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Research Article

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ESCHERICHIA COLI* ISOLATED FROM BOVINE SUBCLINICAL MASTITIC MILK

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ABSTRACT

Antimicrobial drug resistance has become a serious problem leading to treatment failure specifically for multidrug resistant pathogens. The current study was aimed to characterize 50 *Escherichia coli* isolates recovered from 133 bovine subclinical mastitic milk samples. The recovered *E. coli* isolates were subjected to antimicrobial susceptibility testing by agar disk diffusion method. The antibiogram revealed that the most effective antibiotics were chloramphenicol (90%), ampicillin (84%) followed by trimethoprim and gentamicin (82% each), ceftriaxone (76%) and amoxicillin/clavulanic acid (62%). The isolates were found to be highly resistant to erythromycin (80%), oxytetracycline (76%) and cefotaxime (54%). The multidrug resistant (MDR) *E. coli* isolates (n=10) were further subjected to PCR amplification of *uspA*, *blaTEM*, *tetA* and *sul* II genes. All the MDR *E. coli* isolates were found to be positive for *uspA* gene. While, the occurrence of antibiotic resistance genes i.e. *blaTEM*, *tetA* and *sul* II genes was revealed in 20%, 20% and 30% of the isolates, respectively.

Keywords: *Escherichia coli*, Mastitis, Antibiotic susceptibility

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For the prevention and treatment of mastitis, various classes of antimicrobials are used, particularly the β-lactams and fluoroquinolones. However, due to the long term misuse of antibiotics, bacterial drug resistance has become a serious problem leading to treatment failure, especially in multidrug-resistant (MDR) pathogenic bacteria (Yu *et al.*, 2020). Various reports have shown that 20–33% of *E. coli* isolates recovered from raw milk samples obtained from mastitis cases were resistant to at least one antimicrobial agent, and 20% of the isolates were resistant to more than two classes of antimicrobial agents. Various recent studies have highlighted the increasing prevalence of highly resistant extended-spectrum β-lactamase (ESBL)-producing *E. coli*, which have been isolated from food-producing animals in different countries. ESBL producers are generally multidrug-resistant against non β-lactam antibiotics, including fluoroquinolones, aminoglycosides, tetracyclines, sulphonamides and chloramphenicol, which makes the treatment difficult. Antibiotic resistance and virulence genes could be located on the same chromosomal structures or plasmids, which makes the evaluation of these virulence factors and antibiotic resistance genes important. Therefore, surveillance of antimicrobial usage is vital to ensure their prudent use in animal husbandry and reduce the risk for selection and spread of antimicrobial resistance.

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MATERIALS AND METHODS

Antimicrobial susceptibility testing

Modified California mastitis test (MCMT) and somatic cell count (SCC) were performed to detect the bovine subclinical mastitis (SCM). Isolation of *E. coli* from the samples of bovine subclinical mastitic milk was done as per the method described by Quinn *et al.*, 2011. The colonies showing green metallic sheen on eosin methylene blue agar (EMB) were selected for further confirmation. A total of 50 *Escherichia coli* isolates recovered from 133 bovine subclinical mastitic milk were subjected to antimicrobial susceptibility testing by agar disk diffusion method as described by Bauer *et al.* (1966). These milk samples were collected from 10 organized dairy farms of Udaipur district, Rajasthan from June, 2021 and October, 2021. A total of 14 antibiotic discs were placed on two inoculated Mueller-Hinton agar plates (100 × 15 mm) each containing 7 antibiotic discs. After incubation at 37°C for 24 hrs, the diameter of the zone of inhibition were measured and compared with the zone size interpretation chart provided by the supplier so as to determine the susceptibility pattern of the isolates for the respective antibiotics as per CLSI guidelines.

PCR analysis of the isolates

The pure culture of MDR *E. coli* strains obtained on the basis of Gram's staining, morphological study, biochemical characteristics and antibiotic susceptibility test were used for the isolation of DNA by Nucleo-pore

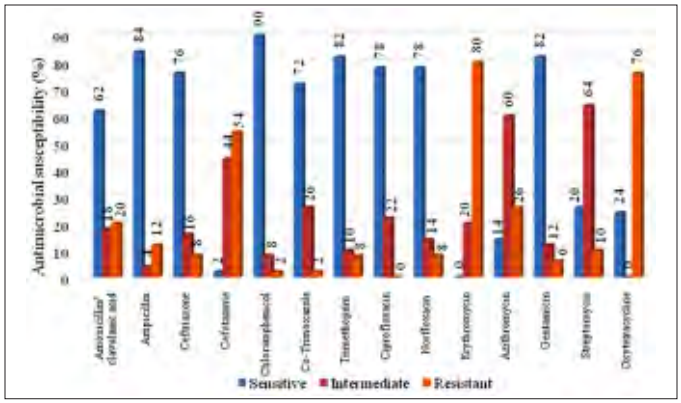

Fig. 1. Antibiogram of *E. coli* isolates (n = 50)

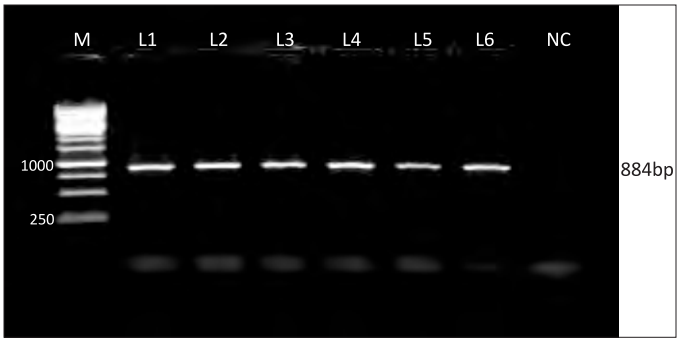

Fig. 2. Agarose gel showing PCR amplified product (884 bp) for uspA gene. M=1kb DNA ladder, positive samples (L1=E1, L2=E2, L3=E3, L4=E4, L5=E5, L6=E6, NC= negative control)

Table 1. The primers used for the confirmation of *E. coli* and screening of antibiotic resistance genes

S.No.	TARGET GENE	SEQUENCE	PRODUCT SIZE (bp)
1.	*uspA* (Osek *et al.*, 2001)	F-CCGATACGCTGCCAATCAGT R-ACGCAGACCGTAAGGGCCAGAT	884
2.	*blaTEM* (Maynard *et al.*, 2003)	F-GAGTATTCAACATTTTCGT R-ACCAATGCTTAATCAGTGA	857
3.	*tetA* (Sengelov *et al.*, 2003)	F-GTAATTCTGAGCACTGTCGC R-CTGCCTGGACAACATTGCTT	956
4.	*sul* II (Boerlin *et al.*, 2005)	F-CGGCATCGTCAACATAACCT R-TGTGCGGATGAAGTCAGCTC	721

Table 2. Detection of *uspA* gene and antibiotic resistant genes (*blaTEM*, *tetA* and *sul* II) in MDR *E. coli* isolates

MDR *E. coli* (n=10) (Isolate ID)	Target genes	Samples positive for *uspA* and antibiotic resistant genes	% Prevalence
(E1), (E2), (E3), (E4), (E5), (E6), (E7), (E8), (E9), (E10)	uspA	(E1), (E2), (E3), (E4), (E5), (E6), (E7), (E8), (E9), (E10)	(100%)10/10
	blaTEM	(E6), (E7)	(20%) 2/10
	tetA	(E4), (E6)	(20%) 2/10
	sulII	(E4), (E6), (E7)	(30%) 3/10

gDNA fungal/bacterial mini kit (Genetix, New Delhi, India). The PCR procedure to screen the species specific gene *uspA* and antibiotic resistance genes *blaTEM*, *tetA* and *sul* II were standardized (Table 1). The reaction mixture was optimized to contain 12.5µl Green Taq PCR master mix, 10 nmol (0.5µl) of each forward and reverse primer, 10.5µl nuclease free water and 1µl of DNA template.

Negative control was run in the last well. The thermal cycling conditions comprised of initial denaturation at 94ºC for 5 min. followed by 30 cycles of denaturation (94º C/1min), annealing (*uspA*; 55º C/1 min., *blaTEM*; 51º C/1 min., *tetA*; 55ºC/1 min. and *sul* II; 52º C/1 min.) and extension (72º C/1-2 min.) with final extension at 72º C for 5 min. The resultant PCR products were viewed using 1.5% agarose gel by electrophoresis which was carried out at 70V for 60 minutes.

RESULTS AND DISCUSSION

Antimicrobial susceptibility pattern of *E. coli* isolates

All the 50 *E. coli* isolates were found to be highly resistant for erythromycin (80%) and oxytetracycline (76%). In total, 54% isolates were resistant for cefotaxime. Ralte *et al.* (2021) found that Cefoperazone and sulbactum combination were effective against *E. coli*. Similar findings among *E. coli* isolates were revealed by El-Zubeir *et al.* (2006) who reported 76.47% isolates as resistant for erythromycin. Ramasamy *et al.* (2021) and Bisht *et al.* (2020) reported high resistance in *E. coli* isolates for tetracycline. Further, 90%, 84%, 82% and 76% of the recovered *E. coli* isolates were found to be sensitive towards chloramphenicol, ampicillin, gentamicin and ceftriaxone, respectively. Mohanty *et al.* (2013) and Liu *et al.* (2014) reported similar findings in which 90% and 91.3% of *E. coli* isolates were found to be sensitive to chloramphenicol, respectively. Also, high susceptibility towards gentamicin and ceftriaxone was reported by Singh *et al.* (2021). Similarly, *E. coli* recovered from bovine mastitis were also found to be sensitive for chloramphenicol and gentamicin (Mittal *et al.*, 2018). The sensitivity pattern of the isolates towards amoxicillin/clavulanic acid was found to be 62% in our study which was in similar lines with the sensitivity rates reported by Vasquezs-Garcia *et al.* (2017). The antimicrobial susceptibility patterns of *E. coli* are shown in Fig. 1. The development of antibiotic resistance can be

attributed to the non-judicious use and non-compliance to the recommended dose regimens of antibiotics. Therefore, the antibiotics should be used prudently to curb this menace of antibiotic resistance. Also, the farmers must be made aware about the necessity of conducting antibiotic sensitivity test on the dairy farms on a regular basis.

Molecular characterization of MDR *E. coli* isolates by targeting *uspA*, *blaTEM*, *tetA* and *sul* II genes

The universal stress protein gene (*uspA*) is used for the molecular confirmation of the *E. coli* isolates (Yarar and Turkyilmaz, 2019). In the present study, all the *E. coli* isolates were found to be positive for *uspA* gene (Fig. 2). Further, all the 10 MDR *E. coli* (resistant to atleast three antibiotic classes) isolates recovered from bovine subclinical mastitis were used for the detection of antibiotic resistance genes (Table 2). The *tetA* gene was detected in 20% of the MDR *E. coli* isolates. Maynard *et al.* (2003) reported 25% isolates as positive for *tetA* gene. While, Abed *et al.* (2021) and Youssif *et al.* (2021) reported contrasting finding in which 100% prevalence of *tetA* gene was observed in the *E. coli* isolates obtained from subclinical mastitis. Further, the prevalence of *sul* II and *blaTEM* genes observed in the present study were found as 30% and 20%, respectively. For *sul* II gene, similar findings were described by Yu *et al.* (2020) who found 32.5% *E. coli* isolates as resistant for sulphonamides based on the molecular confirmation of *sul* II gene. While, Youssif *et al.* (2021) revealed that *blaTEM* genes related to β-lactam resistance were expressed in all the examined *E. coli* isolates.

CONCLUSION

The antibiogram analysis of all the 50 *E. coli* isolates recovered from bovine subclinical mastitic milk samples revealed high resistance to erythromycin (80%), oxytetracycline (76%) and cefotaxime (54%). Also, the occurrence of antibiotic resistance genes i.e. *blaTEM*, *tetA* and *sul* II genes in MDR *E. coli* isolates was 20% (2), 20% (2) and 30% (3), respectively. Therefore, the control strategies should focus on preventing the attainment of resistance towards frequently used antibiotics. Regular screening and antimicrobial susceptibility testing play an important role in deciding the suitable antibiotics. Also, effective extension activities are required to make the dairy farmers aware about the judicious use of antibiotics.

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