Short Communication
The Journal of Animal & Plant Sciences, 24(5): 2014, Page: 1565-1568
ISSN: 1018-7081

STUDIES ON THE ANTIBIOTIC SENSITIVITY PATTERN OF ISOLATES OF *P. MULTOCIDA* FROM BUFFALOES

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ABSTRACT

Antibiotic susceptibility pattern of *Pasteurella multocida* causing hemorrhagic septicemia in buffaloes was determined. The *Pasteurella multocida* (n=11) were isolated from healthy (04) and diseased buffaloes (07) of four districts of Sargodha division. Isolates were identified by cultural, biochemical and serological characteristics, and confirmed by specie specific polymerase chain reaction. All isolates were subjected to antibiotic susceptibility testing against gentamicin, erythromycin, amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, enrofloxacin, sulfadiazine and amikacin by disc diffusion method. Results revealed that *P. multocida* were highly sensitive to enrofloxacin (90.91 %) followed by gentamicin, chloramphenicol, ciprofloxacin and norfloxacin (72.73%). Susceptibility of *P. multocida* isolates was low against ampicillin and amoxicillin (45.45%), amikacin (36.36%), sulfadiazine (18.18%) and erythromycin (18.18%). It was concluded that quinolones (enrofloxacin, ciprofloxacin and norfloxacin) are better choice for the treatment of hemorrhagic septicemia as the level of resistance in *P. multocida* against the macrolides, sulfa drugs and beta lactams has increased enormously.

Key words: Hemorrhagic septicemia, *Pasteurella multocida*, Buffalo, Antibiotic susceptibility, Quinolones.

INTRODUCTION

Pasteurella multocida, a gram-negative bacterium, causes hemorrhagic septicemia (HS) which is an acute, often fatal disease of buffaloes and cattle. *P. multocida* is a natural inhabitant of the nasopharynx and occasional resident of intestines of healthy ruminants (Shivachandra *et al.,* 2011). *P. multocida* are serologically classified as type A, B, C, D and E on the basis of capsular antigen. *P. multocida* serotype B: 2 is major cause of HS in Pakistan and has been involved in all outbreaks in Pakistan (Benkirane *et al.,* 2002, Naz *et al.,* 2012). The incidence of the disease is higher in buffaloes as compared to cattle (De Alwis *et al.,* 1982, Hajikolaei *et al.,* 2008; Khan *et al.,* 2006). The disease process is triggered when host resistance is lowered due to various stress conditions (Naz *et al.,* 2012).

P. multocida is a capsulated, non-motile, non-spore forming, gram negative coccobacilli. A number of serological techniques including agar gel precipitation and mouse protection test are used for confirmation of both vaccine and field isolates of *P. multocida* (Naz *et al.,* 2012). Accuracy of serological identification of microbes is more reliable and specific in comparison with biochemical tests. The reliability of this identification system depends on antiserum used, cross reactivity and differences in immune responsiveness of host animal used to raise serum (Munir *et al.,* 2007)

HS is an acute disease making its treatment extremely difficult (Naz *et al.,* 2012). Immediate and efficient treatment depends on the prior knowledge of recent antibiotic susceptibility patterns of causative agent. Although, antimicrobials have been widely used for the treatment of clinical infections, misuse and over use of antibiotics in clinical cases, veterinary and agricultural set ups pose a high risk for the selection of resistant bacteria (Nawaz *et al.,* 2011). This has resulted in reduced efficacy of the antimicrobial agents that are currently available for the treatment of infections in animals. Emergence of multiple drug resistance and its transfer between microbes of different origins has compounded the problem to its maximum.

Different researchers have studied antibiotic resistance or sensitivity patterns in *P. multocida* isolated from different sources (Babetsa *et al.,* 2012, Hendriksen *et al.,* 2008, Huang *et al.,* 2009, Kroemer *et al.,* 2012, Markowska-Daniel *et al.,* 2010, Mohamed *et al.,* 2012, Shivachandra *et al.,* 2011).Antibiotic resistance pattern of *P. multocida* has also been described in few of the previous studies from different area of Pakistan (Kalhoro *et al.,* 2010, Naz *et al.,* 2012), but there are no reports on the antibiotic susceptibility pattern from the isolates from Sargodha Division of Punjab. Keeping this in mind the present study was designed to isolate, identify and determine the antibiotic susceptibility pattern of *P. multocida* isolates against antibiotics of different classes which are in use against HS.

MATERIALS AND METHODS

Samples: Blood samples from larynx/pharynx of slaughtered buffaloes (Healthy animals) and blood or tracheal swabs and long bones from dead or suspected animals (HS suspected) were collected from different areas of Sargodha division, Punjab. The samples were processed for isolation and identification of suspected etiological agents by standard methods (Carter 1984).

Isolation and identification: Samples were inoculated in Tryptic Soy Broth (TSB) and incubated at 37°C for 24 hours. Broth cultures were inoculated (multiple streaking) on tryptic soy agar (TSA) plate for purification of bacterial culture. The isolates were identified on the basis of colony characteristics, gram reaction, and biochemical characteristics using API (NE-20). The isolates were further confirmed serologically by agar gel precipitation test as described by (Heddleston *et al.,* 1972) and mouse protection tests (Roberts 1947) and specie specific polymerase chain reaction (PCR) (Townsend *et al.,* 2001).

***In vitro* antibiotic sensitivity of isolates:** Antibiotic susceptibility pattern of *P. multocida* isolates against ten commercially available antibiotics (gentamicin, erythromycin, amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, enrofloxacin, sulfadiazine and amikacin) was determined by disc diffusion assay (Bauer *et al*.1966). The inoculums were prepared from fresh cultures of *P. multocida*, resuspended in phosphate buffer saline (0.5 McFarland). Inoculum was evenly spread on TSA plates with cotton swabs. Antibiotic discs were placed on inoculated agar plates and incubated at 37°C for 24 hours. Diameter of the zones of inhibition around each disc was measured and isolates were designated as sensitive, intermediate or resistant on the basis of zone of inhibition.

RESULTS AND DISCUSSION

The *P. multocida* (n=11) were isolated from diseased (07) and healthy buffaloes (04) from Sargodha division. Results of antibiotic sensitivity pattern of isolates are presented in table1.

Sensitivity of *P. multocida* isolates to enrofloxacin was highest (90.91 percent) followed by 72.73% to each of gentamicin, chloramphenicol, ciprofloxacin and norfloxacin. Sensitivity of isolates to ampicillin and amoxicillin was 45.45% while 36.36% isolate were sensitive to sulfadiazine. Lowest sensitivity was observed for to amikacin and erythromycin (18.18%).

High level of antibiotic susceptibility of *P. multocida* to enrofloxacin reported in this study is in agreement with previous studies by Aye *et al.,* (2001), Anwar *et al*. (2000), Kromer *et al* (2012),and Naz *et al.,* (2012). In present study, ciprofloxacin and norfloxacin resistance was also observed in isolates of *P. multocida* which is in agreement with Carty *et al*. (2005). In contrast, Shayegh *et al*. (2009) and Naz *et al.,* (2012) reported the absence of quinolones resistance in *P. multocida*. *P. multocida* were fairly sensitive to gentamicin (72.73%). Similar finding has also been observed by Balakrishnan *et al.,* (2001), Verma *et al.,* (2004) and Naz *et al.,* (2012). Amikacin was one of the least effective antibiotics against *P. multocida* (18.18%). In contrast to our findings, Aye *et al.,* 2001 found that most of the isolates of *P. multocida* were sensitive to amikacin. Susceptibility of *P. multocida* were moderate against beta lactam antibiotics (45.45%), which is higher than previously reported (25%) by Naz *et al.,* (2012) and lower thanShikuma *et al.,* (1985) and Wissing *et al.,* (2001). *P. multocida* were generally resistant to sulfadiazine and erythromycin which is in agreement with Naz *et al.,* (2012). Chlorempheniсol, like quinolones, was also highly effective (72.73%) against *P. multocida*, which is in agreement with Balakrishnan *et al.,* (2001) and Zahoor *et al.,* (2006). In contrast, Naz *et al.,* (2012) observed that 50% isolates of bovine origin were resistant to chloramphenicol.

Table 1: Antibiotic sensitivity pattern of *P. multocida* isolates against commonly used antibiotics

Antimicrobial agent	Concentration µg/disk	Sensitivity pattern, n (%)		
		S	I	R
Ampicillin	10	5 (45.45)	2(18.18)	4(36.37)
Amoxicillin	10	5 (45.45)	3(27.27)	3(27.27)
Amikacin	30	2 (18.18)	1 (9.09)	8 (72.73)
Gentamicin	10	8 (72.73)	0(00)	3 (27.27)
Chloramphenicol	30	8 (72.73)	2 (18.18)	1 (9.09)
Enrofloxacin	5	10 (90.91)	1 (9.09)	0 (00)
Ciprofloxacin	10	8 (72.73)	1 (9.09)	2 (18.18)
Norfloxacin	10	8 (72.73)	1 (9.09)	2 (18.18)
Erythromycin	10	2 (18.18)	6 (54.54)	3 (27.27)
Sulfadiazine	10	4 (36.36)	0 (00)	7 (63.64)

S= Sensitive, R= Resistant, I= Intermediate.

Although isolates of *P. multocida* show resistance against many of the commonly used antibiotics, quinolones and chloramphenicol are still effective. It is insinuated that antibiotic susceptibility patterns *P. multocida* may be determined on larger scale for effective treatment of the HS.

REFERENCES

Anwar, M.A.H., S.U. Rehman and R. Ahmad (2000). Antibiotic susceptibility of *Pasteurella multocida* isolated from cattle and buffaloes. Pak. J. Biol. Sci. 3(4): 392-393.

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.

Babetsa, M., V. Sandalakis, C. Vougidou, A. Zdragas, A. Sivropoulou, A. Psaroulaki and L.V. Ekateriniadou (2012). Tetracycline resistance genes in *Pasteurella multocida* isolates from bovine, ovine, caprine and swine pneumonic lungs originated from different Greek prefectures. Afric. J. Microbiol. Res. 6(17): 3917-3923.

Balakrishnan, G. and M. Mini (2001). Pathogenicity of avian strains of *Pasteurella multocida*. Indian Vet. J. 78: 959-960.

Benkirane, A. and M.C.L. De-Alwis (2002). Haemorrhagic septicemia, its significance, prevention and control in Asia. Vet. Med-Czech. 47(8): 234-238.

Carter, G.R. (1984). Diagnostic procedures in veterinary bacteriology and mycology. (4 ed.): Charles C Thomas Publishing Co., Springfield, IL.

De Alwis, M.C. and M.A. Sumanadasa (1982). Naturally acquired immunity to haemorrhagic septicaemia among cattle and buffaloes in Sri Lanka. Trop. Anim. Health Prod. 14(1): 27-28.

Hajikolaei, M., M. Ghorbanpour, M. Seyfi-Abadshapouri, A. Rasooli, G. Moazeni-Jula and D. Ebrahimkhani (2008). Study on the prevalence of *Pasteurella multocida* carriers in slaughtered cattle and relationship with their immunity status at Ahvaz abattoir. J. Vet. Res. 63: 25-29.

Heddleston, K.L., T. Goodson, L. Leibovitz and C.I. Angstrom (1972). Serological and biochemical characteristics of *Pasteurella multocida* from free-flying birds and poultry. Avian Dis. 16(4): 729-734.

Hendriksen, R.S., D.J. Mevius, A. Schroeter, C. Teale, D. Meunier, P. Butaye, A. Franco, A. Utinane, A. Amado, M. Moreno, C. Greko, K. Stark, C. Berghold, A.L. Myllyniemi, D. Wasyl, M. Sunde and F.M. Aarestrup (2008). Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002-2004. Acta. Vet. Scand. 50: 28.

Huang, T.M., T.L. Lin and C.C. Wu (2009). Antimicrobial susceptibility and resistance of chicken Escherichia coli, Salmonella spp. and Pasteurella multocida isolates. Avian Dis. 53(1): 89-93.

Kalhoro, D.H., A.B. Kalhoro, R. Rind, S. Kalhoro and H. Baloch (2010). In vitro antimicrobial susceptibility of Pasteurella multocida. Pak. J. Agri., Agri. Engg. Vet. Sci. 26(2): 80-86.

Khan, A., U. Saddique, R. Ahmad, H. Khan, Y. Mohammad and M. Zubair (2006). Sero-surveillance of hemorrhagic septicemia in cattle and buffalos in district Malakand, NWFP. J. Agri. Bio. Sci. 1(4): 11-14.

Kroemer, S., D. Galland, V. Guerin-Faublee, H. Giboin and F. Woehrle-Fontaine (2012). Survey of marbofloxacin susceptibility of bacteria isolated from cattle with respiratory disease and mastitis in Europe. Vet. Rec. 170(2): 53.

Markowska-Daniel, I., K. Urbaniak, K. Stepniewska and Z. Pejsak (2010). Antibiotic susceptibility of bacteria isolated from respiratory tract of pigs in Poland between 2004 and 2008. Pol. J. Vet. Sci. 13(1): 29-36.

Mohamed, M.A., M.W. Mohamed, A.I. Ahmed, A.A. Ibrahim and M.S. Ahmed (2012). *Pasteurella multocida* in backyard chickens in Upper Egypt: incidence with polymerase chain reaction analysis for capsule type, virulence in chicken embryos and antimicrobial resistance. Vet. Ital. 48(1): 77-86.

Munir, R., D. Shahwar, U. Farooq, I. Nawaz, I. Shahzad and A. Khanum (2007). Outer membrane protein profiling of *Pasteurella multocida*. Pak. Vet. J. 27(1): 1-4.

Nawaz, M., J. Wang, A. Zhou, C. Ma, X. Wu, J.E. Moore, B.C. Millar and J. Xu (2011). Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr. Microbiol. 62(3): 1081-1089.

Naz, S., A. Hanif, A. Maqbool, S. Ahmed and K. Muhammand (2012). Isolation, characterization and monitoring of antibiotic Resistance in *Pasteurella multocida* isolates from buffalo (Bubalus Bubalis) herds around lahore. J. Anim. Plant Sci. 22(3 Suppl.): 242-245.

Roberts, R.S. (1947). An immunological study of *Pasteurella septica*. J. Comp. Pathol. Ther. 57(4): 261-278.

Shayegh, J., P. Mikaili, J. D. Sharaf, and A. Rastgn (2009). Antimicrobal resistance evaluation of

Iranian ovine and bovine *Pasteurella multocida*. J. Anim. Vet. Adv. 8 (9): 1753-1756.

Shikuma, C.C. and G.D. Overturf (1985). Antibiotic susceptibility of *Pasteurella multocida*. Eur. J. Clin. Microbiol. 4(5): 518-519.

Shivachandra, S.B., K.N. Viswas and A.A. Kumar (2011). A review of hemorrhagic septicemia in cattle and buffalo. Anim. Health Res. Rev. 12(1): 67-82.

Townsend, K., J. Boyce, J. Chung, A. Frost and B. Adler (2001). Genetic organization of *Pasturella multocida* cap loci and development of multiplex capsular PCR typing system. J. Clin. Microbiol. 39: 924-929.

Verma, S.C., N.K. Mahajan, G. Malik and J.P. Dahiya (2004). An epidermiological study on bovine hemorrhagic septicemia in Haryana, Indian J. Anim. Sci. 38: 14–19.

Wissing, A., J. Nicolet and P. Boerlin (2001). The current antimicrobial resistance situation in Swiss veterinary medicine. Schweiz Arch. Tierheilkd. 143(10): 503-510.

Zahoor, M.A. and M. Siddique (2006). Characteristics of *Pasteurella multocida* recovered from avian sources. Pakistan Vet. J. 26(1): 41-43.