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Antimicrobial Resistance Evaluation of Iranian Ovine and Bovine *Pasteurella multocida*

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Abstract: Antibiotic resistance pattern of ovine and bovine *Pasteurella multocida* were evaluated to 16 antimicrobial agents by the disc diffusion method. About 75 and 42.85% of strains were resistant to lincomycin among ovine and bovine isolates, respectively. High level of penicillin resistance from bovine isolates is noticeable. Also, the antibiotic resistance patterns show the slight increase in resistance of other antibiotic and it is necessary to check antibiotic resistance patterns of *P. multocida* time to time in order to make effective decisions in treatment programming against animal disease.

Key words: *Pasteurella multocida*, antimicrobial, ovine and bovine, penicillin, Iran

INTRODUCTION

Pasteurella multocida is the causative agent of cattle and sheep disease that can be a primary or secondary agent involved in pneumonia in cattle and sheep and hemorrhagic septicemia in cattle and buffaloes (Harper *et al*., 2006). This bacterium as normal flora also is present in upper respiratory tract of cattle, sheep and buffaloes that causes disease as opportunistic respiratory pathogen (Shewen and Rice, 1993).

To prevent bovine pneumonic pasterurelosis, application of antibiotics may be performed for therapeutic, prophylactic or metaphylatic purpose (Schwarz and Chaslus-Dancla, 2001). In ovine population, application of antibiotics is similar to cattle.

In other hand, the use of antibiotics is the main cause of selective pressure that determine the rate and extent of the emergence of antimicrobial resistance, because it alters bacterial population through the elimination of susceptible bacteria and the survival of resistance ones. The selection pressures are exerted to commensal bacteria similar to pathogenic bacteria that are clearly the target bacterial population of antibacterial drugs. So, it is important to realize that antimicrobial drugs exert selection pressure on commensal bacteria (Barbosa and Levy, 2000) present in different organs of body such as respiratory tract (Berends *et al*., 2001; Sorum and Sunde, 2001). Since, *P. multocida* isolates are part of the commensal respiratory flora and also opportunistic pathogen, the contrast between pathogenesity and commensalisms is flatten in relation to the disease caused by *P. multocida*. This subject make them remarkable bacteria for research (Shewen and Rice, 1993).

Pneumonic Pasteurellosis is one of the major problems among ovine and bovine respiratory complex syndrome with viral and other bacterial agents in Iran.

The present investigation that are first one in Iran was carried out to study the antibiotic patterns of ovine and bovine *P. multocida* strains to allow selection of suitable antibiotics in order to effective treatment and control.

MATERIALS AND METHODS

Bacterial strain: Thirty seven strains of *P. multocida* (30 strains from sheep and 7 from cattle) were previously obtained from nasal cavity of healthy and diseased animals by our research group (Shayegh *et al*., 2008). The cells were stored in Brain Heart Infusion (BHI) with 30% glycerol at -70°C. Morphological, cultural and biochemical identifications were carried out according to standard test (Barrow and Feltham, 1993). All the strains used in this study have been already confirmed by multiplex PCR (Townsend *et al*., 2001) as *P.multocida* by our group (Shayegh *et al*., 2008). For more confidence, some of isolates were randomly confirmed by multiplex PCR again (Townsend *et al*., 2001).

Antimicrobial susceptibility test: The susceptibilities of the isolates to 16 antimicrobial agents were determined by the disc diffusion method on Muller Hinton agar with 5% blood (Carter and Subronto, 1978).

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The plates were inoculated with a cotton swab dipped into a 0.5 McFarland standard suspension of each isolates, according to the procedures outline in CLSI. Then, the plates were incubated at 37°C for 24 h. The inhibition zone around each disc was measured and compared with standard zone suggested by manufacture recommendation (Pattan-Teb, Tehran, Iran).

RESULTS AND DISCUSSION

Identification of bacterial isolates: Culture and morphological identification of suspected isolates confirm them as *P. multocida*. Small glistening mucoid dewdrop-like colonies were appeared on blood agar plates after incubation at 37°C for 24 h. All isolates were gram-negative coccobacilli and were indole, catalase and oxidase positive. But, citrate, MR, VP and gelatin liquefaction tests of all isolates were negative. Growth test on MacCankey agar was negative with no motile and non-hemolytic effects on blood agar. Multiplex PCR (Townsend *et al*., 2001) result about some strains was in accordance with our previous result (Table 1).

Antimicrobial susceptibility test: The results of *P. multocida* sensitivity pattern against 16 antimicrobial agents presented in Table 2. All of isolates demonstrate sensitivity against Choramphenicol (100%), Ciprofloxacin (100%), Co-trimoxazole (100%), Doxycycline (100%), Enrofloxacin(100%),Nitrofurantein(100%),Oxytetracyclin (100%),Rifampcin(100%), Ampicillin(96%), Carbenicillin (96%), Gentamycin (96%), Streptomycin (96%), Amikacin (83.5%) among ovine isolates. Prevalence of intermediate sensitive strains against penicillin is high. Also, 75% of isolates were resistant to lincomycin.

The result of bovine isolates was similar to ovine ones (Table 2). However, penicillin resistances (82.85%) were high among bovine isolates. About 42.85% of bovine isolates were resistant to lincomycin, too.

Antimicrobial resistance of *Pasteurella* isolates varies according to the host animal species, time, geographical origin and antimicrobial pretreatment of the animals (Kehrenberg *et al*., 2001). The results of this study indicated that the resistance strains of ovine and bovine *P. multocida* has been increased in Iran, following extensive consumption of antimicrobial agent in Iranian herds of sheep and cattle.

Most of the resistance genes to date encountered among *Pasteurella* isolates are associated with either small plasmids or with conjugative and non-conjugative transposons (Kehrenberg *et al*., 2001).

In this study, exception of penicillin resistance among bovine isolates we did not find noticeable resistance against antibiotic agent that other studies had reported resistance to them. Resistance to β-lactams among *P. multocida* isolates is often mediated by small plasmids of 4.1-4.4 kb (Kehrenberg *et al*., 2001).

However, we found important prevalence of lincomycin resistance isolates. Diker *et al*. (1994) reported 100% lincomycin resistance against *P. multocida* isolated from pneumonic ovine lungs in Turkey. The results of this study, showed resistance of lincomycin among ovine and bovine *P. multocida* isolates. Some of the local papers in Iran reported similar resistance against *P. multocida* in Iran. Lincomycin is a broad-spectrum antibacterial agent, active against a variety of bacteria, as well as against mycoplasmas. The drug is completely absorbed after injection and has a particularly good distribution to tissues, where it reaches antibacterial concentrations. In sheep, the drug has been successfully and repeatedly used for the control of foot rot or other disorders of legs (Plenderleith, 1988; Venning *et al*., 1990; Jordan *et al*., 1996), as well as for the treatment of contagious agalactia. Skoufos *et al*. (2006) reported that the Lincomycin is effective against established respiratory mycoplasmal infections of the lambs. Lincomycin has an extensive application in sheep and less than cattle diseases in Iran. It may be the extensive application of lincomycin induces resistance against this antibiotic

Table 1: The list of isolates that are studied in this study

Source	Symptom	Genotype
Cattle	Mastitis	A
Cattle	Pneumonia	A
Cattle	Pneumonia	B
Cattle	Nasal discharge	A
Cattle	Health	A
Buffalo	Health	D
Cattle	Health	D
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	[1]Un
Sheep	Nasal discharge	A
Sheep	Pneumonia	[1]Un
Sheep	Pneumonia	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	[1]Un
Sheep	Nasal discharge	A
Sheep	Nasal discharge	D
Sheep	Nasal discharge	D
Sheep	Nasal discharge	A
Sheep	Nasal discharge	U
Sheep	Nasal discharge	U
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A
Sheep	Nasal discharge	A

[1]Untyping

Table 2: The results of *P. multocida* sensitivity pattern against 16 antimicrobial agents

Antimicrobial agent	Concentration (mcg disc^{-1})	No. strain resistance (%)		No. strain intermediate (%)		No. strain sensitive (%)	
		Cattle	Sheep	Cattle	Sheep	Cattle	Sheep
Ampicillin	10	1 (14.2)	1 (4)	-	-	6 (85.7)	29 (96)
Amikacin	30	-	-	1 (14.2)	5 (16.5)	6 (85.7)	25 (83.5)
Choramphenicol	30	-	-	-	-	7 (100)	30 (100)
Carbenicillin	100	-	-	-	1 (4)	7 (100)	29 (96)
Ciprofloxacin	5	-	-	-	-	7 (100)	30 (100)
Co-trimoxazole	25	-	-	-	-	7 (100)	30 (100)
Doxycycline	30	-	-	-	-	7 (100)	30 (100)
Enrofloxacin	5	-	-	-	-	7 (100)	30 (100)
Gentamycin	10	-	-	-	1 (4)	7 (100)	29 (96)
Lincomycin	10	3 (42.85)	23(75.5)	2 (28.57)	5 (16.5)	2 (28.57)	2 (8)
Nitrofurantein	300	-	-	-	-	7 (100)	30 (100)
Oxytetracyclin	30	-	-	-	-	7 (100)	30 (100)
Penicillin	10 unit disc^{-1}	3 (42.85)	4(13.3)	2 (28.57)	26 (86.7)	2 (28.57)	-
Rifampcin	5	1 (14.2)	-	1 (14.2)	-	5 (71.42)	30 (100)
Streptomycin	10	1 (14.2)	-	-	1 (4)	6 (85.7)	29 (96)
Tetracycline	30	-	-	-	11 (37)	7 (100)	19 (53)

among *P. multocida* isolates as a normal flora. According to our knowledge, there is no study about lincomycine resistance gene in *P. multocida*. Molecular based study of lincomycin resistance are necessary.

CONCLUSION

The antibiotic resistance patterns of present study show the slight increase in resistance of other antibiotic such as amikacine, tetracycline and rifampine among ovine and bovine *P. multocida* isolates. It is presumed that antibiotic resistances in other agents grow, too. In view of these facts, it is necessary to check antibiotic resistance patterns of *P. multocida* time to time in order to make effective decisions in treatment programming against animal disease.

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