobial agents revealed varied multidrug resistant patterns among the *E. coli* isolates.

Keywords: Antibigram, carbapenem resistance, *Escherichia coli*, multidrug resistance

Indiscriminate and extensive use of antibiotics to treat bacterial infections in human beings and animals resulted in enormous antimicrobial pressure on the pathogens, leading to the development of antimicrobial resistance (AMR) (Shaikh *et al.*, 2015). Carbapenems are reserved

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as the last resort in treatment of multi-drug resistant (MDR) infections in human beings. The bacterial infections associated with carbapenem resistant *Escherichia coli* (*E. coli*) strains are emerging as a major public health concern globally (Baran and Aksu, 2016). Production of β -lactamases or β -lactam hydrolysing agents is the most common mechanism of drug resistance; especially in *E. coli* against the β -lactam antibiotics like carbapenems. The spread of these carbapenem hydrolysing enzymes among enteric bacteria in hospital settings is of grave concern. Intestinal microflora of animals carry genes namely, bla_{CTX-M} , bla_{TEM} , bla_{SHV} and integrons, which can act as a possible cause for drug resistance in humans and animals (Tshitshi *et al.*, 2020; Ejaz *et al.*, 2021). Various ESBL genes viz., bla_{SHV} (33.1 per cent), bla_{TEM} (22.5 per cent), bla_{CTX-M} (20.5 per cent) and bla_{OXA} (11.3 per cent) were detected in CRE. It was reported that 76.3 per cent of Gram negative bacteria isolated from dogs were positive for atleast one carbapenemase gene (Sankar *et al.*, 2021).

Carbapenem resistant *Enterobacteriaceae* (CRE) isolates were detected in food-producing animals and companion animals (Taggar *et al.*, 2020). There are reports of identification of carbapenem resistant *E. coli* in cattle and pigs in different parts of the world. But studies carried out in India are rare and no studies have been carried out so far in Kerala. Hence, the present study was undertaken to detect the presence of carbapenem resistant *E. coli* in cattle and pigs. The study also aims at detecting the susceptibility of these isolates to other commonly used antibiotics.

Materials and methods

A total of 100 samples were obtained from 50 cattle and 50 pigs. These included mastitic milk samples [brought to the Department of Veterinary Microbiology, University Livestock Farms (ULF), Mannuthy and Department of Teaching Veterinary Clinical Complex (TVCC)] and faecal samples from cattle [from diarrhoeic and healthy cattle brought to TVCC department and ULF, Mannuthy] and faecal samples [from Centre for Pig Production and Research (CPRR), Mannuthy] and slaughter house

specimens such as lungs, heart, liver and intestine [from Meat Technology Unit (MTU), Mannuthy and authorised slaughter houses in Thrissur district] from pigs during the period of 2017-2019.

The collected samples were immediately plated onto brain heart infusion agar (BHIA) under aseptic conditions and were incubated at 37°C for 24 h. The isolates were analysed for its colony morphology and subjected to Gram's staining. Those colonies, which revealed Gram negative rods were sub-cultured onto Mac Conkey Agar (MCA) and eosin methylene blue (EMB) agar and further subjected to various biochemical tests for the confirmation of the presence of *E. coli*. All the procedures are followed as per Quinn *et al.* (1994).

Antibiogram

Disc diffusion method (CLSI, 2018) was performed on Mueller Hinton Agar (MHA) plates to determine susceptibilities of different β -lactam and non- β -lactam antibiotics. Three carbapenem antibiotics (ertapenem, meropenem, imipenem) and 14 other commonly used antibiotics belonging to various classes with known concentration were analysed.

Polymerase Chain Reaction

The DNA was extracted from *E. coli* isolates using the Genomic DNA Purification Kit (Origin, Kerala) as per manufacturer's instructions. The concentration and purity of DNA were measured spectrophotometrically. The DNA extracted from *E. coli* isolates, showing phenotypic resistance to carbapenems, were subjected to PCR using primers targeting bla_{SHV} , bla_{CTX} (Bora *et al.*, 2014), bla_{TEM} and bla_{NDM} genes (Sankar *et al.*, 2021).

A total volume of 12.5 μ L reaction was prepared. The composition of single reaction mix for amplification was 6.25 μ L of EmeraldAmp® GT PCR Master Mix, 1 μ L each of forward and reverse primer (10 pm/ μ L), 3 μ L of template DNA and 1.25 μ L of nuclease free water. To each reaction tube, 9.5 μ L of reaction mix and 3 μ L of template DNA were added and subjected to initial denaturation (94°C for 5min) followed

by denaturation (94°C for 1 min), annealing (56.9°C, 63.2°C, 65.6°C, 66.7°C for 1 min for *bla*_{SHV}, *bla*_{CTX}, *bla*_{TEM}, *bla*_{NDM}, respectively), extension (72°C for 2 min for *bla*_{SHV}, 72°C for 1 min for *bla*_{CTX}, *bla*_{TEM}, *bla*_{NDM}, respectively), final extension (72°C for 10 min for *bla*_{SHV}, *bla*_{CTX}, *bla*_{NDM}, 72°C for 5 min for *bla*_{TEM}) and hold (4°C for 10 min).

Results and discussion

The presence of *E. coli* was detected in eight samples from cattle (four isolates from mastitis milk and four isolates from diarrhoeic samples). Major pathogen associated with clinical mastitis in dairy cattle was reported to be *Staphylococcus aureus* followed by *E. coli* (Sumathi *et al.*, 2008). In a study conducted by Ranjan *et al.* (2011) on aetiological agents associated with bovine mastitis in Jharkhand, *E. coli* could be isolated from only 8.95 per cent. Lakshmi and Jayavardhanan (2016) reported that 27 per cent of isolates from mastitic milk

samples of cattle in organised farms in Kerala were *E. coli*. Fifteen *E. coli* isolates were obtained from pigs (10 isolates from faecal samples and 5 isolates from intestine). There were reports of isolation of *E. coli* from more than 50 per cent of faecal samples collected from pigs (Kagambega *et al.*, 2012; Lalzampua *et al.*, 2013). Individual animals shed *E. coli* in different concentrations due to several factors such as pathogen, host and environment and *E. coli* might not be isolated from all the faecal samples (Stein *et al.*, 2017).

The carbapenem resistance pattern of *E. coli* isolates obtained from cattle and pigs were analysed against three carbapenem discs using disk diffusion method (CLSI, 2018) on MHA plates. Antibiotic susceptibility of the isolates to 14 commonly used antibiotic discs were also analysed. The varying degrees of resistance and susceptibility were observed (Table 1).

Table 1. Antibiotic sensitivity pattern of *E. coli* isolates from cattle and pigs

| Species | Sample | <i>E. coli</i> isolate | Sensitivity to Antibiotics* | | | | | | | | | | | | | | Sensitivity to Carbapenem** | | |
|---------|-----------|------------------------|-----------------------------|-----|-----|-----|-----|----|-----|----|-----|-----|-----|-----|-----|-----|-----------------------------|-----|-----|
| | | | CTX | CTR | AMX | CIS | CEC | EX | CIT | CN | GEN | PIT | TET | CPD | CAZ | COT | ETP | IPM | MRP |
| Cattle | Milk | C1 | R | S | R | R | R | S | R | S | S | S | S | R | S | S | S | S | S |
| | | C2 | R | R | S | R | R | R | R | S | IN | R | S | R | S | S | S | S | S |
| | | C3 | R | S | S | R | R | R | R | R | S | R | R | R | S | S | S | S | S |
| | | C4 | R | S | R | R | R | R | S | S | R | R | R | R | S | S | S | S | S |
| | Faeces | C5 | R | R | R | R | R | R | R | S | S | IN | R | R | S | S | R | R | R |
| | | C6 | R | S | S | R | R | R | R | IN | IN | IN | S | R | S | S | R | R | R |
| | | C7 | R | IN | S | R | R | S | R | S | S | IN | S | R | S | S | R | R | R |
| | | C8 | R | R | R | R | R | S | R | R | S | R | S | R | S | S | R | R | R |
| Pig | Faeces | P1 | R | S | R | R | R | S | R | S | S | R | S | R | S | S | S | S | S |
| | | P2 | R | S | S | R | R | R | R | R | S | R | S | R | R | R | R | S | S |
| | | P3 | R | S | R | R | R | S | S | IN | IN | S | R | R | IN | S | R | S | R |
| | | P4 | R | R | IN | R | R | R | R | S | S | R | S | R | IN | S | S | S | S |
| | | P5 | R | S | S | R | R | S | R | S | S | R | S | R | IN | R | R | S | S |
| | | P6 | R | S | R | R | R | S | R | S | R | S | S | R | S | S | S | R | S |
| | | P7 | R | R | S | R | R | R | R | S | S | R | R | R | IN | R | S | S | S |
| | | P8 | R | S | S | R | R | IN | S | S | IN | S | S | R | S | S | S | R | S |
| | | P9 | R | S | R | R | R | S | S | S | S | R | S | R | S | S | S | S | S |
| | | P10 | R | S | S | R | R | R | R | S | R | S | R | R | R | R | R | R | S |
| | Intestine | P11 | R | R | R | R | R | R | S | R | R | R | R | R | R | R | S | R | S |
| | | P12 | R | S | S | R | R | R | R | R | R | R | R | R | R | S | S | S | S |
| | | P13 | R | S | R | R | R | R | S | IN | R | S | R | R | R | R | S | S | S |
| | | P14 | R | IN | R | S | R | R | S | IN | S | R | R | R | R | R | S | S | S |
| | | P15 | R | R | R | S | R | S | R | R | R | IN | R | R | R | R | S | S | S |

*Cefotaxime (CTX) - 30mcg, Ceftriaxone (CTR) - 30mcg, Amoxicillin (AMX) - 30mcg, Ceftriaxone/Sulbactam (CIS) - 30/15mcg, Cefotaxime/Clavulanic acid (CEC) - 30/10mcg, Enrofloxacin (EX) - 10mcg, Ceftriaxone/Tazobactam (CIT) - 30/10mcg, Cefalexin (CN) - 30mcg, Gentamicin (GEN) - 30mcg, Piperacillin/Tazobactam (PIT) - 100/10mcg, Tetracycline (TET) - 30mcg, Cefpodoxime (CPD) - 10mcg, Ceftazidime (CAZ) - 30mcg, Co-trimoxazole (COT) - 25mcg.

**Ertapenem (ETP) - 10mcg, Imipenem (IPM) - 10mcg, Meropenem (MRP) - 10mcg

All the *E. coli* isolates obtained from faecal samples collected from cattle were found to be resistant to all the three carbapenems (ertapenem, meropenem and imipenem) and all the four isolates found from milk were observed to be sensitive to all the three (Fig. 1). Similar results were made by Papp-Wallace *et al.* (2011) and Chika *et al.* (2017) who reported the presence of carbapenem resistant *E. coli* in cattle. However, in the present study, no difference in the resistance could be observed among the three carbapenems.

All the ten *E. coli* isolates from faecal

sample from pigs showed different resistance patterns. Four isolates showed resistance to ertapenem and three were resistant to imipenem; only one isolate showed resistance to meropenem. Among five isolates obtained from intestine, only one isolate was resistant to ertapenem and imipenem where no isolate showed resistance to meropenem (Fig. 2).

The study revealed the presence of carbapenem resistant *E. coli* in cattle and pigs. Out of 23 *E. coli* isolates obtained from cattle and pigs, 12 isolates were found to be resistant to atleast one of the carbapenem antibiotics.

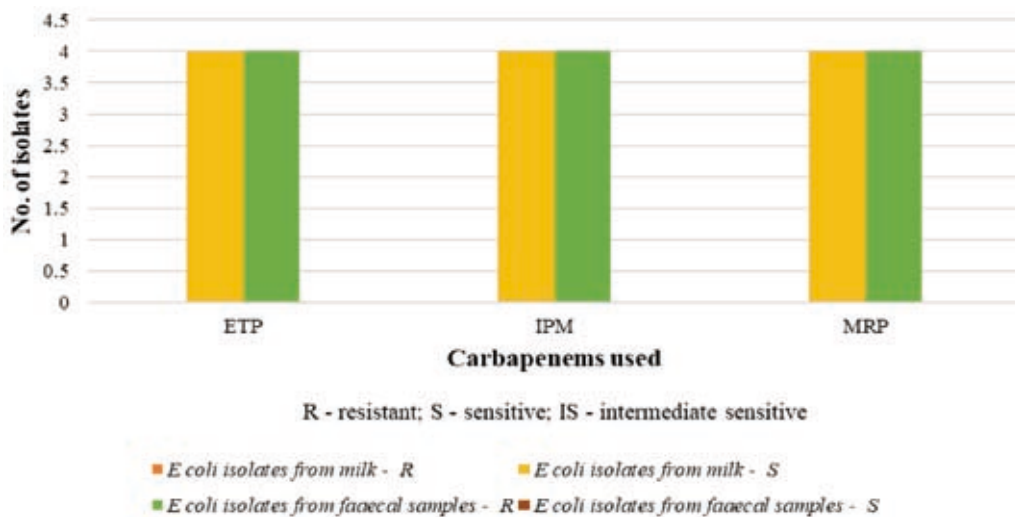


Fig. 1. Susceptibility of *E. coli* isolates from cattle to carbapenems

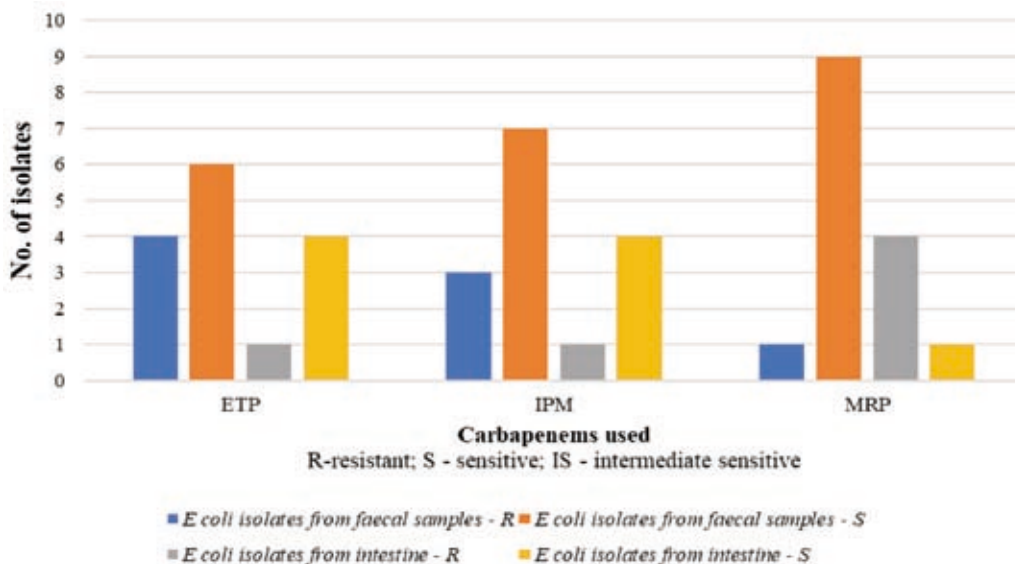


Fig. 2. Susceptibility of *E. coli* isolates from pigs to carbapenems

These 12 isolates were subjected to PCR, targeting resistance genes such as *bla*_{SHV}, *bla*_{CTX}, *bla*_{NDM} and *bla*_{TEM} using specific primers. Seven were positive for *bla*_{SHV}, four were positive for *bla*_{CTX} (Fig. 3 and 4) and all were negative for *bla*_{TEM} and *bla*_{NDM}.

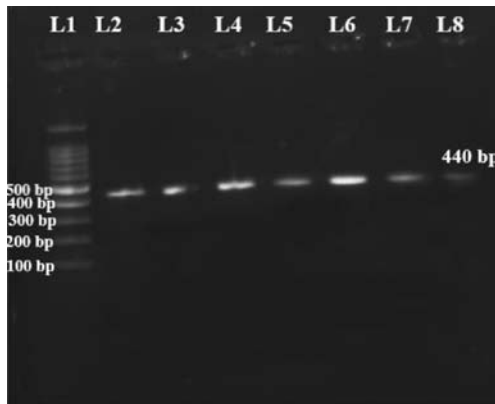


Fig. 3. Amplification of *bla*_{SHV} gene in *E. coli* L1 – 100bp Ladder L2 – L8 – Positive samples (Sample size- 440bp)

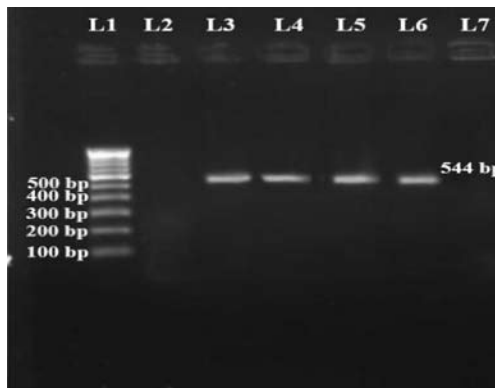


Fig. 4. Amplification of *bla*_{CTX} gene in *E. coli* L1 – 100bp Ladder L2 and L7 – Negative samples L3 – L6 – Positive samples (Sample size- 544bp)

Rita *et al.* (2021) confirmed the presence of *bla*_{CTX} gene in one *E. coli* isolate out of 27 isolates obtained from cattle. However, there are various reports on detection of *bla*_{SHV} and *bla*_{CTX} from *E. coli* isolates from humans (Gondal *et al.*, 2022). The transmission of these resistant genes from humans to animals and vice-versa pose a major threat to control of infection using these antimicrobials. However, further detailed surveillance of the resistant genes in personnel in contact with these animals is needed.

Carbapenem resistant *E. coli* were reported from pigs (Singh *et al.*, 2017). In the present study, among the ten isolates from faecal samples from pigs, six (60 per cent) were resistant to at least one of the carbapenem drugs. This indicates that the personnel working in these pens with animals harbouring resistant microbes may have a chance to get horizontal transmission. From the five isolates from slaughter house specimens, two (40 per cent) were carbapenem resistant. None of the isolates were resistant to all the three as against Pruthvishree *et al.* (2016), who reported that 30 per cent of the carbapenem resistant *E. coli* were resistant to all the three carbapenems.

Antibiotic susceptibility testing using commonly used antibiotics revealed that all the eight isolates from cattle were found to be multidrug resistant. The four isolates from milk were resistant to cefotaxime, ceftazidime/sulbactam, cefotaxime/clavulanic acid and ceftazidime. Among them, three were resistant to enrofloxacin (C2, C3 and C4), ceftazidime/tazobactam (C1, C2 and C3) and piperacillin/tazobactam (C2, C3 and C4). Two isolates showed resistant to amoxicillin (C1 and C4) and tetracycline (C3 and C4). Only one isolate showed resistance to ceftazidime (C2), cefalexin (C3) and gentamicin (C4). All the four isolates were found to be sensitive to ceftazidime and co-trimoxazole (Fig. 5).

Antibiogram results showed that all the four isolates from faecal samples were resistant to cefotaxime, ceftazidime/sulbactam, ceftazidime/tazobactam, cefotaxime/clavulanic acid and ceftazidime. Two isolates showed resistance to ceftazidime (C5 and C8), amoxicillin (C5 and C8) and enrofloxacin (C5 and C6). Only one isolate showed resistance to piperacillin/tazobactam (C8), cefalexin (C8) and tetracycline (C5). All the four isolates were found to be sensitive to gentamicin, ceftazidime and co-trimoxazole (Fig. 5).

There are several reports on resistance of *E. coli* isolated from cattle to a variety of antimicrobials including penicillin, amoxicillin, tetracycline, oxytetracycline, methicillin, amoxycloxacillin, ceftazidime, gentamicin and enrofloxacin (Chandrasekaran

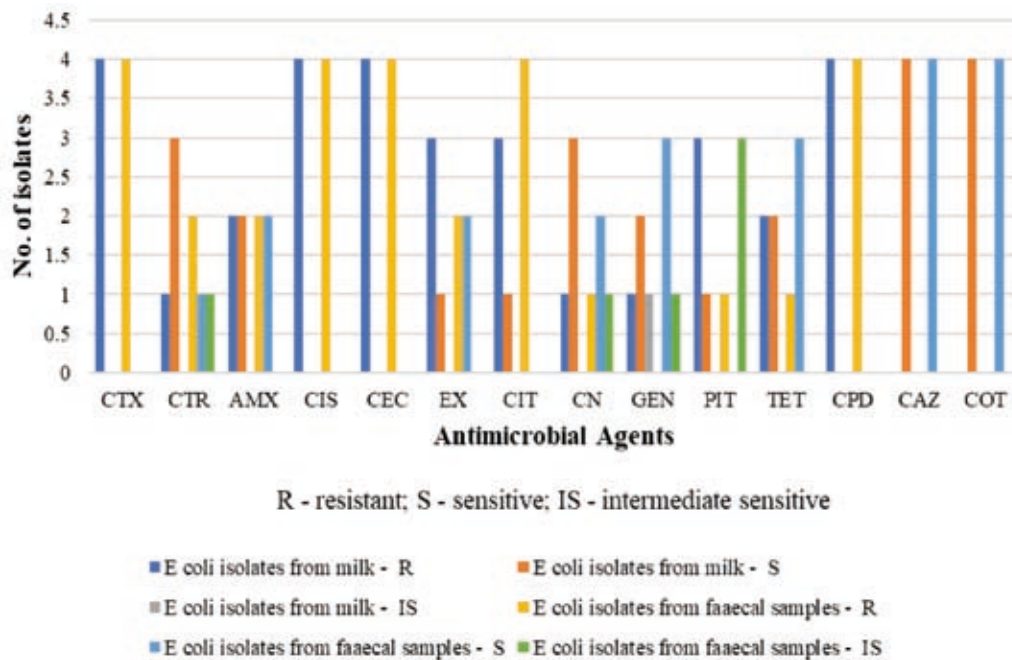


Fig. 5. Antimicrobial susceptibility pattern of *E. coli* isolates from cattle

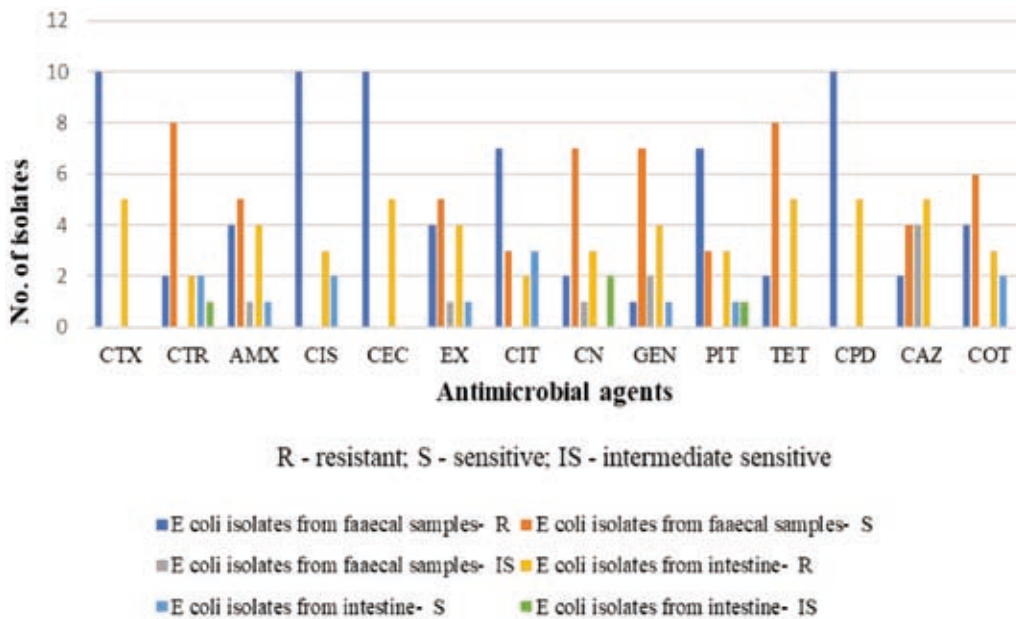


Fig. 6. Antibiotic susceptibility pattern of *E. coli* isolates from pigs

et al., 2014; Das *et al.*, 2017, Verma *et al.*, 2017; Revathi, 2018; Xavier *et al.*, 2020). However, Sumathi *et al.* (2008) observed that gentamicin was found to be the most effective drug. All the eight isolates were found to be sensitive to co-trimoxazole. Revathi (2018) also obtained similar result.

All the fifteen isolates from pigs were found to be multidrug resistant. Antibio gram results showed that the ten isolates from faecal samples were resistant to cefotaxime, ceftriaxone/sulbactam, cefotaxime/clavulanic acid and cefpodoxime. Seven of the isolates were resistant to ceftriaxone/tazobactam (P1, P2, P4, P5, P6, P7 and P10) and piperacillin/

tazobactam (P1, P2, P4, P5, P7, P9 and P10). Four isolates showed resistance to amoxicillin (P1, P3, P6 and P9), enrofloxacin (P2, P4, P7 and P10) and co-trimoxazole (P2, P5, P7 and P10). Two isolates were found to be resistant to ceftriaxone (P4 and P7), cefalexin (P2 and P10), tetracycline (P3 and P7) and ceftazidime (P2 and P10). Only one isolate was resistant to gentamicin (P6). All four isolates were found to be sensitive to ceftazidime and co-trimoxazole (Fig. 6).

Antibiogram results showed that all five isolates from the intestine of pig were resistant to cefotaxime, cefotaxime/clavulanic acid, tetracycline, cefpodoxime and ceftazidime. Four of the isolates were resistant to amoxicillin (P11, P13, P14 and P15), enrofloxacin (P11, P12, P13 and P14) and gentamicin (P11, P12, P13 and P15). Three isolates showed resistance to ceftriaxone (P11, P12 and P13), cefalexin (P11, P12 and P15), piperacillin/tazobactam (P11, P12 and P14) and co-trimoxazole (P11, P14 and P15). Two isolates were found to be resistant to ceftriaxone (P11 and P15) and ceftazidime/tazobactam (P12 and P15) (Fig. 4).

E. coli in pigs were reported to be resistant to antibiotics like amoxicillin, oxytetracycline, trimethoprim, sulphonamide, streptomycin, cefixime, cefazolin, cephalixin, ceftriaxone, cefotaxime, enrofloxacin, ampicillin, chlortetracycline, erythromycin and lincomycin (van den Bogaard *et al.*, 2000; Enne *et al.*, 2007; Lalzampaia *et al.*, 2013). The present study revealed varying degrees of resistance and susceptibility of the isolates to the antimicrobial agents in the study.

Conclusion

The reports on detection of carbapenem resistant bacteria are rare in cattle and pigs. The occurrence of these bacteria in livestock has serious consequences and may affect therapeutic options in human medicine, since carbapenems are the last resort of antibiotic therapy. A key finding of this study was that 100 per cent of *E. coli* isolates recovered from faecal samples from cattle were resistant to all three carbapenems, ertapenem, meropenem and imipenem, which are not used in the livestock in Kerala. The high

rate of resistance may reflect the increased clinical use of these antimicrobials among humans, which needs a thorough investigation. Hence, constant monitoring of carbapenem susceptibility among enteric bacteria of livestock is warranted. The bacteria were found to have various mechanisms conferring decreased sensitivity. The necessity for the study of antibiotic resistance and the associated genetic mechanisms is highly essential. Since a dependable substitute to carbapenems is not there, rationalisation of use of these antibiotics in both humans and animals is needed. The infection control measures should be meticulous whenever carbapenem resistance is detected and the active surveillance of carbapenemase-encoding genes are of the extreme importance. The tracing of the source of infection in livestock is important and all the steps should be taken to prevent the spreading of bacteria.

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Conflict of interest

The authors declare that they have no conflict of interest.

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