ANTIBACTERIAL SUSCEPTIBILITY PROFILES OF *COLIFORMS* ISOLATED FROM BOVINE SUBCLINICAL AND CLINICAL MASTITIS AGAINST FLUOROQUINOLONES

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ABSTRACT

The primary objective of this study was to assess the antibacterial sensitivity pattern of Coliforms isolates from clinical and sub-clinical mastitis to quinolones. Forty coliforms isolates from bovine clinical and subclinical mastitis were tested for antimicrobial susceptibility by measuring MIC by Resazurin dye broth microdilution method. Of the 27 E. coli isolates tested, only 7.4% of the isolates were found to be susceptible to ofloxacin and 7.37, 55.55 and 59.25% of isolates were found to be resistant to ciprofloxacin, enrofloxacin and ofloxacin, respectively. The proportion of coliform isolates other than E. coli resistant to fluoroquinolones tested were 30-47%. The result of this study indicates the presence of high level of resistance of coliforms against fluoroquinolones. This necessitates routine monitoring of resistance pattern among field isolates of mastitis and to develop suitable antimicrobial usage policy.

Key Words: Mastitis **-** Coliforms- *Escherichia coli* - Antimicrobial resistance- Fluoroquinolones

INTRODUCTION

Bovine mastitis is one of the most important diseases of high yielding dairy animals and is responsible for heavy economic losses. It has been reported that the annual economic losses due to bovine mastitis increased 114 folds in about 4 decades from 1962 (INR 529 million/ annum) (Dhanda and Sethi, 1962) to 2001 (INR 60532 million/annum) (Sharma et al., 2012).

Coliforms such as *Escherichia coli*, *Klebsiella* Spp., *Enterobacter* spp., *Citrobacter* spp. are frequently reported in cases of bovine mastitis (Dembele et al., 2006). Antimicrobial therapy is the primary tool for controlling mastitis. The main reason for low efficacy of antibiotic treatment of mastitis cases is the resistance of the bacteria to antimicrobials (Gitau et al., 2011). Continuous monitoring of resistance pattern of isolates is important to select the antimicrobials for successful

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treatment as well as to take initiatives to prevent development of resistant isolates. Hence the present study was designed with the following objective of studying the antibacterial sensitivity pattern of coliform isolate from clinical and sub-clinical mastitis to fluoroquinolones and assessing resistance pattern of coliforms against quinolones.

MATERIALS AND METHODS

Bacterial Isolates

Forty coliforms (*Escherichia coli*, *Enterobacter* spp and *Klebsiella* Spp) isolates from bovine clinical and subclinical mastitis were used in this study. All strains were identified by standard procedures and maintained frozen at 20°C in the Dept. of Veterinary Pharmacology and Toxicology, Madras Veterinary College.

Drugs and Chemicals

Antibacterial susceptibility of isolates were analyzed for three fluoroquinolones: Ciprofloxacin (Hi Media Laboratories, Mumbai), Enrofloxacin (Hi Media Laboratories, Mumbai) and Ofloxacin (M/s Microlabs Pvt Ltd., Bangalore). Antibacterial stock solutions (1 mg/ml) were prepared by initially dissolving 10 mg of antibiotics in 10 ml of respective solvent and further diluting it in distilled water. Ciprofloxacin and Enrofloxacin were initially reconstituted in 0.1 N NaOH whereas ofloxacin was reconstituted in 0.1 N HCl.

Antimicrobial susceptibility testing

The MICs of antimicrobials were determined by a Resazurin dye broth microdilution method (Sarker et al., 2007). Serial two fold dilutions of drugs in nutrient broth (50 µl) were prepared in 96 well ELISA plates. Resazurin dye (10 µl), nutrient broth (30 µl) and bacterial suspension (10 µl) adjusted to 5 x 10^6 CFU/ml was added in the order to all the wells. Sterile control without bacteria and growth control without drug were also included. Plates were wrapped and incubated at 37°C for overnight. The lowest concentration which inhibited visible growth (persistence of blue color) of bacteria was recorded as MIC. The MIC_{50} and MIC_{90} are parameters determined as the MICs at which 50% and 90% of the isolates tested were inhibited and are useful clinically to arrive at the therapeutic concentration. The MIC_{50} and MIC_{90} were arrived by arranging MICs for individual isolates on ascending order and then identifying the concentration at which MICs 50% and 90% of isolates, respectively, were inhibited. All the experiments were performed in triplicate. MIC results were interpreted in accordance with MIC interpretive criteria recommended by NCCLS. The MIC breakpoints indicating susceptibility, moderate susceptibility (intermediate), and resistance to ciprofloxacin were ≤1, 2, and ≥ 4 ìg/ml (Brown-Elliott *et al*, 2002), those for enrofloxacin were ≤ 0.5, 1-2, and ≥ 4 ìg/ml (Boothe, 2006) and those for ofloxacin were ≤ 2, 4, and ≥8 ìg/ml (Fuchs *et al*., 1989)

RESULTS AND DISCUSSION

The MIC values of *E. coli* and coliforms other than *E. coli* are presented in Table 1 and 2, respectively. The MIC of ciprofloxacin, enrofloxacin and ofloxacin against *E. coli* isolates ranged from 1.22-312.5 µg/ml,

1.22-62.5 µg/ml and 0.61-156.25 µg/ml, respectively.

Of the 27 isolates tested, only two isolates (7.4%) were susceptible to ofloxacin with an MIC of 0.61 and 1.22 µg/ml. Similarly, eight (29.62 %), 12 (44.44%) and nine (33.33%) of the 27 isolates showed moderate susceptibility to ciprofloxacin, enrofloxacin and ofloxacin, respectively. A total of 70.37% of isolates were resistant to ciprofloxacin compared with 55.55% and 59.25% for enrofloxacin and ofloxacin, respectively. Watts et al., (1997) reported MIC value of ≥0.015–0.03 ìg/ml for enrofloxacin and ≥0.015 ìg/ml for ciprofloxacin against *E. coli* isolate from bovine mastitis. Martin *et al.*, (2003) reported MIC value of 0.062-16.0 ìg/ml for enrofloxacin against *E. coli* isolate from mastitis. Jousimies-Somer et al. (1996) reported MIC of 0.25–165 ìg/ml for Ofloxacin against Gram negative bacteria from bovine summer mastitis. For the coliforms other than *E. coli (Enterobacter* sp. and *Klebsiella* sp.), 7 of 13 (53.84%), 9 of 13 (69.23%), 4 of 13 (30.76%) isolates showed moderate susceptibility to ciprofloxacin, enrofloxacin and ofloxacin, respectively. In the present study, 46.15, 30.76 and 30.76% of coliforms other than *E. coli (Enterobacter* sp. and *Klebsiella* sp.) were found to be resistant to ciprofloxacin, enrofloxacin and ofloxacin, respectively.

The fluoroquinolones are an important and highly used group of antimicrobial drugs in human and veterinary medicine for a wide variety of microbial infections. In our study, we found that the frequencies of resistance of *E. coli* strains to enrofloxacin, ciprofloxacin and ofloxacin were very high. However, the proportion of *Enterobacter* and *Klebsiella* isolates that showed resistance to fluoroquinolones tested were less when compared to *E. coli*. Hendriksen *et al.* (2008) reported higher levels (35%) of resistance to fluoroquinolones among *E. coli* strains isolated from cattle in European countries.

In the present study, the levels of resistance of coliforms other than *E. coli* to fluoroquinolones tested were similar. Compared to this, the level of resistance of *E. coli* to enrofloxacin was similar to the level of resistance to ofloxacin. This is due to the development of cross-resistance to one of the fluoroquinolones generally

Enrofloxacin and other fluoroquinolones are commonly used for the treatment of mastitis. Indiscriminate and unwarranted usages are the contributing factors for development of high level of resistance. Fluoroquinolone resistance is usually chromosomal-mediated arising from mutations in *gyr A* (DNA gyrase) and *par C* subunits (topoisomerase IV). Resistance to fluoroquinolones can also be mediated by plasmids that produce Qnr protein, which binds to and protect DNA gyrase and topoisomerase IV from inhibition by quinolones. Qnr by itself produces only low-level resistance but facilitates the selection of higher-level resistance mutations, thus contributing to the alarming increase in resistance to quinolones (Jacoby, 2005). The present study provides preliminary evidence about fluoroquinolone resistance in coliforms and necessitates the monitoring of resistance pattern among field isolates of mastitis.

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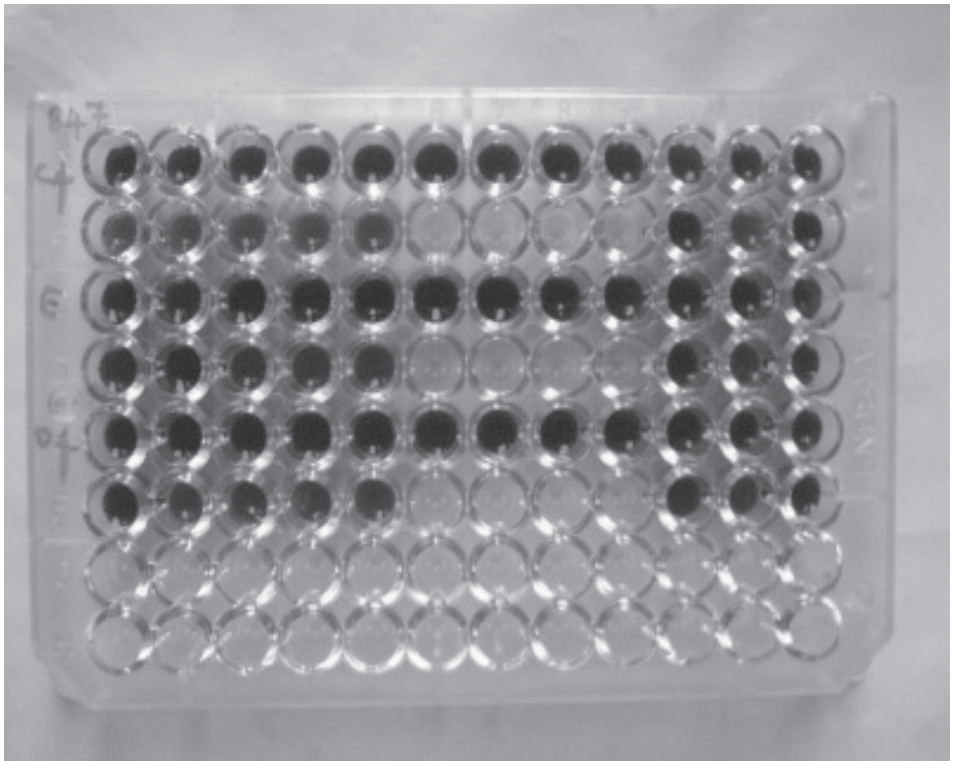
Table 1 MIC results for *E.coli* isolates (n= 27) from bovine mastitis

Parameter	Ciprofloxacin	Enrofloxacin	Ofloxacin
MIC range (ìg/ml)	1.22 – 312.5	1.22 – 625	0.61 – 156.25
MIC (ìg/ml, Mean ± SE)	36.32 ± 12.65	59.83 ± 25.63	23.21 ± 7.74
MIC_{50} (ìg/ml)	9.76	4.88	9.76
MIC_{90} (ìg/ml)	78.25	156.25	39.06
% Resistant	70.37	55.55	59.25
% Susceptible	-	-	7.40
% Intermediate susceptible	29.63	44.45	99.98

Table 2 MIC results for coliforms other than *E. coli* (n=13)

Parameter	Ciprofloxacin	Enrofloxacin	Ofloxacin
MIC range (ìg/ml)	2.44 – 312.5	0.61 – 312.5	1.22 – 78.25
MIC (ìg/ml, Mean ± SE)	27.98 ± 23.72	32.07 ± 24.09	10.81 ± 5.81
MIC_{50} (ìg/ml)	2.44	1.22	4.88
MIC_{90} (ìg/ml)	9.76	78.125	19.53
% Resistant	46.15	30.76	30.76
% Susceptible	-	-	38.46
% Intermediate susceptible	53.84	69.23	30.76

Fig. 1 MIC result of *Coliform*



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