

IN VITRO ANTIMICROBIAL SUSCEPTIBILITY OF *PASTEURELLA MULTOCIDA*

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ABSTRACT

Antimicrobial sensitivity of *Pasteurella multocida* was determined. Different media, Muller Hinton agar, Nutrient agar and Brain Heart Infusion agar were used to study the suitable medium for the antibiotic sensitivity. All media were observed equally good for the use with various representative antibiotics. Of these seven from quinolones group, four from cephalosporins group, four from penicillin group, three from aminoglycoside group, one from tetracycline group, and eight from miscellaneous group were tested. *Pasteurella multocida* was sensitive to enrofloxacin, oxytetracycline, chloramphenicol, ampicillin and their zones of sensitivity against the *Pasteurella multocida* was recorded as 22, 22, 21 and 20 mm in diameter, respectively.

Keywords: Antimicrobial susceptibility, Enrofloxacin, *Pasteurella multocida*.

INTRODUCTION

Pasteurella multocida is a non-motile, gram-negative, coccobacillus that is found in the nasopharynx and gastrointestinal tract of many wild and domesticated animals. It is divided into various serotypes on the basis of nomenclature. The serotypes are cause of pasteurellosis in cattle, buffalo, sheep, goat, wild animals and fowl cholera in poultry. The causative agent plays a role in the respiratory disease complex of cattle (Rimler and Glisson, 1997). *Pasteurella multocida* toxin acts intracellularly and inhibits differentiation, particular in bone cells, where it prevents the formation of mineralized bone nodules in-vitro. The toxin is the causative agent of a porcine disease that is characterized by bone resorption. Injection of very low doses of toxin leads to proliferative effects, but at higher dose it is lethal (Lax and Grigoriadis, 2001).

Haemorrhagic septicaemia is a highly contagious fatal disease of cattle and buffaloes with great economic importance in tropics and sub-tropics, including

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Pakistan. It is an acute inflammatory disease of cattle, but buffaloes are more susceptible. In the absence of proper treatment and preventive measures, it gives classical losses to farmers as it has high morbidity and mortality in bovines. Most cases are acute resulting in death within 8 to 24 hours after onset. Animal first evinces dullness, reluctant to move, fever, salivation, and serous nasal discharge. Oedematous swelling is frequently seen in the throat region and spreading to the parotid region, neck and brisket and animal dies within hours due to respiratory distress. Because of rapid course (8 to 24 hours) of disease it is important to use antimicrobial agents which are very effective. Various sulphonamides, tetracycline, penicillin and chloramphenicol are effective if administered early (Aiello, 1998).

Berge *et al.* (2006) studied the antimicrobial susceptibility of isolates of *Pasteurella multocida* (n = 28) and *Mannheimia haemolytica* (39) to amoxicillin-clavulanic acid, ceftiofur, ciprofloxacin, florfenicol, and tetracycline and minimum inhibitory concentrations of florfenicol. All isolates were susceptible to amoxicillin-clavulanic acid, ceftiofur, ciprofloxacin, and florfenicol. Only 5% (4/77) of isolates were resistant to tetracycline.

Stefan *et al.* (2004) investigated 154 *Pasteurella multocida* and 148 *Mannheimia haemolytica* strains from respiratory tract infections in cattle for their MICs of spectinomycin and other antimicrobial agents. A total of 93.5% of the *Pasteurella multocida* and 98.6% of the *Mannheimia haemolytica* strains were susceptible to spectinomycin.

The proposed study was designed to determined susceptibility of *Pasteurella multocida* to various antibiotics.

MATERIALS AND METHODS

Pasteurella multocida type B-6, the causative agent of hemorrhagic septicemia was obtained from Veterinary Research Institute (VRI) Lahore to investigate antimicrobial susceptibility. The glassware like test tubes, flasks, petri dishes were placed in sterilizing oven at 180°C for 2 hours. Instruments such as inoculating loops, scissors and forceps were sterilized by flaming with the help of gas burner. Furthermore, media and reagents were also sterilized in an autoclave at temperature of 121°C with 15lb pressure for 15 minutes. Different media, Muller Hinton agar, Nutrient agar and Brain Heart Infusion agar were used to study the suitable medium for the antibiotic sensitivity.

Drug susceptibility was adopted as proposed by Bauer *et al.* (1966). The antibiotics used were from five groups, Quinolones, Cephalosporins, Pencillins, Amino glycosides, Tetracycline and Miscellaneous group.

Table-1. Influence of media on zone of inhibition (diameter in mm) of various antibiotics used against *Pasteurella multocida*.

Antibiotics used		Code	Zone of inhibition (mm)		
			MHA	BHI	NA
A. Quinolones					
1	Ciprofloxacin	CIP5	24	16	18
2	Oflaxacin	OFX5	20	18	16
3	Rifampicin	RD5	20	16	14
4	Lincomycin	MY10	07	08	09
5	Bacitracin	B10	07	08	06
6	Enrofloxacin	ENR5	22	20	24
7	Nalidixic acid	NA30	20	20	17
B. Cephalosporins					
8	Cepharadin	CE30	16	14	12
9	Cefuroxime sodium	CXM30	22	17	18
10	Cefepime	FEP30	16	12	13
11	Cephalexin	CL30	12	14	11
C. Pencillins					
12	Pencillin-G	P10	15	11	08
13	Amoxycillin	AML25	20	17	14
14	Ampicillin	AMP25	22	18	20
15	Cloxacillin	OB5	09	08	07
D. Aminoglycosides					
16	Kanamycin	K30	10	07	10
17	Gentamycin	CN10	12	08	10
18	Amikacin	AK30	10	07	09
E. Tetracycline					
19	Oxytetracycline	OTC30	25	22	19
F. Miscellaneous					
20	Metronidazole	MTZ25	R	R	R
21	Erythromycin	E15	06	09	12
22	Colistin sulphate	CT10	09	09	08
23	Vancomycin	VA30	10	12	10
24	Ampiclox	AX30	20	17	14
25	Furozolidone	FR15	12	08	10
26	Chloramphenicol	C30	23	19	21
27	Sulphamethoxazole/trimet hoprim	SXT25	14	12	10

MHA = Muller Hinton Agar

BHI = Brain Heart Infusion Agar

NA = Nutrient Agar

Table 2. The mean sensitivity of *Pasteurella multocida* type 1(B6) against various antibiotics observed during study.

Antibiotics used		Code	Zone of inhibition (mm)	%age
			Type 1(B6)	
A. Quinolones				
1.	Ciprofloxacin	CIP5	19	86.36 %
2.	Oflaxacin	OFX5	18	81.81 %
3.	Rifampicin	RD5	17	77.27%
4.	Lincomycin	MY10	08	36.36%
5.	Bacitracin	B10	07	31.81%
6.	Enrofloxacin	ENR5	22	100%%
7.	Nalidixic acid	NA30	19	86.36%
B. Cephalosporins				
8.	Cephradin	CE30	14	63.63%
9.	Cefuroxime sodium	CXM30	19	86.36%
10.	Cefepime	FEP30	14	63.63%
11.	Cephalexin	CL30	12	54.54%
C. Pencillins				
12.	Pencillin-G	P10	11	50%
13.	Amoxycillin	AML25	17	77.27%
14.	Ampicillin	AMP25	20	90.90%
15.	Cloxacillin	OB5	08	36.36%
D. Aminoglycosides				
16.	Kanamycin	K30	09	40.90%
17.	Gentamycin	CN10	10	45.45%
18.	Amikacin	AK30	10	45.45%
E. Tetracycline				
19.	Oxytetracycline	OTC30	22	100%
F. Miscellaneous				
20.	Metronidazole	MTZ25	R	00%
21.	Erythromycin	E15	09	40.90%
22.	Colistin sulphate	CT10	09	40.40%
23.	Vancomycin	VA30	11	50%
24.	Ampiclox	AX30	17	77.27%
25.	Furozolidine	FR15	10	45.45%
26.	Chloramphenicol	C30	21	95.45%
27.	Sulphamethoxazole/trimethoprim	SXT25	12	54.54%

MHA = Muller Hinton Agar
 BHI = Brain Heart Infusion Agar
 NA = Nutrient Agar

Drug susceptibility method

Three to five colonies of *Pasteurella multocida* were selected and picked-up by sterilized wire loop and inoculated into 5ml of normal saline. Sterile cotton swab was dipped into the suspension and was rotated several times with a firm pressure on the inside wall of the tube and swab was streaked-over the entire agar surface in three different directions to ensure an even distribution of the inoculum. After drying the plates for 3-5 minutes, the antibiotics disks were placed and gently pressed to ensure complete contact with the agar surface. Plates were inverted and were incubated 37 °C for 24 hours. After 24 hours of incubation, plates were examined and diameters of the zones of inhibition was measured. The disks not inhibiting growth were recorded as resistant while those inhibited growth were recorded as sensitive. (Bauer, 1966).

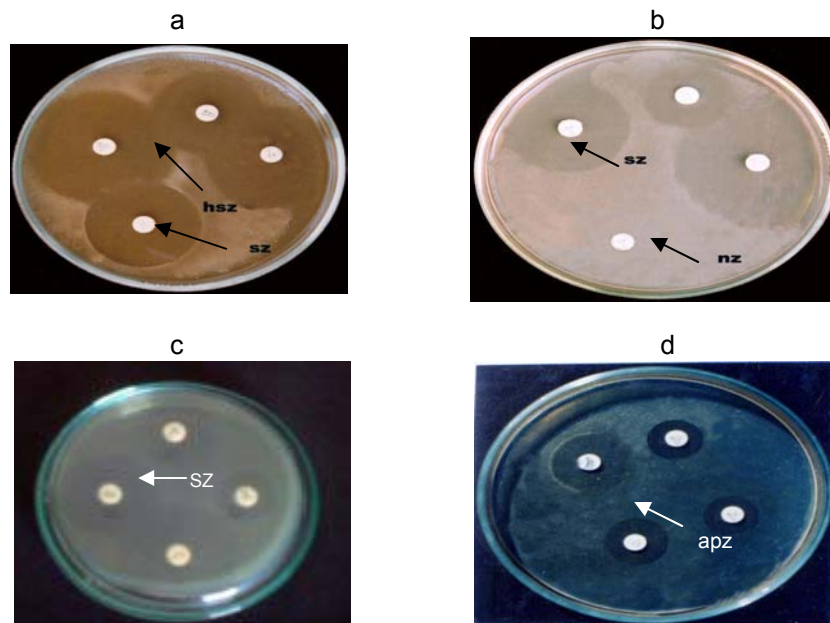


Plate 1: Shows antimicrobial sensitivity zones on different media

- a. SZ = Sensitive zone Hhsz = Highly sensitive zone
- b. SZ = Sensitive Zone nz = No zone,
- c. SZ = Sensitive Zone,
- d. apz = All positive zones

RESULTS AND DISCUSSION

During present study *Pasteurella multocida* was observed highly sensitive to enrofloxacin, oxytetracycline, chloramphenicol, ampicillin, nalidixic acid cefuroxime sodium, ciprofloxacin and ofloxacin. Results of *in-vitro* susceptibility of

Pasteurella multocida type 1(B6) against various antibiotics are summarized in Tables 1 and 2, Plate 1. Diker *et al.* (1994) investigated the *in-vitro* susceptibility of *Pasteurella multocida* and found sensitive to chloramphenicol and resistance to lincomycin. Harmansdorfe *et al.* (1998) who tested antimicrobial agents against *Pasteurella multocida* and found it highly sensitive to enrofloxacin, chloramphenicol, ampicillin, penicillin G, and cephalothin, by agar diffusion and recorded them as most effective drugs against strains of *Pasteurella multocida*. Mortensen *et al.* (1998) performed *in-vitro* activity of *Pasteurella multocida* and found cefuroxime, ampicillin and amoxicillin as effective drugs against *Pasteurella multocida*. Hanan *et al.* (2000) also tested antibiotics against *Pasteurella multocida* and observed ciprofloxacin to be highly effective drug. Shivachandra *et al.* (2004) tested twenty antibiotics against *Pasteurella multocid* and found highly susceptible to enrofloxacin, chloramphenicol, lincomycin and doxycycline. It is very clear from the present study that the findings recorded about the antimicrobial sensitivity of the *Pasteurella multocida* are in accordance to the results demonstrated by above workers in their studies.

CONCLUSION

On the basis of the present study, the author conclude that *Pasteurella multocida* was observed highly sensitive to enrofloxacin, oxytetracycline, chloramphenicol, ampicillin and their zone of sensitivity was recorded as 22, 22, 21 and 20 mm in diameter, respectively.

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(Received 09 December, 2010; Revised 03 August, 2011)