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CHARACTERISTICS OF *PASTEURELLA MULTOCIDA* RECOVERED FROM AVIAN SOURCES

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ABSTRACT

Fowl cholera (FC) is still a major threat for poultry industry in Pakistan. Despite the extensive vaccination programme, outbreaks continue to occur. Six *Pasteurella multocida* isolates were recovered from several farms in Faisalabad with outbreak of FC. The isolates were characterized by biochemical profiles, Analytical Profile Index (API) and antimicrobial susceptibility testing. Isolates were found resistant to streptomycin and cephradine and susceptible to ciprofloxacin and chloramphenicol. API resulted in 86.1 to 98.6% identification. The results indicated that API system and antimicrobial susceptibility testing were helpful for the quick diagnosis and selection of appropriate antimicrobial agents, respectively. Furthermore, both the systems were quick and inexpensive.

Key words: Fowl cholera, antimicrobial, *Pasteurella multocida.*

INTRODUCTION

Fowl cholera (FC), a contagious disease of domestic and wild avian species, is caused by *Pasteurella multocida* (Glisson *et al*., 2003; Kwon and Kang, 2003). FC has been recognized as an important disease in domestic poultry for more than 200 years that causes devastating economic losses to poultry industry world wide (Aye *et al*., 2001). The infected birds remain carriers up to 9 weeks after infection (Christiansen *et al*., 1992). It usually occurs as primary disease that does not require predisposing factors, but these factors may increase the severity of the disease. The disease has two forms, an acute septicemia with high morbidity and mortality rates and a chronic localized infection of joints and sinuses. FC can affect birds of any age, but it rarely occurs in commercial poultry of less than 8 weeks of age (Rimler *et al*., 1998). Previous work in Pakistan has shown that *P. multocida* serotype 1 is most frequently isolated serotype, followed by serotype 3, 4 and serotype 12 (Arshed, 2002).

At present, there is very little information on the antimicrobial susceptibility and Analytical Profile Index (API) for *Pasteurella multocida* isolates in Pakistan. The present study was conducted to determine the variation among different field isolates of *Pasteurella multocida* by using API system and to find susceptibility to various antimicrobial agents that could help in appropriate antimicrobial selection.

MATERIALS AND METHODS

Liver tissue samples from 43 birds were collected from several poultry farms with outbreaks of FC and located in and around Faisalabad, Pakistan. The samples were inoculated on nutrient agar and brain heart infusion agar (Difco, Inc, USA). The suspected colonies were stained by using the Giemsa's staining for the demonstration of characteristic bipolar staining. Biochemical tests were performed, as described previously (Morishita *et al*., 1996). All the isolates were further confirmed by using Analytical Profile Index to generate the API using the commercially available kit (API 20-E kit, BioMerieux, France) according to the manufacturer's instructions. Briefly, a single, well isolated colony of the bacterium was made into homogenous suspension in 5 ml of sterile distilled water, and inoculated in the tubes of API- Kit. The suspension of each positive culture was subjected to antimicrobial susceptibility testing, using the standard method of National Committee for Clinical Laboratory Standards (NCCLS, 1990). The turbidity of the inoculum was adjusted to 0.5 McFarland opacity standards and medium used was Mueller Hinton agar. The antimicrobials Ciprofloxacin, Cephridine, Chloramphenicol, Neomycin, Streptomycin, Trimethoprim-Sulpha, Nitrofuratoin and Novobiocin were used. The results were interpreted as resistant (R), intermediate (I) and susceptible (S).

RESULTS AND DISCUSSION

Six *Pasteurella multocida* isolates were recovered from 43 liver samples. The isolates were named according to the area of sample collection. Biochemical characteristics of these isolates are described in Table 1. By using the API kit, a numerical number was generated that indicated 86.1 to 98.6% identification (Table 2). All the isolates were found resistant to Streptomycin and Cephradine but susceptible to

Table 1: Biochemical characteristics of *Pasteurella multocida* isolates

Sr. No.	Source of isolates	Gelatin liquefaction	Catalase test	H_2S production	Nitrate reduction	Methyl red test	Voges-Proskaur test	Urease production test	Indole production test
1	Summundri Road (SR)	-	+	+	+	-	-	-	+
2	Jaranwala Road (JR)	-	+	-	+	-	-	-	+
3	Barnala & Adda Joal (BA1)	-	+	-	+	-	-	-	+
4	Barnala & Adda Joal (BA2)	-	+	+	+	-	-	-	+
5	Barnala & Adda Joal (BA3)	-	+	-	+	-	-	-	+
6	Satiana Road (S1)	-	+	-	+	-	-	-	+

Ciprofloxacin and Chloramphenicol. Summundri Road (SR) isolate was found susceptible to Novobiocin, while rest of the isolates were found resistant. Trimethoprim Sulpha, Neomycin and Nitrofurantoin gave variable results for all the isolates as susceptible, intermediate and resistant (Table 2).

Analytical Profile Index results of the isolates are in accordance with Waltman and Horne (1993) i.e. excellent to good identification. Furthermore, the system was found quick and easy to perform, as it took less than 24 hrs for biochemical as well as sugar fermentation tests on a single strip.

Antimicrobial susceptibility results were observed with different patterns showing variable responses to different antimicrobial agents. In previous studies, antimicrobial susceptibility of various *Pasteurella multocida* isolates has been described (Aye *et al*., 2001; Waltman and Horne, 1993; Shivachandra *et al*., 2004), showing that the isolates are absolutely resistant to Trimethoprim in combination with Sulphadiazine, as intermediate susceptible to resistant results were observed in the present study. Morishita *et al*. (1996) reported that *Pasteurella multocida* isolates were susceptible to Trimethoprim-Sulfamethoxazole. All the six isolates were found susceptible to Chloramphenicol and Ciprofolxacin and resistant to Cephradine and Streptomycin. These results are not in agreement with previous studies (Morishita *et al*., 1996) which described that the isolates were intermediate sensitive to resistant. Majority of the isolates were susceptible to Neomycin, as described by Waltman and Horne (1993).

Antibiogram profiles obtained from the present work revealed that a variable pattern of drug susceptibility existed among field isolates of *P. multocida* belonging to different areas. It might be due to the emergence of new strains or variants. Similar results about the emergence of multidrug-resistant strains of *P. multocida* among different isolates have been described by Shivachandra *et al*. (2004). It is suggested that birds suffering from fowl cholera may preferably be treated with Ciprofloxacin and Chloramphenicol.

REFERENCES

Arshed, M. J., 2002. Studies on immunoprophylaxis of fowl cholera. PhD Thesis. Dept. Vet. Microbiol., Univ. Agri. Faisalabad, Pakistan.

Table 2: Antibiogram and API- 20 E No. of *Pasteurella multocida* Isolates

Sr. No.	Isolates	API-20 E No.	Identification (%)	ST	CIP	NB	TMS	CH	N	C	Ne
1	SR	0040020	86.1	R	S	S	R	R	S	S	I
2	JR1	0044520	98.1	R	S	R	I	R	R	S	S
3	BA1	0040520	86.5	R	S	R	I	R	I	S	S
4	BA2	0044520	98.1	R	S	R	R	R	I	S	S
5	BA3	0040520	86.5	R	S	R	I	R	S	S	R
6	S1	0040024	98.6	R	S	R	R	R	I	S	S

C= Chloramphenicol, CH= Cephridine, CIP= Ciprofolxacin, N= Nitrofuranoin, NB= Novobiocin, Ne= Neomycin, St= Streptomycin, TMS= Trimethoprim-Sulphadiazine, S=Susceptible, I=Intermediate and R=Resistant.

Aye, P. P., E. J. Angrick, T. Y. Morishita and B. S. Harr, 2001. Prevalence and characteristics of *Pasteurella multocida* in commercial turkeys. Avian Dis., 45(1): 182-190.

Christiansen, K. H., T. E. Carpenter, K. P. Snipes and D. W. Hird, 1992. Transmission of *Pasteurella multocida* on California turkey premises in 1988-89. Avian Dis., 36: 262-271.

Glisson, J. R., C. L. Hofacre and J. P. Christensen, 2003. *In*: Diseases of Poultry, 11th Ed. Saif, Y. M., H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald and D. E. Swayne, (Eds), Iowa State Univ. Press, Ames, Iowa, USA, pp: 658-676.

Kwon, Y. K and M. I. Kang, 2003. Outbreak of fowl cholera in Baikal teals in Korea. Avian Dis., 47(4): 1491-1495.

Morishita, T. Y., L. J. Lowenstine, D. C. Hirsh and D. L. Brooks, 1996. *Pasteurella multocida* in raptors: prevalence and characterization. Avian Dis., 40: 908-918.

NCCLS, 1990. Performance Standards for Antimicrobial Disk Susceptibility Tests. 4th Ed., Villanova, PA, USA.

Rimler, R. B., T. S. Sandhu and J. R. Glisson, 1998. In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens, 4th Ed. Swayne, D. E., J. R. Glisson, M. W. Jackwood, J. E. Pearson and W. M. Reed. (Eds.) Amer. Assoc. Avian Pathol., Pennsylvania, USA, pp: 17-28.

Shivachandra, S. B., A. A. Kumar, R. Gautam, V. P. Singh, P. Chaudhuri and S.K. Srivastava, 2004. PCR assay for rapid detection of *Pasteurella multocida* serogroup A in morbid tissue materials from chickens with fowl cholera. Vet. J., 168(3): 349-352.

Waltman, W. D and A. M. Horne, 1993. Characteristics of fowl cholera diagnosed in Georgia during 1989-1991. Avian Dis., 37: 616-621.