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In vitro Efficacy for Some Local Antimicrobial Products Against *E. coli* K12

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Abstract: The aim of this study was to assess the *in vitro* activity of a number of antimicrobial agents (Sultrim, Oxytetracycline, enrofloxacin and florfenicol) produced by different Iranian manufacturers against *E. coli* K12. Comparison of the MICs of reference antimicrobials with Disk Diffusion (DD) results of different products made by Iranian manufacturers revealed that all products of tested antimicrobials seem to be in synergy with their reference MIC evaluation. Present data indicate that *E. coli* K 12 is resistant to Enrofloxacin (MIC value of 6.25 μg mL^{-1}) by both MIC and DD evaluation.

Key words: Antimicrobial products, Iranian manufacturers, MIC evaluation, disk diffusion, *E. coli* K12

INTRODUCTION

Microorganisms have developed unlimited resistance to antibiotics since their introduction into clinical practice. The emergence of antibiotic-resistant strains among these organisms has resulted in a major public health concern worldwide (Mosland *et al*., 2006).

Essentially there are two methods of Antimicrobial Susceptibility Testing (AST): Agar diffusion methods and antimicrobial dilution tests. Quantitative methods include agar dilution and broth dilution and the commercially available E-test Minimum Inhibitory Concentration (MIC) strips. The MIC is the minimum inhibitory concentration of antibiotic that inhibits growth of the organism (King and Brown , 2001). Qualitative methods include disk diffusion test which is a qualitative method since the organism is classified as susceptible (S), intermediate (I), or resistant (R) to the antibiotic (NCCLS, 1997). Several antimicrobials, such as sultrim, oxytetracycline, enrofloxacin and florfenicol are manufactured by different Iranian companies.

Since some of these products are exported to regional countries, to have an idea about the quality and performance of these products, the current study was undertaken to evaluate the susceptibilities of *E. coli* K12 to 5 reference antimicrobial agents as control, using MIC method and their products produced by Iranian manufacturers using the disk diffusion method (NCCLS, 1997).

MATERIALS AND METHODS

The study was conducted through October 2006 to March 2007 in Microbiology Lab. of the Faculty of Veterinary Medicine of Shahrekord University in Shahrekord, Iran.

The minimum inhibitory concentration was selected as the reference method for this study (Saiman *et al*., 1999).

Sultrim, oxytetracycline, enrofloxacin and florfenicol from different manufacturers were the agents assayed. (Table 1).

The following drugs were obtained from ICN biochemical's (sulfadiazine + trimetoprime = sultrim, oxytetracycline), glaxo (enrofloxacin) and padtanteb (florfenicol). The MICs of these drugs were assayed and used as control for their relative products. MICs were determined by a two- fold serial dilution method using Mueller-Hinton broth according to the National Committee for Clinical Laboratory Standards recommendations (NCCLS, 1997). One milliliter of 200 μg mL^{-1} concentration of each drug was added to first serial tubes of tested antimicrobials. The MIC endpoint was the lowest concentration of an antimicrobial that completely visibly inhibited the growth of *E. coli* K12 in a tube.

For disk diffusion, concentrations were in accordance with the NCCLS (1997) recommendations. The ranges of concentrations tested by the disks for each of the

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tested drugs were 23.75, 30, 5 and 30 μg for Sultrim, oxytetracycline, enrofloxacin and florfenicol, respectively.

The same concentrations were used for relative products of mentioned antimicrobials produced locally.

Testing strain was *E. coli* RTCC 2310 (K12) from Razi institute incubated over night in TSB (Tryptonic Soy Broth), at 37 degree centigrade and then adapted with 0.5 McFarland turbidity, adjusted to final concentration of 1.5×10^8 colony-forming units mL^{-1} according to Carter and Chengappa (1991) method.

The plates were then incubated for 20 h at 37°C after which interpretation was performed using a ruler for measuring inhibition diameters according to the criteria recommended by NCCLS (2002a).

RESULTS AND DISCUSSION

MIC of the *E. coli* K12 to tested antimicrobials for Sultrim, Oxytetracycline, Enrofloxacin and Florfenicol were 1.5625, 0.7812, 6.25 and 3.125 μg mL^{-1} respectively. Based on these MICs *E. coli* K12 was resistant to Enrofloxacin but sensitive to others. Zone diameter of reference Enrofloxacin (21 mm) and its products (19- 21 mm) are very near and are near optimum. For sultrim, oxytetracycline and florfenicol zone diameter of reference drugs were 18, 25 and 24 mm respectively, while for their products ranges between 17-20, 22-26 and 22-24 mm, respectively. For Sultrim products these results seems to be the optimum but for others are linear across the range. The results for disk diffusion of these drugs and their products are summarized in Table 1, as it appeared the results for DD of the above antimicrobials and their local products are just as the results of MICs of reference drugs.

The aim of this study was to compare the reference group of antimicrobials and their locally produced products against *E. coli* K12 a representative of gram negative bacteria to have an idea about the quality and performance of these products.

Animal species-bacterial pathogen breakpoints and zone interpretive criteria for *in vitro* susceptibility testing were as outlined in the NCCLS (2001).

Our data indicate that *E. coli* K12 is resistant to enrofloxacin (MIC value of 6.25 μg mL^{-1}) by both MIC and DD evaluation. No remarkable difference between reference and local products of this drug were observed. It therefore seems likely that the reduced susceptibility to fluoroquinolones of this bacteria is sufficient to allow it to survive antibiotic challenge in chickens or animal infections.

Based on MIC of reference florfenicol (3.125 μg mL^{-1}) and DD inhibition zone diameter (NCCLS, 2002b) of related products (22-24 mm), Table 1, tested *E. coli* was sensitive to this synthetic antibiotic and florfenicol commercial products seem to have acceptable anti *E. coli* effect. However there are no approved NCCLS breakpoints for MIC of *E. coli* currently available, the resistance breakpoint of Florfenicol for other gram negative bacteria (i.e., *Pasteurella multocida*) is suggested from 8 μg mL^{-1} (David *et al*., 2000) to 0.12-4 μg mL^{-1} (Sung *et al*., 2005). In an earlier study, Rajaian *et al*. (2003) examined the concentration of active ingredient of several common veterinary antimicrobial products by disk diffusion method using *Staphylococcus aureus* as tested bacteria. They reported high quality of oxytetracycline products from Iranian manufacturer and 10 to 100% impurities in other antimicrobial products. In

Table 1: Tested antimicrobial agents by disk diffusion method (NCCLS, 2002), their pnrity and relative sources

Antimicrobial agents	Commercial name	Manufactnrer	Zone diameter (mm)	Sensitivity	Pnrity
Sultrim (Sulfadiazine + Trimetoprime)	Chimiaprime	Chimiafam	19	S	80 + 400 mg mL^{-1}
	Sulfabehrood	Behrood	20	S	80 + 400 mg mL^{-1}
	Trisol	Dam loran	18	S	80 + 400 mg cc^{-1}
	Sulfa prime	Aras bazar	17	S	80 + 400 mg mL^{-1}
	Trimetoprime	ICN Biochemical's (USA)	18	S	100%
	Sulfadiazine	ICN Biochemical's (USA)			100%
Oxytetracycline (powder)	Chimiacyclin 50	Chimiafam	22	S	50%
	Chimiacyclin 20	Chimiafam	26	S	20%
	Oxytilin	Dam loran	26	S	20%
	Oxybehrood	Behrood	23	S	50%
	Oxytetracycline	ICN Biochemical's (USA)	25	S	100%
Enrofloxacin	Enrofloxacin	Chimiafam	20	NS	10%
	Enrokin	Dam loran	19	NS	10%
	Enrobeh rood	Behrood Attack	19	NS	10%
	Enrofloxacin	Glaxo (UK)	21	NS	100%
Florfenicol	Florfenicol	Afagh	24	S	10%
	Floracid	Aras bazar	23	S	10%
	Noflosep	Sepideh Dehdasht	22	S	10%
	Florfenicol	Padtanteb	24	S	100%

S = Sensitive, NS = Non Sensitive

this study the data for Oxytetracycline products are in across the range and so in agree with that study but for other products are not in agree. There are some differences between susceptibilities of *E. coli* and *S. aureus* to different antimicrobials and this can be one explanation for this difference. In our study *E. coli* K12 was used as a representative of gram negative bacteria, however all gram negative bacteria have not the same antimicrobial susceptibility patterns, more over this pattern for gram positive bacteria may be completely different. So a complete study on some gram positives and some other gram negative bacteria is essential to achieve a satisfying idea about these products. But as a pilot study we can conclude that, all products of tested antimicrobials seem to be in synergy with their reference MIC evaluation against *E. coli* K12 and can be supposed to be reliable.

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