Haryana Vet. (June, 2024) **63(1),** 16-20

Research Article

IN VITRO EFFECT OF SPICES ON ESBL PRODUCING *E. COLI* BACTERIA ISOLATED FROM MILK OF APPARENTLY HEALTHY CATTLE

R. POOJITHA, A. SHRIVASTAV*, N. SHRIVASTAVA[1], N. KUMAR, A.K. JHA[2], S.K. SINGH, R. RANJAN, RINESH KUMAR[3] and BALESHWARI DIXIT[4]

Department of Veterinary Pharmacology & Toxicology, [1]Department of Veterinary Microbiology, [2]Department of Animal Breeding and Genetics, [3]Department of Veterinary Parasitology, [4]Department of Veterinary Public health & Epidemiology, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, India

Received: 26.05.2023; Accepted: 17.07.2023

ABSTRACT

The present study was undertaken to observe the effect of spices on the extended-spectrum beta- lactamase enzyme of ESBL producing *E. coli* bacteria isolated from milk of apparently healthy cattle. Out of 100 samples collected randomly from various dairy farms located at different areas of Rewa, Madhya Pradesh, 14 samples were found to be ESBL positive yielding a positivity rate of 14%. Positive isolates were further characterized by the phenotypic standard methods, such as combine disc diffusion test (CDDT), double disc synergy test (DDST) and enzyme MIC strip method. Inhibitory potential and per cent inhibition of beta lactamase enzyme were observed against dry powder of *Methi* seeds (*Trigonella foenum-graecum*), dry *zinger*, (*Zingiber officinale*), *Ajwain* seeds (*Trachyspermum ammi*) *Kalonji* seeds (*Nigella sativa*), *Black pepper* (*Piper nigrum*), *Clove* bud (*Syzigium aromaticum*) (each in a concentration of 10 mg/ml) and one test drug Tazobactum by Iodometric method. Maximum effect was observed for *Zingiber officinale* (9.10±0.40) and the minimum inhibitory potential has been shown by *Syzgium aromaticum* (15.67±0.45). The zone of inhibition observed for *Piper nigrum* and *Nigella sativa* (12.21±0.47, 11.95±0.45) gave comparatively lower per cent inhibition. The result exhibited that spices contain phytochemicals, which have both antimicrobial and antioxidant properties. The findings of the present study recommended use of spices for controlling over-usage of antibiotics and could serve as a better alternative for antimicrobials.

Keywords: Antimicrobial resistance, ESBL, Milk, *Nigella sativa*, *Syzygium aromaticum*, *Trachyspermum ammi*, *Zingiber officinale*, *Trigonella foenum-graecum*

How to cite: Poojitha, R., Shrivastav, A., Shrivastava, N., Kumar, N., Jha, A.K., Singh, S.K., Ranjan, R., Kumar, R. and Dixit, B. (2024). *In vitro* effect of spices on ESBL producing *E. coli* bacteria isolated from milk of apparently healthy cattle. *Haryana Vet.* **63(1)**: 16-20.

Milk is a large part of the human food plan and it has a crucial role to play in food. The presence of pathogenic microorganism in milk is of significant public health challenge. Milk is an ideal medium for the speedy multiplication of microorganism, particularly in unhygienic manufacturing and storage. *Escherichia coli* and *Klebsiella pneumoniae* are people and animals' opportunistic pathogens, liable for a huge variety of infections. Since 2000, the European Antimicrobial Resistance Surveillance community has stated a constant boom in the number of invasive *E. coli* and *Klebsiella pneumonia* isolates resistant against third and fourth-generation cephalosporins. Beta-lactams antibiotics forms one of the crucial groups of antimicrobial agents in veterinary and human therapeutics and are the drug of choice for some established practices, together with dry cow therapy in dairy animals. Presence of ESBL producing Enterobacteriaceae bacteria in milk may become an important issue promoting the emergence, selection and spreading of antibiotic-resistant microorganisms in veterinary and human medication. The expansion of bacterial resistance because of the misuse of antibiotics especially inside the dairy animals can carry perilous outcomes for the animals in addition to people therefore new safe biological alternatives to antibiotics are urgently needed. The most successful options include antimicrobial proteins, bacteriophages, probiotics, and plant-based materials (Kovacevic *et al.*, 2021) Spices, herbs, and their components are usually known as safe (GRAS) and approved through several regulatory agencies consisting of US food and Drug Act, the European Union standards, Codex Alimentarius, and food safety and standards Authority of India (Dhiman *et al.*, 2015).

Several spices, such as clove, turmeric, oregano, thyme, cinnamon, fenugreek seeds are used to treat infectious conditions, due to their antimicrobial activities against morbific and spoilage fungi and bacteria. Furthermore, the secondary metabolites of the spices are recognized as antimicrobial agents, therefore, spices could be used to develop new antimicrobial agents against animals and human pathogens. Looking into the present scenario this study was undertaken.

MATERIAL AND METHODS

Isolation of ESBL producing *E. coli* bacteria

An over-all of 100 milk samples were collected from apparently healthy cattle of various dairy farms for isolation of ESBL producing *E. coli* bacteria. The milk samples were selectively enriched in tryptone soy broth (9 ml TSB per 1 ml milk sample; supplemented with cefotaxime and aztreonam 2 µg/ml ,4 µg/ml, respectively).

*Corresponding author: arpitavet@gmail.com

Overnight culture was streaked on selective agar media (Tryptone bile glucuronic acid, cefotaxime 2 µg/ml and aztreonam 4 µg/ml) to isolate ESBL producing *E. coli* bacteria. The plates were incubated overnight at 42 °C. Presence of *E. coli* was further confirmed through 16s r*RNA PCR*.

Procedure for 16s r*RNA* PCR

The single blue green colony was picked up and isolation of DNA was done using Instagene Matrix as per the manufacturer's instruction. The *E. coli* was identified using 16S r*RNA* primers as per the method of Shailesh *et al.* (2013). The 50 µl PCR reaction was performed using 2X DreamTaq green master mix with 25 pmol of primers and 2 µl of DNA. The PCR amplification conditions were; denaturation for 10 min at 94° C, 30 cycles of 40 s at 94° C, 40 s at 60° C and 1 min at 72° C then final extension of 7 min at 72° C. 5 µl of the amplified PCR product was electrophoresed on a 2% agarose gel in Tris-borate-EDTA buffer (TBE) containing ethidium bromide (0.5 µg/ml). Primers used were F-TGGGAACGGCGAGTCGGA ATAC; R -GGG CGC AGG GGA TGA AAC TCA AC and product size of 1467bp.

Phenotypic Characterization

Double Disc Synergy Test (DDST) - Amoxicillin-clavulanic acid disc (Hi media) was placed in between two cefotaxime disc next to a distance of 20 mm from centre in the agar plates. Augmentation of the inhibition zones around any of the cefotaxime discs towards amoxicillin-clavulanic acid indicated a positive result (Garrec *et al.*, 2011).

Ezy MIC strip test - Cefotaxime and Cefotaxime and clavulanic acid E strip (Hi media make) were used. In case of MIC ratio ≥ 8 or deformed ellipse present around Cefotaxime + clavulanic acid, test was positive (Garrec *et al.*, 2011).

Combination Disc Diffusion Test (CDDT) - cefotaxime 30 µg discs (Himedia) and in combination with clavulanic acid (30-10 µg placed at a particular distance as per the method described by Garrec *et al.* (2011).

Inhibitory potential of spices on extended spectrum beta lactamase enzyme

Positive isolates were later subjected to the inhibitory potential study of spices on the beta-lactamase enzyme isolated as previously described by Yang *et al.* (2009) with slight modifications.

Isolation of beta lactamase enzyme:

Bacterial culture kept overnight inoculated in broth for 2 h at 35° C in a rotary shaker, further incubated for an additional 4 h. after addition of Inducer (Penicillin-G 400 µg/ml). After centrifugation cell pellets resuspended and washed with potassium phosphate buffer (0.05 M, pH 7.0) at 4° C. Bacteria in the cell pellet was again centrifuged and then re-suspended in the same buffer with 10-fold concentration and then disruption of bacteria by 5 minutes sonication in an ice bath. Cellular debris separated with 10000 rpm centrifugation for 4 minutes at 4° C. Supernatants containing beta lactamase enzyme were further stored in -20° C until further use

Iodometric method/ Starch-iodine agar plate method

Inhibitory potential and per cent inhibition of beta lactamase enzyme were observed against dried powder of Methi seeds (*Trigonella foenum-graecum*), dry zinger, (*Zingiber officinale*), Ajwain seeds (*Trachyspermum ammi*) Kalonji seeds (*Nigella sativa*), Black pepper (*Piper nigrum*), Clove bud (*Syzigium aromaticum*). Dry powder of each spice was freshly prepared in triple glass distilled water (100 mg/ml), filter sterilized with 0.2 micron filter further diluted to get a final concentration of 10 mg/ml.

Preparation of Starch iodine plate- Plates were prepared by pouring 60 ml of hot starch agar solution composed of 1.2 g of agar powder, 300 mg of soluble starch and 60 ml of distilled water, to hot agar solution 0.5 ml of iodine solution (5 g iodine,15 g potassium iodide and 100 ml of distilled water) was immediately added into the plate (iodine in the presence of beta lactamase enzyme and penicillin form colourless complex in the form of zone of inhibition). 5 mm diameter 4 wells were punched in the starch-iodine plates with sterile stainless-steel cork borer (Himedia). One well filled with 100 µl of phosphate buffer solution (0.05 M, pH 7.0) as negative control. Second well with 50 µl of crude β-lactamase solution (as mentioned previously) with 50 µl of phosphate buffer solution (0.05 M, pH 7.0) served as a positive control, 50 µl of crude β-lactamase solution and 50 µl of Tazobactum in the third well (standard β-lactamase inhibitor), while fourth well contained 50 µl of crude β-lactamase solution and 100 µl of spices solution (10 mg/ml). In each well 100 µl penicillin G (50 mg/ml) was added after 10 minutes as an enzyme inducer. After incubation at 35° C for 30 min the diameter (mm) of the transparent zones was measured including the diameter (5 mm) of well. The experiments were carried out for 6 samples in triplicates with all the spices individually as well as in combination.

MIC of the spices powder

MIC of the spices powder was determined using serial tube dilution technique as per the method described by CLSI guidelines (CLSI, 2019), for antibiotics. Aqueous solution (containing distilled water) of the spice powders were prepared and serially diluted in the range of 1 mg/ml to 10 mg/ml. After inoculation of the tubes with 100 µl of bacterial culture, the density of selected bacteria was adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml) with sterile distilled water before use. Negative and Positive control was used simultaneously each time. Tazobactum was used as the standard drug for comparison. All the experiments were carried out in triplicates. The minimum inhibitory concentration was taken as the lowermost

Table 1. Inhibitory Effect of Spices on ESBL Enzyme by Iodometric method

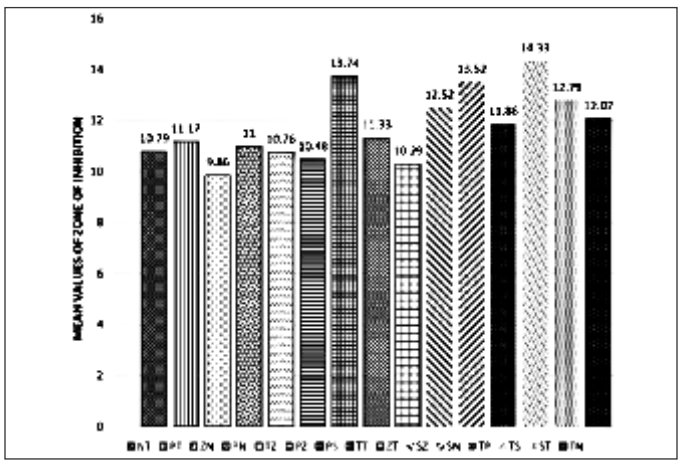
Samples	*T. ammi*	*P. nigrum*	*S.aromaticum*	*T. graecum*	*N. sativa*	*Z. officinale*
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
1.	12.67±0.67	11.33±0.33	15.33±0.67	12.00±0.58	11.67±0.67	9.33±0.33
2.	12.33±0.33	11.67±0.67	16.00±0.58	12.67±0.33	11.67±0.33	9.00±0.58
3.	12.67±0.33	12.33±0.33	17.00±0.58	12.33±0.33	11.67±0.33	9.33±0.33
4.	13.33±0.33	12.67±0.33	16.33±0.88	12.67±0.33	12.33±0.33	9.00±0.58
5.	12.67±0.88	11.33±0.33	16.00±0.00	12.33±0.88	11.67±0.67	9.00±0.58
6.	12.67±0.33	12.33±0.33	16.33±0.67	12.67±0.33	12.33±0.67	8.33±0.33
7.	13.67±0.33	11.67±0.33	15.67±0.67	13.00±0.58	12.67±0.88	9.33±0.33
8.	13.67±0.33	11.67±0.33	15.33±0.33	11.67±0.33	11.67±0.67	10.00±0.58
9.	15.67±1.67	15.67±1.86	16.33±0.33	11.67±0.33	13.67±0.33	9.67±0.33
10.	14.33±0.33	13.33±0.33	16.67±0.33	11.67±0.33	11.67±0.33	9.33±0.33
11.	15.00±2.00	11.67±0.33	15.33±0.33	11.33±0.33	11.67±0.33	8.67±0.33
12.	15.33±0.33	11.33±0.33	14.67±0.33	12.33±0.33	11.67±0.33	8.33±0.33
13.	14.33±0.88	11.33±0.33	14.67±0.33	12.00±0.58	11.67±0.33	8.67±0.33
14.	15.33±0.88	12.67±0.33	13.67±0.33	11.33±0.33	11.33±0.67	9.33±0.33
Mean±SE	13.83^{b}±0.69	12.21^{c}±0.47	15.67^{a}±0.45	12.12^{c}±0.42	11.95^{d}±0.49	9.10^{d}±0.40

Mean with different superscript differ significantly ($p<0.05$).

concentration of spices that totally led to inhibition of bacterial growth in the tube as detected by unaided eye.

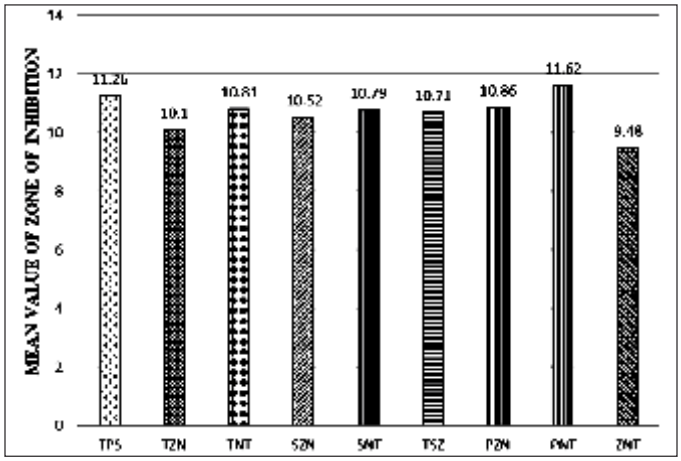
RESULTS AND DISCUSSION

Out of 100 samples, 14 samples were positive for ESBL producing bacteria in the initial screening giving a positivity rate of 14%. ESBL producing bacteria were phenotypically characterized as per the method described by Garrec *et al.* (2011). Only *E. coli* was observed in the findings confirmed by 16s *rRNA* PCR. Correlating with the study done by Batabyal *et al.* (2018) in West Bengal wherein, ESBL producing *E. coli* observed prevalence rate of 12% in healthy animals. Kamaruzzaman *et al.* (2020) also reported occurrence of ESBL *E. coli* in dairy cattle, milk, and farm surroundings in Malaysia, wherein only 0.4% samples were ESBL-producing *E. coli* positive in faeces of lactating cow, 6.5% in farm samples and 16.9% positive in milk samples. Another study, in eastern plain zone of Uttar Pradesh (India) reported 59.58% isolates including 53.33% *E. coli* and 6.25% *Klebsiella* spp. through PCR, out of which 41.25% isolates were ESBL producers including 36.66% *E. coli* and 4.58% *Klebsiella* species by phenotypic confirmation (Yadav *et al.*, 2021). In this context Bairwa *et al.* (2023) also reported serious problem of antimicrobial resistance resulting in treatment failure of bovine subclinical mastitis. Possible reasons of positivity rate of ESBL *E. coli* in our research could have been credited to various reasons, mostly related to milking hygiene and sanitation of the surroundings, proper hygiene and good nutritional level of mother as well as calves. Milk provide an ideal medium for the transmission of diseases caused by *E. coli* bacteria, its source of contamination could be from the milk handling system, health of udder, udder surface, teat and milk storing utensils. Antibiotic dry



NT- *N. sativa+T. ammi*; PT- *Piper nigrum+ T. ammi*; ZN- *Z. officinalis + N. sativa*; PN- *P. nigrum+ N. sativa*; PZ - *P. nigrum + Z. officinale*; TZ- *T. ammi+ Z. officinalis*; PS- *P. nigrum+ S. aromaticum*

Fig. 1. Inhibitory Effect of Combination of Various Spices on ESBL Enzyme



TPS - *T. ammi+ P. nigrum+ S. aromaticum*; TZN- *T. ammi+ Z. officinalis+ N. sativa*; TNT-*T. ammi+N. sativa+T. graecum*; SZN- *S. aromaticum+Z. officinalis+N. sativa*; SNT- *S. aromaticum+N. sativa+T. ammi*; TSZ - *T. graecum+S. aromaticum+N. sativa*; PZN- *P. nigrum+Z. officinalis+N. sativa*; PNT- *P. nigrum+N. sativa+T. graecum*; ZNT- *Z. officinalis+N. sativa+T. graecum*

Fig. 2. Inhibitory Effect of Combination of three Spices on ESBL enzyme

cow management (selective dry cow therapy), mastitis treatment (high self-recovery) are therefore taken as high risk factors allied with incidence of ESBL *E. coli* in cattle.

As per the CLSI and EUCAST most of the ESBL producers were confirmed by three standard methods i.e. Combined Disc Diffusion Test (CDDT) method (Linscott and Brown, 2005), double disc synergy test method (DDST) gave less percent isolation of positive samples, whereas Ezy MIC strip method gave the least positive results correlating with the study of Keite *et al.* (2012) who also reported sensitivity of DDST as 89.2% and of CDDT as 94%. Similar findings were noted by Taslima (2012) and Rahman *et al.* (2004), who found that DDST method only revealed 54% isolation, the CDDT method was able to isolate the largest amount of ESBLs (80%). *Salmonella* species. House flies infesting Kerala farm and non-farm regions revealed presence of ESBL *E. coli*, providing resistance to a variety of antibiotics (Manoj *et al.*, 2023).

Size of zone of inhibition is inversely related to the inhibitory potential and per cent inhibition ability of the spice. Beta lactamase inhibitors like Tazobactum showed no transparent zone inhibiting the activity of β-lactamase completely, on the other hand, the various spices showed small transparent zone around the well, partly inhibiting the beta lactamase activity. *Z. officinale* had the greatest effect (9.10±0.40), whereas *S. aromaticum* and *P. nigrum* had the lowest inhibitory potential (15.67±0.45 and 12.21 ±0.47), respectively. *T. graecum* exhibited a marginally greater inhibitory potential (12.12±0.42) which was statistically significant ($p<0.05$). The zone of inhibition for *T. ammi* is 13.83±0.69, which is less than that of *P. nigrum*, *N. sativa* and *Z. officinale*. *N. sativa* demonstrated moderate inhibition (11.95±0.45). Between *N. sativa* and *Z. officinale*, no significant difference was found ($p>0.05$) (Table 1). Sarowa *et al.* (2023) findings which support our research, also noted the significance of *T. foenum-graecum* seeds for buffalo milk production, highlighting the value of spices. The dry spices powder was used in combination of two spices in equal concentration to see if there is any potentiation effect (Fig. 1). Combination of *N. sativa* and *Z. officinale* gave maximum inhibitory effect (9.86±0.42) and the minimum effect was shown by *T. ammi* and *S. aromaticum* (14.33 ± 0.55). *N. sativa* and *T. graecum* zone of inhibition observed was 10.79± 0.33, whereas the effect of *T. graceum* with *Piper nigrum* was better (11.17± 0.87). The effect of *P. nigrum* in combination with *N. sativa* (11± 0.52) yielded higher inhibitory effect. *P. nigrum* with *Z. officinale* gave higher inhibitory effect than with *N. sativa* with zone of inhibition of Mean±SE (10.48±0.45). Minimum inhibitory potential was observed by *S. aromaticum* (*Clove* bud) powder with largest zone of inhibition (15.67±0.45) in water which differ from the earlier study conducted by Shrivastav *et al.* (2016) in which *S. aromaticum* oil showed maximum effect against ESBL isolates of poultry caeca. Faujdar *et al.* (2020) also reported high antibacterial potential of *Clove* extract (ethanol) for almost all the gram-negative urinary pathogens. *Clove* activity for specific strain was similar between beta-lactamase producing and non- producing isolates.

P. nigrum and *S. aromaticum* in combination showed low inhibitory effect (13.74±0.60). Inhibitory potential of *N. sativa* and *S. aromaticum* (13.52±0.43), *Z. officinale* and *S. aromaticum* (12.5±0.61), *P. nigrum* and *T. ammi* (11.86±0.48), *T. ammi* and *S. aromaticum* (14.33±0.55) was significantly different ($p<0.05$). No significant difference was observed between *T. Ammi*, *Z. officinale*, *N. sativa* and *T. foenum-graecum* (10.76±0.42, 10.79±0.33), respectively (Fig. 1). Combination of *Z. officinale* and *T. foenum-graecum* also showed good inhibitory potential against ESBL enzyme. Similar studies conducted by Kaveri and Joseph (2019) on *E. coli* and *S. aureus* also observed antibacterial activity by mixture of these two spices (1 mg/ml). Fenugreek exhibited very less inhibitory effect against *Escherichia coli* and *Staphylococcus aureus* bacteria when compared to *Z. officinale* which further correlates with our findings (9.10±0.40, 12.12± 0.45) (Fig. 1). Perusal of their data revealed frequently used herbs and spices like *garlic*, *black cumin*, *cloves*, *cinnamon*, *thyme*, *bay leaves*, *mustard* and *rosemary*, hold antimicrobial properties and in specific cases could be used therapeutically (Lai and Roy, 2004).

Combination of three spices in equal proportion with final concentration of 10 mg/ml were evaluated for their antibacterial activity. Among the various combinations *Z. officinale*, *T. foenum-graecum* and *N. sativa* gave the maximum inhibitory effect (9.48±0.88). Whereas, the combination of *P. nigrum*, *N. sativa* and *T. foenum-graecum* together showed the minimum inhibitory effect (11.62±0.62) against ESBL *E.coli* bacteria. *Z. Officinale*, *T. foenum-graecum* and *N. sativa* (9.48±0.88) and *P. nigrum*, *N. sativa*, *T. faenum-graecum* (11.62 ± 0.62) showed significant difference in their inhibitory potential ($p<0.05$).

T. ammi, *P. nigrum*, *S. aromaticum* (10.26±0.42) *T. ammi*, *Z. officinale*, *N. sativa* (10.10±0.37) and *T. ammi*, *N. sativa*, *T. foenum-graecum* (10.81±0.37) showed significant difference in their inhibitory potential ($p<0.05$). Whereas, *T. ammi*, *N. sativa*, *T. foenum-graecum* (10.81±0.37), *S. aromaticum*, *Z. officinale*, *N. sativa* (10.52± 0.59); *S. aromaticum*, *N. sativa*, *T. foenum-graecum* (10.79±0.38); *T. ammi*, *S. aromaticum*, *Z. officinale* (10.71±0.34); *Piper nigrum*, *Z. officinale* , *N. sativa* (10.86±0.43) showed no significant difference in their inhibitory potential (Fig. 2).

In another study Kumar *et al.* (2023) also described antibacterial and antioxidant activity of oregano and lime essential oil against both Gram negative as well as Gram positive isolates. The tannins, saponins, phenolic compounds, essential oil, and flavonoids present in the plants are supposed to be responsible for its antibacterial potency

(Serrano *et al.*, 2009).

MIC values for the aqueous solution of the spices were observed by tube dilution method. The MIC of *Z. officinale* was found to be 0.7 mg/ml, while *N. sativa* showed minimum inhibitory concentration at 0.8 mg/ml, MIC value of *T. foenum-graecum*, *P. nigrum* and *T. ammi* seed powder in water was observed as 0.9 mg/ml, 1 mg/ml and 0.9 mg/ml and highest MIC value was observed for *S. aromaticum* (2 mg/ml). Tazobactum used as the standard drug showed MIC of 8 µg/ml. The higher MIC values in some species specify restricted antibacterial property.

CONCLUSION

The present work observed ESBL producing *E. coli* in approximately 14% of the milk samples from apparently healthy cattle which may be of great health concern from both veterinary and public health aspect. The inhibitory effect of spices observed in our study on the activity of beta lactamase enzyme was maximum with *Z. officinale* and minimum with *S. aromaticum*. Combination of one or more spices gave better results than individual spices. In future, *in vivo* studies of these spices could be recommended in animals which could further reduce selection pressure of the routinely used antibiotics an important precondition for antimicrobial resistance.

REFERENCES

Bairwa, V.C., Gaurav, A., Sharma, D.K., Chavhan, D.M. and Doot, M. (2023). Antibiotic susceptibility pattern of *Escherichia coli* isolated from bovine subclinical mastitis milk. *Haryana Vet.* **62(1)**: 13-15.

Batabyal, K., Banerjee, A., Pal, S., Dey, S., Joardar, S.N., Samanta, I. and Singh, A.D. (2018). Detection, characterization, and antibiogram of extended-spectrum beta-lactamase *Escherichia coli* isolated from bovine milk samples in West Bengal, India. *Vet. World.* **11(10)**: 1423.

CLSI. (2019). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals (4th Edn). 950 West Valley Road, Suite 2500, Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute.

Dhiman, R., Aggarwal, N.K. and Kaur, M. (2015). Comparative evaluation of antimicrobial activities of commonly used Indian spices against microbes associated with juices. *Res. J. Microbiol.* **10(4)**: 170-180.

Faujdar, S.S., Bisht, D. and Sharma, A. (2020). Antibacterial activity of *Syzygium aromaticum* (clove) against uropathogens producing ESBL, MBL, and AmpC beta-lactamase: Are we close to getting a new antibacterial agent? *Fam. Med. Prim. Care Rev.* **9(1)**: 180.

Garrec, H., Drieux-Rouzet, L., Golmard, JL., Jarlier, V. and Robert, J. (2011). Comparison of nine phenotypic methods for detection of extended-spectrum β-lactamase production by enterobacteriaceae. *J. Clin. Microbiol.* **49(3)**: 1048-1057.

Kamaruzzaman, E.A., Abdul, Aziz, S., Bitrus, A.A., Zakaria, Z., Hassan, L. (2020). Occurrence and characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* from dairy cattle, milk, and farm environments in peninsular Malaysia. *Pathogens.* **9**:1007.

Kaveri, C.M., Sincy, J. (2019). Comparative analysis of phytochemical and antibacterial activity of ginger (*Zingiber officinale*) and fenugreek (*Trigonella foenumgraecum*). *Int. J. Botany Stud.* **4(2)**: 2455-541.

Keite da Silva, Nogueira-Miranda, Jussara Kasuko Palmeiro, Danieli Conte, Fernanda Valverde Maia, Iara Taborda de Messias Reason, Cristina Leise Monteiro and Libera Maria Dalla- Costa (2012). Detection of extended-spectrum β-lactamase in *Enterobacter* spp. evaluation of six phenotypic tests. *Microb. Drug Resist.* pp. 66-70.

Kovacevic, Z., Radinovic, M.C., Abarkapa, I., Kladar, N. and Bozin, B. (2021). Natural agents against bovine mastitis pathogens. *Antibiotics.* **10**: 205.

Kumar, D., Mehta, N., Panwar, H., Chatli, M.K. and Malav, O.P. (2023). *In-vitro* evaluation of antibacterial and antioxidant activity of essential oils from oregano (*Origanum vulgare*) and lime (*Citrus aurantifolia*). *Haryana Vet.* **62(1)**: 101-105.

Lai, P.K. and Roy, J. (2004). Antimicrobial and chemo preventive properties of herbs and spices. *Curr. Med. Chem.* **11(11)**: 1451-1460.

Linscott, A.J. and Brown, W.J. (2005). Evaluation of four commercially available extended spectrum beta-lactamase phenotypic confirmation tests. *J. Clin. Microbiol.* **43(3)**: 1081-1085.

Manoj, Prejit, M., Vergis, J., Vinod, V. K., Asha, K. and Yadav, V. (2023). Occurrence of extended spectrum beta-lactamase producing *Escherichia coli* and *Salmonella* spp. in houseflies infesting farm and non-farm premises in Wayanad, Kerala, India. *Haryana Vet.* **62(1)**: 42-45.

Rahman, M.M., Haque, J.A., Hossain, M.A., Sultana, R., Islam, F., AHM. and Islam, S. (2004). Prevalence of extended spectrum beta lactamase-producing *Escherichia coli* and *Klebsiella* pneumoniae in an urban hospital in Dhaka Bangladesh. *Int. J. Antimicrob. Agents.* **24**: 508-510.

Sarowa, A., Chhikara, S.K., Sahu, S., Yadav, D.C., Amandeep, Ghotar, R. and Komal (2023). Effect of fenugreek (*Methi*) supplementation on milk production, milk composition and haemato-biochemical parameters in Murrah buffaloes. *Haryana Vet.* **62(1)**: 1-4.

Serrano, J., Puupponen-Pimia, R., Dauer, A., Aura, A.M. and Saura-Calixto F. (2009). Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Mol. Nutr. Food Res.* **53**: 310-29.

Shailesh, K., Shahi, Vinay, K., Singh and Ashok Kumar (2013). Detection of *Escherichia coli* and Associated b-lactamases genes from diabetic foot ulcers by multiplex PCR and molecular modeling and docking of SHV-1, TEM-1 and OXA-1 b-lactamases with Clindamycin and Piperacillin-Tazobactam. *Plos One.* **8(7)**: 1-13.

Shrivastav, A., Sharma, R.K., Sahni, Y.P., Shrivastav, N., Gautam, V. and Jain, Sachin. (2016). Study of antimicrobial resistance due to extended spectrum beta lactamase producing *Escherichia coli* in healthy broilers of Jabalpur. *Vet. World.* **9(11)**: 1259-1263.

Taslima, Y. (2012). Prevalence of ESBL among *E. coli* and *Klebsiella* spp. in a tertiarycare hospital and molecular detection of important ESBL producing genes by multiplex PCR. *Int. J. Antimicrob. Agents*. **45**: 678-681.

Yadav, V., Joshi, R.K., Joshi, N., Kumar, A. and Singh, S. (2021). Status of multidrug resistance among ESBL producing *E. coli* and *Klebsiella* spp. isolates of buffalo origin in eastern plain zone of Uttar Pradesh. *Haryana Vet.* **60(2)**: 208-212.

Yang, Z., Xiaosheng, Y. and Yule Niu. (2009). A novel and rapid method to screen for β-lactamase inhibitors from extracts of herbs. *Int. J. Integr. Biol.* **6(2)**: 62-64.