

Original Research Article

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Capsular Typing and Antibiogram Study of *Pasteurella multocida* Isolates of Rabbit Origin

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ABSTRACT

Keywords

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Pasteurella multocida causes upper respiratory syndrome or snuffles in rabbits. The present study was done to characterize the *P. multocida* isolates of rabbit origin by capsular typing and to carry out the antimicrobial sensitivity testing using different antibiotics in order to know the prevalent phenotypes of *P. multocida* strains causing rabbit pasteurellosis in India. We observed that, capsular types A and B are associated with snuffles in rabbits. Antibiogram study revealed that great majority of the isolates was resistant to streptomycin, pefloxacin, ciprofloxacin, kanamycin and tetracycline, while amoxicillin/clavulanic acid, gentamicin, ceftriaxone, cefepime and ampicillin were found to be effective against the isolates. Emergence of multidrug resistant strains of *P. multocida* of rabbit origin is a matter of concern in India.

Introduction

Pasteurella multocida is a Gram-negative, cocco-bacillary, non-motile, non spore-forming, capsulated, facultative anaerobic bacterium, which belongs to the family Pasteurellaceae. As a primary and secondary pathogen, *P. multocida* is associated with wide variety of diseases in animals and birds. Based on the capsular antigens, serologically 5 capsular types A, B, D, E and F have been recognised in *P. multocida* (Rimler and Rhoades, 1987). It causes Haemorrhagic septicaemia in cattle and buffaloes, pneumonic pasteurellosis in sheep and goats, fowl cholera in poultry and wild birds and snuffles in rabbits. Mostly snuffles, an upper respiratory tract infection in rabbits is caused

by capsular types A and B and often lead to more serious complications resulting in huge economic loss in terms of high morbidity and mortality in rabbitries in India and abroad. The prevalence of upper respiratory tract infection in rabbits due to *P. multocida* is higher than other respiratory diseases (Anina *et al.*, 2009). Apart from upper respiratory syndrome, chronic infection of internal organs and tissues has been reported due to *P. multocida* in rabbits resulting in high mortality (Petrov *et al.*, 2012). Although, *P. multocida* are generally susceptible to majority of commonly used antibiotics, the increasing rates of antimicrobial resistance may dramatically reduce the efficacy of the

antimicrobial agents used to control infections due to *P. multocida* (Kehrenberg *et al.*, 2001). Moreover, the antimicrobial resistance data of these organisms is scanty in India. Hence, the aim of the present study was to characterize the *P. multocida* isolates by capsular typing and to carry out the antimicrobial sensitivity testing of the isolates obtained from rabbits in India, which will assist the farmers or veterinary practitioners in treating rabbit pasteurellosis in India.

Materials and Methods

Bacterial strains

A total of 15 freeze dried cultures of *P. multocida* isolated from rabbits and maintained in the culture repository of All India Network Programme on Haemorrhagic Septicaemia (AINP-HS), Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar were used in the study.

Revival and confirmation of *P. multocida* isolates

The cultures were revived by inoculating in brain heart infusion (BHI) broth and incubating at 37 °C for 24 h. These broth cultures were then inoculated on 5% sheep blood agar in order to study their morphology and cultural characteristics. The genomic DNA of the isolates was extracted by CTAB method (Wilson *et al.*, 1987). The isolates were then confirmed by *P. multocida* specific PCR (PM-PCR) using set of primers as reported by Townsend *et al.*, (1998) and capsular serogrouping was done using capsular PCR as described by Townsend *et al.*, (2001). The PCR reaction mixture and cycling conditions for PM-PCR and capsular PCR were same as described earlier (Townsend *et al.*, 2001). The PCR products were subjected to agarose gel electrophoresis

using 1.5% agarose (Sigma, USA) and then visualised by UV gel documentation system (Alpha Imager, Germany).

Antibiotic sensitivity test

Antibiotic susceptibility testing was performed by the Kirby – Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008). All the isolates were tested for their susceptibility pattern using 16 different antibiotics (Hi-Media laboratories, Mumbai, India). The list of antibiotics and their concentrations used in the study are presented in the Table 1. For performing antibiogram, a single colony of each isolate was inoculated into BHI broth and incubated for a period of 10 – 12 h. The culture was then spread on Muller-Hinton agar medium and allowed to adsorb for 10 min. Later, the antibiotic discs were placed on to the plate at an appropriate distance from each other and the plates were incubated aerobically at 37°C for 24 h.

The diameters of the zone of inhibition surrounding the antibiotic discs were measured. The isolates were interpreted as sensitive, intermediately sensitive and resistant based on the manufacturer's interpretative criteria.

Results and Discussion

In the present study all the isolates were found to be pure on blood agar. The colonies were small, non-hemolytic and dewdrop like in appearance. Also, small coccobacillary organisms suggestive of *P. multocida* were found by Gram's staining of the single colony. Further, all the isolates gave an amplicon of 460 bp in agarose gel electrophoresis in PM-PCR assay (Fig. 1A) confirming the presence of *P. multocida* (Dutta *et al.*, 2001; Dey *et al.*, 2007; Kumar *et al.*, 2009). In capsular PCR assay, 7 (46.6%)

isolates were found to be capsular type A with an amplicon of ~1044 bp and 8 (53.3%) isolates were of type B with an expected amplicon of ~ 760 bp (Fig. 1B). The absence of other capsular types D, E and F in these isolates suggests that the capsular types A and B are mainly associated with disease in rabbits. The amplification of ~ 1044 bp and ~760 bp products using capsular primers is specific for the serogroups A and B, respectively, of *P. multocida* (Townsend *et al.*, 2001).

All the isolates of *P. multocida* from rabbits in Mexico were found to be capsular type A (Vargas *et al.*, 2012). Seventy nine per cent of the isolates from rabbits were found to be type A and 21% were untypable (Tayeb *et al.*, 2004). *P. multocida* type F (33.3 %) have been reported to be associated with rabbit pasteurellosis in Czech Republic apart from

type A (58.4%) and type D (8.3%) isolates (Jaglic *et al.*, 2004). The results of the antibiotic sensitivity test showed that the isolates were resistant to streptomycin, tetracycline, pefloxacin, kanamycin, ciprofloxacin and chloramphenicol in the order 66.6%, 60%, 53.3%, 53.3%, 53.3% and 40%, respectively (Table 1). Erythromycin and enrofloxacin showed an intermediate sensitivity of 73.3% and 60% respectively. The most commonly used antibiotic for pasteurellosis, sulfamethaxazole trimethoprim was effective only to 53.3% of the isolates. Hence, these antibiotics may be of little value in treating rabbit pasteurellosis in India. Similar to our results, antibiogram study of the *P. multocida* isolates from rabbits in Brazil revealed highest resistance to sulfonamides and cotrimoxazole, followed by erythromycin, penicillin, and amoxicillin (Ferreira *et al.*, 2012).

Table.1 Antimicrobial sensitivity pattern of 15 isolates of *P. multocida* against 16 antibiotics

S.No	Antimicrobial agent	Symbol	Concentration (µg)	Resistant N (%)	Intermediate N (%)	Sensitive N (%)
1	Ampicillin	AMP	10	4 (26.7)	0 (0)	11 (73.3)
2	Amoxycillin	AMC	30	0 (0)	0 (0)	15 (100)
3	Chloramphenicol	C	30	6 (40)	0 (0)	9 (60)
4	Ciprofloxacin	CIP	5	8 (53.3)	0(0)	7 (46.7)
5	Cefepime	CFM	5	2(13.3)	0 (0)	13 (86.7)
6	Cefoperazone	CPZ	75	0 (0)	4 (26.7)	11 (73.3)
7	Ceftriaxone	CTR	30	1 (6.7)	0 (0)	14 (93.3)
8	Erythromycin	E	15	0 (0)	11 (73.3)	4 (26.7)
9	Enrofloxacin	EX	10	0 (0)	9 (60)	6 (40)
10	Gentamicin	GEN	10	0 (0)	1 (6.7)	14 (93.3)
11	Kanamycin	K	30	8 (53.3)	2 (13.3)	5(33.3)
12	Pefloxacin	PF	5	8 (53.3)	0 (0)	7 (46.7)
13	Streptomycin	S	10	10 (66.6)	0 (0)	5 (33.3)
14	Sulfa/trimethoprim	SXT	23.75/1.25	5 (33.3)	2 (13.3)	8 (53.3)
15	Spectinomycin	SPT	100	0 (0)	3 (20)	12 (80)
16	Tetracycline	TE	30	9 (60)	0 (0)	6 (40)
N= number of isolates resistant to particular antibiotic						

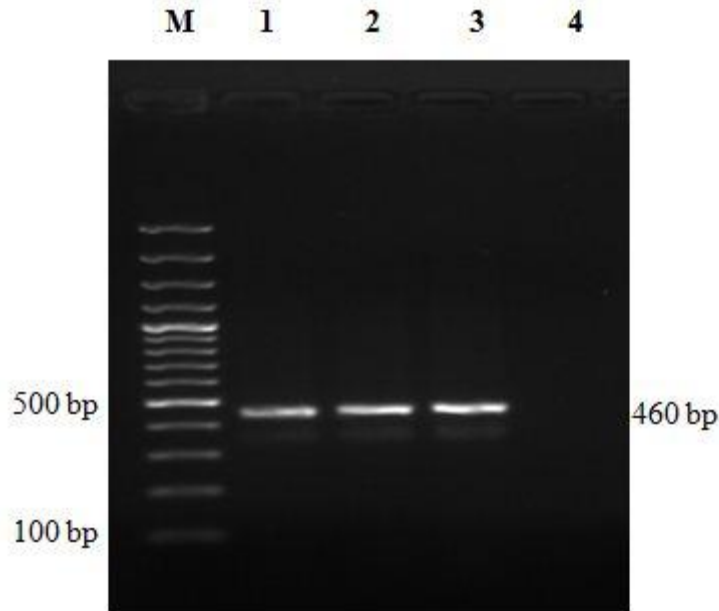


Fig. 1A *Pasteurella multocida* specific PCR of isolates
Lane M – 100 bp marker, 1,2,3 – positive isolates (460 bp)
4 – negative control

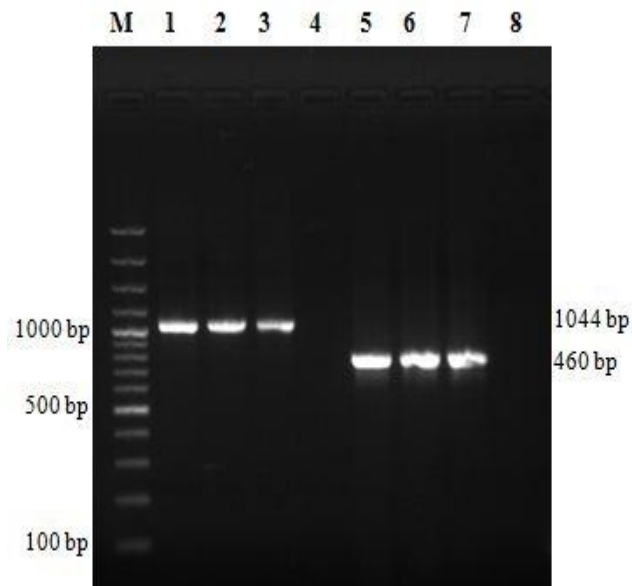


Fig. 1B Capsular PCR of *Pasteurella multocida* isolates
Lane M – 100 bp marker, 1,2,3 – Type A isolates (1044 bp),
5,6, 7- Type B isolates (760 bp), 4, 8- Negative controls

In another study in Brazil 12.1% isolates were susceptible to all tested drugs, and 87.8% were resistant to at least one drug tested. Also, the resistance was more frequent to trimethoprim-

sulphamethoxazole (75.6%), followed by sulfizoxazole (60.9%) (Ferreira *et al.*, 2015). Martino and Luzi (2008) observed the resistance to chloramphenicol and amikacin

(85.71%), gentamicin, kanamycin and cotrimoxazole (57.1%), enrofloxacin (42.8%) and the organisms were sensitive to doxycycline (85.71%) and marbofloxacin (71.4%). In contrast to our findings, low levels of resistance were found to rabbit isolates from Italy (Cucco *et al.*, 2017).

Most of isolates from rabbits in Bulgaria were susceptible to chloramphenicol (92.8%) and streptomycin (77.4%). Resistance to amoxicillin was 12.2%, to doxycycline 14.3%, to 11.9% and to enrofloxacin 14.8% (Petrov *et al.*, 2012).

In this study, the isolates were 100% sensitive to amoxicillin/clavulanic acid, followed by gentamicin and ceftriaxone (93.3%), cefepime (86.7%). The antimicrobial resistance varies according to the host animal species, time and geographical origin of the animals (Naz *et al.*, 2012) and hence regular monitoring of antimicrobial resistance is important to know the prevalent phenotypes so that appropriate treatment strategy could be employed.

In the present study, 8 isolates (60%) were found to be resistant to more than 3 antibiotics used in the study. Similarly, all *P. multocida* isolates from Rabbits in New Zealand were found to be resistant to at least one of the antibiotics used in the study and majority were resistant to penicillin, tetracycline, streptomycin and sulphonamide (Jones *et al.*, 1988). The high and indiscriminate usage of antibiotics both for treatment and prevention could be the reason for the emergence of multidrug resistance strains of *P. multocida*. In conclusion, the present study documents that *P. multocida* type A and type B are associated with snuffles in rabbits and the emergence of multidrug resistant strains of *P. multocida* necessitates that appropriate antibiotic should be used only after performing antibiotic sensitivity test.

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