

ANTIBIOGRAM OF *E.coli* SPP INVOLVED IN BOVINE MASTITIS IN AND AROUND PRODDATUR REGION

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Abstract: The present study was aimed to study antimicrobial susceptibility pattern of *E.coli* spp involved in mastitis. Mastitis milk samples were collected aseptically and inoculated in to BHI broth and *E.coli* spp were isolated using MCA agar and EMB agar. Biochemical characterization was carried out for its identification and finally ABST was performed for isolated *E.coli* following standard protocols. On ABST *E.coli* spp were highly resistant towards pencillin-G (100%), cephalixin(100%), cephalothin (100%), cefotaxime (100%), followed by ceftazidime (72%), ceftriaxone (52%), Cefepime (48%), Cefoxitin (40%), and Cefuroxime (32%). Intermediate resistance was observed against Cefuroxime(48%), ceftriaxone (32%), followed by Cefepime(28%), Cefoxitin(20%). In the present study *E.coli* spp were highly susceptible to Imipenem (56%) followed by Cefoxitin(40%) etc. Antimicrobial analysis clearly explains that multidrug resistant *E.coli* spp were involved in mastitis.

Keywords: Mastitis, multidrug resistance, B-lactamases.

Introduction

Mastitis is a multifactorial, economically important infectious disease affecting dairy industry throughout the world. Mastitis due to *E. coli*, *Klebsiella* spp. and *Enterobacter* spp is known as coliform mastitis, among which *E.coli* is frequently involved [1]. Un hygiene managmental practices is the major predisposing factor for coliform mastitis [2]. Antibiotic treatment in case of coliform mastitis is not completely effective because of emergence of multidrug resistance *E.coli* strains [3]. Beta-lactam group of antibiotics are commonly used for treatment in veterinary due to their efficacy, low cost and minimal side effects. Because of common & indiscriminate use of B-lactam group of antibiotics leads to emergence of resistance among coliforms mediated by expression of B-lactamases [4].

Materials and Methods

Mastitis milk samples were collected aseptically following standard aseptic procedures [5].

Isolation and Identification

The milk samples was inoculated in to BHI broth and incubated at 37⁰c for 24 hrs. After

incubation loop full of culture was streaked on to MacConkey agar (MCA agar), followed by Eosin methylene blue agar (EMB agar). Finally biochemical tests like catalase, oxidase, indole, methyl red, vogesproskauer and citrate tests were performed according to standard protocols [6]. After confirmation, antimicrobial sensitivity test is carried out for *E.coli* spp.

Kirby-Bauer disc diffusion method

After confirmation by biochemical tests, individually each *E.coli* isolate was inoculated in to nutrient broth and incubated at 37⁰c for 4-6 hours. Finally antibiotic susceptibility test is performed on Mueller-Hinton agar plates as per standard protocols [7].

Results

On gram staining pink color medium sized rods, Pink color colonies on MCA agar and metallic sheen colonies were observed on EMB agar indicates *E.coli*. The biochemical tests catalase- positive, oxidase- Negative, IMVC (++-) and reaction on TSI agar slant (Y/Y/H₂S-ve) confirms that isolated bacteria as *E.coli* spp. The antimicrobial discs (Himedia), their concentrations, and their susceptibility patterns (%) were mentioned in **Table-1**.

Table 1: Antimicrobial susceptibility patterns of *E.coli* spp (%)

Antibiotic disc	Symbol	Potency µg per disc	Sensitivity (%)	Intermediate (%)	Resistance (%)
Pencillin -G	P	10 units	0	0	100
Cephalexin	CP	30 µg	0	0	100
Cephalothin	CEP	30 µg	0	0	100
Cefuroxime	CXM	30 µg	20	48	32
Ceftriaxone	CTR	30 µg	16	32	52
Cefotaxime	CTX	30 µg	0	0	100
Ceftazidime	CAZ	30 µg	8	20	72
Cefepime	CPM	30 µg	24	28	48
Cefoxitin	CX	30 µg	40	20	40
Imipenem	IPM	10 µg	56	28	16

Discussion

Mastitis is a serious problem to dairy industry. Poor management and unhygienic milking practices mostly lead to coliform mastitis. In the present study about fifty *E.coli* spp were isolated from hundred and fifty milk samples indicating that *E.coli* spp is one of the

major causative agent of mastitis which is in accordance with previous studies [8&9]. Multi drug resistance is a common burning problem facing today. Inadequate use of antibiotics leads to development of multidrug resistant *E.coli*. All *E.coli* isolates obtained in the present study were totally resistant to penicillins, cephalosporins, cephalothin and cefotaxime indicating existence of B-lactamases. In the present study *E.coli* organisms were highly susceptible to imipenem followed by cefuroxime, ceftazidime and ceftipime indicating that carbapenems is a drug of choice against multidrug resistant *E.coli*. Resistance to B-lactam group of antibiotics was already reported in several studies [10&11].

Conclusion

The above study concludes that multidrug resistant *E.coli* were commonly involved as mastitis causative agents. Carbapenem group of antibiotics are useful to some extent for treatment. Further studies should be carried out for confirmation of ESBL producers which impose a major threat.

References

- [1] Eberhart R J, Natzke RP and Newbould F H S 1979 Coliform mastitis: A review. J. Dairy. Sci. **62**: 1-22.
- [2] Radostits O M, Blood D C, Gay CC, Blood D C and Hinchkliff K W 2000 Veterinary Medicine. 9th Edn, ELBS-Bailliere Tindal, London pp: 563-618.
- [3] Unakal CG and Kaliwal BB 2010 Prevalence and antibiotic susceptibility of *Staphylococcus aureus* from bovine mastitis. Vet. World. **3**: 65-67.
- [4] Babic M, Hujer A M and Bonomo R A 2006 .what's new in antibiotic resistance? Focus on beta-lactamases. Drug Resistance Updates : Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. **9**(3): 142-56.
- [5] Marimuthu M, Abdullah M, Jesse F F, Mohammed K, Adamu L, Osman A Y, Abba Y and Tijjani A 2014 Prevalence and antimicrobial resistance assessment of subclinical mastitis in milk samples from selected dairy farms. Am. J. Anim. Vet. Sci. **9**(1): 65-70.
- [6] Markey B K, Leonard F C, Archambault M, Cullinane A and Maguire D 2013 Clinical Veterinary Microbiology, 2nd edition.
- [7] Clinical and Laboratory Standards Institute 2014. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI document M100-S24.
- [8] Ranjan R, Gupta M K and Singh K K 2011. Study of bovine mastitis in different climatic conditions in Jharkhand, India. Veterinary World **4**(5): 205-208.

- [9] Elango A, Doraisamy K A, Rajarajan G and Kumaresan G 2010 Bacteriology of sub clinical mastitis and antibiogram of isolates recovered from crossbred cows. *Indian J. Anim. Res.* **44**(4): 280–284.
- [10] Weiner M, Rozanska H, Kubajka M, Szolowski K, Krajewska M and Wasinski B 2015 Occurrence and characterization of MRSA and extended-spectrum β -lactamases producing *Escherichia coli* isolated from mastitis cow's milk. *Bull. Vet. Inst. Pulawy.* **59**: 191-195.
- [11] Su Y, Yu C Y, Tsai Y, Wang S H, Lee C and Chu C 2014 Fluoroquinolone resistant and extended spectrum β -Lactamase (ESBL) producing *Escherichia coli* from milk of cow with clinical mastitis in southern Taiwan. *Journal of Microbiology, Immunology and Infection.*