MINIMUM INHIBITORY CONCENTRATION OF METRONIDAZOLE AGAINST VACCINAL STRAIN OF *PASTEURELLA MULTOCIDA*

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ABSTRACT: *Minimum inhibitory concentration was performed against metronidazole that allowed the growth of Pasteurella multocida type1 (B6) but inhibited the growth of Escherichia coli which is capable of spoiling vaccine during its production and showed no growth in concentration of 3.2 µg/ml. Metronidazole is an antibiotic of choice in confirming the purity of the cultures of vaccinal strain of Pasteurella multocida during vaccine production process*.

Keywords: Pasteurella multocida, Vaccinal strain, MIC, Metronidazole

INTRODUCTION

Pasteurella multocida is Gram negative, nonmotile, facultative anaerobic, coccobacillus. *Pasteurella* infections are usually characterized by local intense cellulitis, purulent discharge, and lymphangitis 12 to 24 hours after the infection [1-3]. The most avoidable mean by which antibiotics become resistant is the misuse of prescribed medication. The proper use of antibiotics should be enforced. Also new products need to be developed including vaccines and diagnostic devices. Most important is that people need to be educated that all the products must be labeled properly for consumers [4]. Minimum inhibitory concentrations against *Pasteurella multocida* isolated from animals was performed. Oxytetracycline, doxycycline, tilmicosin and thiamphenicol for porcine were higher than bovine isolates. MIC of Enrofloxacin was 90 % of the isolates (MIC_{90}) of 0.05 μg/ml for isolates from bovine and porcine. Whereas, MIC_{90} of > 100 μg/ml was recorded for aminoglycosides [5]. Minimum inhibitory concentrations (MICs) of antibacterial agents against *Mannheimia haemolytica* and *Pasteurella multocida* was investigated and no resistance was detected for ceftiofur and florfenicol. Three strains showed an intermediate susceptibility to tilmicosin. Resistance of *Mannheimia haemolytica* and *Pasteurella multocida* to neomycin, gentamicin, spectinomycin, flumequine, enrofloxacin, and chloramphenicol was from 2% to 16% [6].

Antibiotics can be very well used as identification marker in vaccine production process [7]. The technology can be well exploited to monitor vaccinal strain purity throughout the vaccine production processes. Thus the undesirable microbial species can be eliminated or separated from vaccinal strains. The proposed study envisages antibiogram of *Pasteurella multocida* under varying conditions of growth along with determination of minimal inhibitory concentration (MICs) of selected antibiotics. Our main aim was to determine antibiotics for the purity of cuture during vaccine production process.

MATERIALS AND METHODS

To conduct the study different preliminary steps were taken like sterilization of glassware, media preparation and stains preparation. For this purpose, Nutrient agar, MacConkey's agar, Brain Heart Infusion Agar Muller Hinton Agar, blood agar and peptone agar were prepared. Vaccinal strain of *Pasteurella multocida* type 1 B6 was obtained from Veterinary Research Institute (VRI) Lahore. The bacteria was inoculated over these media and incubated for 24 hours. After getting the colonies of bacteria, the staining characteristics were studied by grams's staining; further confirmation of bacterium was conducted by the biochemical tests [8].

Macrodilution broth susceptibility test

Muller Hinton broth was used for this purpose, medium was prepared in ten flasks, each flask was marked as 0.4 µg/ml , 0.8 µg/ml, 1.6 µg/ml, 3.12 µg/ml,6.25 µg/ml,12.5 µg/ml,25 µg/ml,50 µg/ml,100 µg/ml and control (C).The medium was then incubated over night to check sterility. Metronidazloe was added in above mentioned nine flasks according to the dilution marked on them and tenth was kept as control.Twenty test tubes measuring 13x100mm, plugged with cotton were placed in hot air oven for 2 hours at 170 oC. These test tubes were grouped into three, each containing ten tests tubes. The test tubes were then filled with medium containing antibiotic at the rate of 5ml per tube. Next day single colony from *Pasteurella multocida and Escherichia coli* was inoculated into the test tubes of each group. The test tubes were then incubated over night and growth was observed.

RESULTS AND DISCUSSION

Pasteurella multocida was identified by morphological and biochemical characteristic during present study. Minimum Inhibitory Concentration of Metronidazole against *Pasteurella multocida* was performed by serially diluting antibiotic metronidazole as 0.4 µg, 0.8 µg, 1.6 µg/ml, 3.2 µg/ml, 6.4 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml. *Pasteurella multocida* type 1(B6) and isolated extraneous contaminant *Escherichia coli* that was identified in this study was inoculated in said dilutions of antibiotic concentrations. *Pasteurella multocida* type 1(B6) grew in all concentrations while contaminant *Escherichia coli* in concentrations of 0.4 µg/ml , 0.8 µg/ml and 1.6 µg/ml, but same *Escherichia coli* showed no growth in concentrations of 3.2 µg/ml, 6.4 µg/ml, 12.5 µg/ml , 25 µg/ml ,50 µg/ml and 100 µg/ml. (Table 1). Minimal inhibitory concentrations of *Pasteurella multocida* were investigated and found highly resistant for spiramycin

and fosfomycin [9]. Whereas, minimum inhibitory concentrations of doxycycline and oxytetracycline against *Pasteurella multocida* recorded MIC of doxycycline 1 mu g/ml against *Pasteurella multocida* [10]. Studies on efficacy of antimicrobial agents against *Pasteurella multocida was investigated by* agar diffusion method that showed high sensitivity to various antibiotics including enrofloxacin, chloramphenicol, ampicillin, penicillin G and cephalothin [11]. MICs of spectinomycin and other antimicrobial drugs against *Pasteurella multocida* and *Mannheimia haemolytica* strains from cattle with respiratory infections were performed. Results showed that spectinomycin was highly sensitive strains of *Pasteurella multocida* (93.5%) and *Mannheimia haemolytica* (98.6%) [12]. MIC determination against cefovecin and other reference antimicrobial agents was investigated. The MIC90 of cefovecin against *Staphylococcus intermedius* was 0.25 microg/ml, for *Escherichia col was* 1.0 microg/ml and 0.06 microg/ml for *Pasteurella multocida* [13]. Present study would help in better understanding of the identification procedures for the organisms of *Pasteurella* species, especially *Pasteurella multocida* during laboratory identification and vaccine production.

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CONCLUSIONS

It is concluded that contamination usually occurs during vaccine manufacturing process. The main contaminant was *Escherichia coli* in the present study. *Escherichia coli* were highly susceptible to meteronidazloe that can be used for purity of the Haemorrhagic septecaemia vaccine

Table 1. MIC of Metronidazole against Vaccinal strain of *Pasteurella multocida* type 1(B6) and *Escherichia coli*.

S.No	Species	Concentrations of Metronidazole (µg per ml of media)									
		0.4	0.	1.6	3.2	6.4	12.5	25	50	100	Control
1.	*Pasteurella multocida* Type 1(B6)	+	+	+	+	+	+	+	+	+	+
2.	*Escherichia coli*	+	+	+	-	-	-	-	-	-	+

+ = Growth - = No growth

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