

Drug sensitivity profile of various capsular types of *Pasteurella multocida* in Himachal Pradesh

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ABSTRACT

An experiment was carried out to study the antibacterial sensitivity of various capsular types of *Pasteurella multocida* with the purpose of controlling and treating pasteurellosis in different species of animals in Himachal Pradesh. A total of 144 strains of *Pasteurella multocida* isolated from different species of 144 animals {cattle (44), buffaloes (49), sheep (6) goats (7), rabbits (29), poultry(8) and canine (1)}, from different parts of the State were subjected to drug sensitivity test using 30 different antimicrobials. A total of 27 isolates were of Capsular serotype A, while, 117 isolates were identified as Capsular serotype B. Capsular serotype A of *Pasteurella multocida* was found to be highly sensitive to ceftriaxone, cefotaxime, chloramphenicol, enrofloxacin, ciprofloxacin, ofloxacin, nitrofurantoin and norfloxacin, while capsular serotype B of *Pasteurella multocida* were mostly sensitive to the antimicrobial agents like ceftriaxone, cefotaxime, cephalixin, enrofloxacin, chloramphenicol, ciprofloxacin, ofloxacin, nitrofurantoin and norfloxacin. Both Capsular type A and type B were found to be highly resistant to ceftiofur, cefuroxime, ampicillin, streptomycin, chlortetracycline and clotrimazole.

Key words: Antibiotic sensitivity, Capsular serotypes, *Pasteurella multocida*

In many Asian and African countries, pasteurellosis still remains a major cause of high morbidity and mortality among cattle, buffaloes, sheep, goats, poultry, rabbits, pigs and poultry (AHIS 1997, Singh *et al.* 1996, Carigan *et al.* 1991, and Sharma *et al.* 2004). Singh *et al.* (2008) calculated the annual economic losses in India due to *Pasteurella multocida* infection alone to the tune of Rs 225 millions. Treatment of pasteurellosis has traditionally been done with the administration of antibacterial drugs with varying success depending more often on the kind of drug used by the veterinarian (Rimler and Glisson 1997). However, as with any other disease or infection, the prolonged and indiscriminate use of antibiotics has resulted in the development of resistance among various strains of these organisms (Arora *et al.* 2005) with multidrug resistant forms of *Pasteurella multocida* emerging at regular intervals (Shivchandra *et al.* 2004). Adding to this factor, the number of serotypes encountered in an outbreak of pasteurellosis in India are frequently different. This generally warrants periodic assessment of sensitivity/resistance pattern to enable sensitive, effective and judicious use of antimicrobial agents.

There is paucity of literature in the country on the antibiotic susceptibility pattern for different capsular types of *Pasteurella multocida*. The present study reveals the variation among different capsular strains of *Pasteurella multocida* among various livestock species and their susceptibility to various antibacterial drugs in Himachal Pradesh. The investigation will help to implement effective treatment and control measures for the disease.

MATERIALS AND METHODS

Bacterial strains (144) of *Pasteurella multocida* were isolated from different parts of the State and from different animals and birds, viz. cattle (44), buffaloes (49), sheep (6), goats (7), rabbits (29), canine (1) and poultry (8). All 144 *Pasteurella multocida* strains were confirmed culturally, morphologically and biochemically as per Cowan and Steel (1970) and Cruickshank *et al.* (1975). All the strains were also subjected to polymerase chain reaction (PCR) as reported by Townsend *et al.* (2001) for confirmation and serotype identification.

All the 144 strains were then subjected to drug sensitivity against 30 commercially available antimicrobial agents in the market for the treatment of haemorrhagic septicaemia. Out of these 30 antimicrobials, amoxicillin, ampicillin,

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cephalothin, gentamicin, tetracycline, sulphonamide, cotrimoxazole, erythromycin, enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin have also been recommended by OIE for the treatment of pasteurellosis.

Antibiotic susceptibility test of *Pasteurella multocida* was performed on Mueller- Hinton agar medium with standardized commercially available antimicrobial discs by the disc diffusion method (Bauer *et al.* 1966). The inhibitory

effect of the antibacterial to the growth of the culture was recorded and subsequently matched with standard inhibition zones. Test cultures were classified as resistant, intermediate sensitive and highly sensitive.

RESULTS AND DISCUSSION

All the 144 *Pasteurella multocida* strains isolated from different species of animals and birds of different parts of

Table 1. Details of antibiogram analysis of different capsular types of *Pasteurella multocida*

List of antibiotics		Sensitivity per cent	Capsular type A	Capsular type B	List of antibiotics		Sensitivity per cent	Capsular type A	Capsular type B
Amoxycillin	Resistance	56	19	61	Erythromycin	Resistance	33	10	37
	Intermediate	35	6	45		Intermediate	37	12	41
	High	09	2	11		High	30	5	39
Ampicillin	Resistance	62	21	68	Enrofloxacin	Resistance	06	5	4
	Intermediate	26	4	33		Intermediate	28	5	35
	High	12	2	16		High	66	17	78
Amikacin	Resistance	52	16	59	Gentamicin	Resistance	43	12	50
	Intermediate	35	9	41		Intermediate	31	6	38
	High	13	2	17		High	26	9	29
Cephalexin	Resistance	22	18	13	Kanamycin	Resistance	57	18	64
	Intermediate	26	6	31		Intermediate	41	8	51
	High	52	3	73		High	2	1	2
Cefamandole	Resistance	13	2	17	Nalidixic acid	Resistance	21	4	26
	Intermediate	56	16	64		Intermediate	38	11	43
	High	31	9	36		High	42	12	48
Ceftriaxone	Resistance	10	1	13	Neomycin	Resistance	13	6	13
	Intermediate	13	5	14		Intermediate	40	9	49
	High	77	21	90		High	47	12	55
Cefotaxime	Resistance	14	2	18	Nitrofurantoin	Resistance	15	4	18
	Intermediate	17	7	17		Intermediate	34	8	41
	High	69	18	82		High	51	15	58
Ceftiofur	Resistance	85	24	98	Norfloxacin	Resistance	15	2	19
	Intermediate	13	2	16		Intermediate	38	11	44
	High	02	1	3		High	47	14	54
Cephadroxil	Resistance	13	2	17	Ofloxacin	Resistance	15	3	18
	Intermediate	56	16	64		Intermediate	35	9	41
	High	31	9	36		High	50	15	58
Cefuroxime	Resistance	81	23	93	Oxytetracycline	Resistance	61	18	70
	Intermediate	12	3	14		Intermediate	29	6	35
	High	07	1	10		High	10	3	12
Cloxacillin	Resistance	19	4	23	Penicillin	Resistance	20	6	23
	Intermediate	44	12	51		Intermediate	46	17	49
	High	37	11	43		High	34	4	45
Chloramphenicol	Resistance	08	1	11	Spectinomycin	Resistance	24	4	31
	Intermediate	34	8	41		Intermediate	37	10	43
	High	58	18	65		High	39	13	43
Chlortetracycline	Resistance	64	22	70	Spiramycin	Resistance	17	7	17
	Intermediate	28	3	37		Intermediate	40	12	46
	High	08	2	10		High	43	8	54
Ciprofloxacin	Resistance	11	3	13	Streptomycin	Resistance	74	19	87
	Intermediate	37	8	45		Intermediate	18	5	21
	High	52	16	59		High	8	3	9
Co-trimoxazole	Resistance	83	23	97	Tetracycline	Resistance	24	3	32
	Intermediate	13	2	17		Intermediate	52	17	58
	High	04	2	3		High	24	7	27

Himachal Pradesh were confirmed in the laboratory on the basis of cultural, morphological and biochemical characteristics.

All these bacterial strains positive by conventional method also gave an amplified product of ~ 460 bp corresponding to the product specific for the *Pasteurella multocida* by PM-PCR assay. Out of these 144 isolates, 27 were found positive for capsular serotype A and 117 were positive for capsular serotype B by Multiplex-PCR as per Townsend *et al.* (2001).

The results of sensitivity analysis of different capsular types of the *Pasteurella multocida* strains against 30 antimicrobial agents are given in Table 1.

The present study revealed high resistance against ceftiofur, cefuroxime, ampicillin, amoxicillin, chlortetracycline, clotrimazole and streptomycin. Similar resistance to amoxicillin, ampicillin, amikacin, ceftiofur, cefuroxime, chlortetracycline, cotrimoxazole, gentamicin, kanamycin, oxytetracycline and streptomycin have also been reported earlier by Shivchandra *et al.* (2004) and Kumar *et al.* (2009) that is in accordance with our studies.

Higher overall efficacy of the following antimicrobials like ceftriaxone, cefotaxime, chloramphenicol, ciprofloxacin, enrofloxacin, ofloxacin, norfloxacin, nitrofurantoin, spectinomycin and neomycin against *P. multocida* isolates have been found in this study. Sharma *et al.* (2005) and Kumar *et al.* (2009) also reported similar efficacy of cephalixin, Cloxacillin, cefamendol, erythromycin, nalidixic acid, penicillin, spiramycin, tetracycline against *P. multocida* infections.

In the present study, cephelexin, cephadroxil, gentamicin, kanamycin, nalidixic acid, penicillin, spiramycin and tetracycline were found to be moderately effective, however, earlier reports by Dey *et al.* (2007) and Verma *et al.* (2004) suggest higher efficacy of these drugs that is in variance with our study. It was observed that gradual increase in resistance among field strains of *Pasteurella multocida* against these drugs develop as these are commonly and widely used in therapeutics. Low efficacy of ampicillin, amoxicillin, tetracycline, cotrimoxazole is in accordance with previous report (Arora *et al.* 2005 and Kumar *et al.* 2009).

Antibacterials having higher efficacy against capsular serotype A are: ceftriaxone, cefotaxime, chloramphenicol, enrofloxacin, ciprofloxacin, ofloxacin, nitrofurantoin, norfloxacin and spectinomycin in descending order of sensitivity pattern. Ceftriaxone, cefotaxime, cephalixin, enrofloxacin, chloramphenicol, ciprofloxacin, ofloxacin, nitrofurantoin and norfloxacin, were highly efficacious against capsular serotype B.

Antimicrobials that showed highest efficacy against both capsular serotypes A and B were: ceftriaxone, cefotaxime chloramphenicol, enrofloxacin, ciprofloxacin, ofloxacin, norfloxacin and nitrofurantoin.

In the present study, both A and B capsular types showed maximum resistance against drugs like ceftiofur, cefuroxime,

co-trimoxazole, chlortetracycline, streptomycin, ampicillin and oxytetracycline.

Highly effective antimicrobials in cattle were: ceftriaxone, cefotaxime, chloramphenicol, enrofloxacin and ciprofloxacin; in buffaloes: ceftriaxone and cefotaxime; in sheep: cefotaxime, cephalothin and ceftriaxone; in goats: cefotaxime, ceftriaxone, enrofloxacin, ciprofloxacin, chloramphenicol, amikacin and cephalothin; in poultry: ceftriaxone, cefotaxime, chloramphenicol, enrofloxacin and ciprofloxacin, in rabbits: enrofloxacin, ciprofloxacin, norfloxacin, amikacin, chloramphenicol and ofloxacin. Similar observations have also been reported earlier by Rahman *et al.* (2004) and Kumar *et al.* (2009).

High resistance of the local isolates of *Pasteurella multocida* was noticed for ceftiofur, cefuroxime cotrimoxazole, streptomycin, chlortetracycline and oxytetracycline which are commonly used drugs against Pasteurellosis. It is interesting to note here that Ceftiofur is a new drug that has been marketed for controlling various types of infections. The high resistance of *Pasteurella multocida* isolates to this drug reveals that the drug should not be used in a clinical condition.

It is suggested that the most effective antibiotics encountered in this investigation, like ceftriaxone and cefotaxime should be used in cases of suspected outbreaks pending serotype identification and antibiogram profile studies.

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REFERENCES

- Animal Health Information Services (AHIS). 1997. Deputy Commissioner (LH). Department of Animal Health, Ministry of Agriculture, Government of India, New Delhi, India.
- Arora A K, Virmani S, Jand S K and Oberoi M S. 2005. Isolation, characterization and antibiogram of *Pasteurella multocida* isolates from different animal species. *Indian Journal of Animal Sciences* 75: 749–52.
- Bauer A W, Kirby W M M, Sherris J C and Turek N. 1996. Antibiotic susceptibility testing by standardizes single disc method. *American Journal of Clinical Pathology* 45: 493.
- Carigan M J, Dawkins H J S, Cockram E A and Hansen A T. 1991. *P. multocida* septicaemia in fallow deer. *Australian Veterinary Journal* 68: 201.
- Cowan S T and Steel K J. 1970. *Manual for the Identification of Medical Bacteria*. (Cambridge University Press, Cambridge).
- Cruickshank R, Duguid J P, Marmion B P and Seain R H A. 1975. *Medical Microbiology*. 2nd edn, (Churchill Livingstone, Edinburgh).
- Dey S, Singh V P, Kumar A A, Srivastava S K and Singh N. 2007. Molecular identification of Indian isolates of *Pasteurella*

- multocida* serotype B: 2 by different PCR assays. *Indian Journal of Animal Sciences* **77**: 317.
- Kumar P, Singh V P, Agrawal R K and Singh S. 2009. Identification of *Pasteurella multocida* isolates of ruminant origin using polymerase chain reaction and their antibiogram study. *Tropical Animal Health and Production*. **41**: 573.
- Rahman M A, Samad M A, Rahman M B and Kabir S M L. 2004. Bacterio-pathological studies on salmonellosis, colibacillosis and pasteurellosis in natural and experimental infections in chickens. *Bangladesh Journal of Veterinary Medicine* **2**: 99.
- Rimler R B and Glisson J R. 1997. Fowl cholera. *Diseases of Poultry*, 10th edn, pp. 143–59. (Eds.) Calnek B W, Barnes H J, Beard C W, McDougald L R, and Saif YM. Iowa State University Press, Ames Iowa, USA.
- Sharma S P, Sharma R K and Rahman H. 2004. Isolation, serotyping and antibiogram of *Pasteurella* from pigs of North-east India. *Indian Journal of Animal Sciences* **74**: 905.
- Sharma P C, Jindal N and Khokhar R S. 2005. Mortality in sheep due to pneumonic pasteurellosis and haemonchosis. *Haryana Veterinarian* **4**: 73.
- Shivchandra S B, Kumar A A, Biswas A, Ramakrishnan M A, Singh V P and Srivastava S K. 2004. Antibiotic sensitivity patterns among Indian strains of avian *P. multocida*. *Tropical Animal Health and Production* **36**: 743.
- Singh V P, Kumar A A, Srivastava S K and Rathore B S. 1996. Significance of H S in Asia: India. *International Workshop on Diagnosis and Control of H.S.* Bali, Indonesian Department of Agriculture, Indonesia, 28–30 May 1999, Pp. 16.
- Singh V P, Sinha D K, Gupta S K, Chauhan R S. 2008. Prevalence of H.S. in India. *Vth Annual Scientist Meet on Haemorrhagic Septicaemia*. 5–6th September. Guwahati, Asom.
- Townsend K M, Boyce J D, Chung J Y, Frost A J and Adler B. 2001. Genetic organization of *Pasteurella multocida* cap loci and development of a multiplex capsular PCR typing system. *Journal of Clinical Microbiology* **39**: 924.
- Verma S C, Mahajan N K, Malik G and Dahiya J P. 2004. An epidemiological study on bovine haemorrhagic septicaemia in Haryana. *Indian Journal of Animal Sciences* **38**: 14–19.