Multi Drug Resistance of *Pasteurella* spp. Isolated from Sheep and Goats in Iran

Yahya Tahamtan and Masoumeh Hayati
Department of Bacteriology, Razi Vaccine and Serum Research Institute, Shiraz, Iran

*Corresponding Author, Yahya Tahamtan, Razi Vaccine and Serum Research Institute, Shiraz, Sanaye Sq, Shiraz, Iran Tel: 0987116240331 Fax: 0987116240201*

ABSTRACT

One of the most frequent causes of respiratory infection and death in sheep and goats is *Pasteurella multocida*. Among the antibiotics, penicillin is the important member of the beta-lactam group has a wide spectrum of activity against *Pasteurella* strain. Although, *Pasteurella* usually susceptible to penicillin, tetracycline or chloramphenicol, during the several years ago, drug-resistant to *Pasteurella* strains have been encountered. The aim was evaluation of resistance pattern of *Pasteurella* to antimicrobial agents. A collection of 64 *P. multocida* strains were evaluated against 23 antimicrobial agents using two methodology, E test and disk diffusion. Nearly fifteen percent of *P. multocida* was significantly resistance to penicillin. Multi drug resistance was also observed among the isolates. E test MICs results were always more susceptible than those obtained by disk diffusion at the end of the MIC ranges. Penicillin resistance of *P. multocida* isolated from sheep and goats and also resistant to clindamycin and oxacillin have been reported. Eventually, use of Penicillin could potentially give rise to antibiotic resistance in *P. multocida* and should be considered as a risk factor for treatment of human pasteurellosis.

**Key words:** *Pasteurella*, antibiotic resistant, sheep, goats, Iran

INTRODUCTION

Antimicrobial drugs have been widely used in human and veterinary medicine for more than 50 years with benefits to both human and animal health. Antimicrobial medicines are used against bacteria in human and animal food product during the 20th century (FDA, 2010). The introduction of antibiotics was one of the most important developments in modern medicine (Gottlieb and Nimmo, 2011). Following the use of antibiotic treatment in food animal such as cattle, sheep and goats developed with more than half (52%) of those bacteria resistant to antibiotics (Van Looveren et al., 2001). International and US public health agencies have targeted antibiotic resistance as an emerging public health concern and one of the greatest threats to human health worldwide (Barza and Travers, 2002; Spellberg et al., 2008).

Resistance which enables microbes to escape being killed by antimicrobial (including antibacterial, antiviral, antifungal, etc.) drugs, undermines physicians’ ability to treat serious and life threatening infections. Antibiotic resistant usually microorganisms are able alive after exposure to antibiotics, therefore physicians are not able to treatment dangerous infection illness (Spellberg et al., 2008; Boucher et al., 2009).
Veterinary Investigation Disease Analysis (VIDA) statistics have revealed that, over the past ten years or so, one of the most commonly diagnosed infectious causes of disease or death in sheep, in descending order, have been pneumonia due to the Mannheimia spp./Pasteurella spp. Veterinary Investigation Disease Analysis (VIDA) have indicated during ten years ago Mannheimia spp./Pasteurella spp. are the most representation causes of pneumonia in sheep and goats (Brogdan et al., 2001). Many animals including sheep and goats are able to become infected from this particular disease leading to pneumonia (Kilonzo-Nthenge et al., 2008). Young lambs are the most susceptible animal to infection from. The most susceptible animal to infection is young lamb because of weak immune system (Bruere et al., 2002).

Antimicrobials such as Penicillin are still the drug of choice to control Pasteurella (P) multocida and P. hemolytica infection in animals (Kehrenberg et al., 2001a; Schwarz et al., 2004). It is the important members of beta-lactam group of antibiotics licensed to treat diseases related to Pasteurella sp. in the sheep and goats in Iran (Tehrani et al., 2004).

Low doses of antibiotics in animal feed over a long period of time contribute to the growth of antibiotic resistant bacteria that can be transferred to humans and is a greater risk to public health. Exposing bacteria at low doses of antibiotic for long periods growth promoting antibiotic resistant and is a human health risk (Prescott et al., 2000; Ismail, 2004). Generally, P. multocida isolates are susceptible to most of the widely used commercial antimicrobial agents but their excessive and unjustified use accelerates the emergence of resistant strains. Antimicrobial therapy is effective tool for P. multocida infection, but using too much enhanced the risk of resistant stains (Ismail, 2004; Selleyi et al., 2009). In the last few years, many animal isolates of Pasteurella spp. were found to be resistant to antibiotics. It has been found over the last few years, resistant to antibiotics were found in many animal isolates of Pasteurella spp. (Selleyi et al., 2009). Resistance to beta-lactam groups has occasionally been reported (Fales et al., 1982; Kehrenberg et al., 2001b). Due to increasing antibiotic resistance, particularly major bacterial pathogens, accurate antimicrobial susceptibility testing results are vital for both animal care and public health surveillance (Kiehlbauch et al., 2000; Reynolds et al., 2003).

There are several established methods of antibiotics susceptibility tests used in different studies and different countries (Reynolds et al., 2003). Many of them, including disk diffusion, agar dilution, broth micro dilution and antibiotic gradient disks (Jorgensen, 1993; Donabedian et al., 2003). E test is convenient and widely used for susceptibility testing of several antibiotics on a large number of bacterial isolates in a short time. E test is appropriate used for study large number of antibiotics on bacterial isolates at one time (CLSI, 2009). Disk diffusion (Kirby-Bauer) is one of the most commonly used antimicrobial susceptibility testing methods among diagnostic laboratories. Disk diffusion (Kirby-Bauer) is most used to determine antibiotic sensitivity testing in diagnostic laboratories (Jorgensen and Ferraro, 2009; Van Belkum and Dunne, 2013). These methods are a well-established procedure and are accepted by the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

We investigated the antibiotic susceptibility against a very large contemporary collection of P. multocida in sheep and goats. We also evaluated the correlation between the susceptibility testing results generated by disk diffusion and Etest (AB Biodisk, Solna, Sweden) for 23 antimicrobials. In the current study the antibiotic resistant were evaluated by disk diffusion method and compared with E test for 23 antimicrobial agents. The aim was also to evaluate
whether and how the new species related breakpoints may influence the detection decrease antibiotic susceptibility among Pasteurella strains.

**MATERIALS AND METHODS**

**Sample:** A collection of 64 P. multocida strains isolated from sheep and goats in different parts of Iran were examined.

**PCR assay:** This collection has been well characterized by PCR relation to kmt1 (all pass), hydD-hydC (Cap A), ddbP (Cap D) and toxA (Dermo Necrotic Toxin) genes in our previous study (Sahragard et al., 2011). All isolates harboured toxA gene and belonged to capsular type A.

**Antibiotic test:** In addition, all P. multocida strains were evaluated by the E test methodology against Penicillin, Streptomycin, Gentamicin, Enrofloxacine, Ceftriaxone, Ampicillin, Flumequine, Lincopectin, Tetracycline, Oxacillin, Carbenicillin, Cloxacillin, Cefotaxime, Furaladone, Neurofloxacine, Clindamycin, Neomycin, Nalidixic acid, Amoxicillin, Cefotin, Cephalexin, Erythromycin and Oxytetracycline using two inocula (0.5 and 2 McFarland standards) (CLSI, 2009). E test strips were used in accordance to manufacturer’s practice. Mueller-Hinton agar plates were inoculated with a 0.5 McFarland standard of the P. multocida isolates. Four E test strips were applied to the surface of the plate in an equidistance radial manner, with the lowest concentration toward the centre. Plates were incubated under the same condition as for agar dilution. Minimum inhibitory concentrations (MICs) were read directly from the test strip at the point where the zone of inhibition intersected the MIC scale on the strip. The concentration gradient of each antimicrobial agent on the E test strips was 0.016-256.0 mg L⁻¹. Disk diffusion tests with 30 μg of all antibiotics disks were performed on all 64 isolates according to the method published by the CLSI for 100 mm Mueller-Hinton agar plates (CLSI, 2006, 2009). The quality control strains, PMSHI (Accession No.: JF694003.1), were tested along with every set of tests.

**Data analysis:** MIC agreement between the two methods was defined. The following MIC breakpoints defined by the NCCLS for the Pasteurelasciae were employed.

**RESULTS**

None of the isolates showed resistance to 20 antibiotics (Table 1). The percentages of isolates resistant to antibiotics were 15.62% to Penicillin, 34.37% to Clindamycin and 28.12% to Oxacillin. A scatter gram illustrating the disk diffusion zone diameter results for antibiotics is shown in Fig. 1. Fifty four strains were considered no susceptible to penicillin when tested by the disk diffusion method using the current CLSI interpretation criteria. However, these isolates had inhibition zone diameters of ≥ 16 mm and were considered susceptible according to CLSI breakpoints for the disk diffusion test (≥ 15 mm). A scatter gram illustrating demonstrated the inhibitory zone diameter varied from 10 to 44 mm among the 64 P. multocida strains.

Based on MICs from E test, Pasteurella isolates (n = 64) demonstrated the significantly high resistance rate (p<0.05) to Clindamycin (34.37%), Oxacillin (28.12%) and Penicillin (15.62%) (Fig. 2). Multidrug resistance was also observed among the Pasteurella isolates (Fig. 3).

For each antimicrobial agent, E test MICs were always one in two dilutions lower than those obtained by disk diffusion at the susceptible end of the MIC ranges. On the other hand, the E test tended to yield much higher resistant MICs than those measured by disk diffusion at the resistant
end of the MIC ranges (p<0.05). The overall agreement of MICs between the two methods was 79.8%, ranging from 45% with Erythromycin to 92.4% with Penicillin. Oxacillin and Clindamycin MIC agreement between the two methods was 56.3 and 61%, respectively.

Fig. 1: Thirty two isolates were shown the diameter zone between 10 to 20 mm while 32 of them resistant to Penicillin. Scattergram showing the disk zone diameter results. Thirty two isolates were shown the diameter zone between 10 to 20 mm while 32 of them resistant to Penicillin

Fig. 2: Antibiotic susceptibility of 64 isolates of *P. multocida*. Penicillin was shown resistant to more than 50% of isolates. Antibiotic susceptibility of 64 isolates of *P. multocida*. Fifty percent of isolates were resistant to Penicillin. But around 30% were resistant to Clindomycin and Oxacillin

Fig. 3: Multi drug resistance to *P. multocida*, bar 1: Ten isolates resistant to one antibiotic, bar 2: Eight to two, bar 3: Five to three and bar 4: Four to four. Multi drug resistance of *P. multocida* to three antibiotics
DISCUSSION

Although, the frequency of resistance varies by area and country, several investigators have been reported a frequency of Penicillin resistance in Pasteurella (Okamoto et al., 2002; Citron et al., 2005). Consequently, it has been recognized that there is a need to test Pasteurella and other unusual organisms for antimicrobial resistance. Therefore, there is necessary to evaluate the antimicrobial resistant pattern of Pasteurella (Citron et al., 2005). Multidrug-resistance (Penicillin, Erythromycin, Oxaclillin and Clindamycin) was observed in P. multocida that was isolated from sheep and goats in southern Iran. Recent reports have cited evidence for an increase in the incidence of Penicillin resistant throughout the world (Kilonzo-Nthenge et al., 2008). The Antibiotic resistance in Pasteurella in food animals is leading to increased for treatment of human pasteurellosis and increased public health threat (Graham et al., 2007).

However, there were no significant differences noted on disk diffusion method and E test, but the disk diffusion was an easy and inexpensive method for the susceptibility testing. All 64 P. multocida strains were highly susceptible to at least one antimicrobial agents with MIC<0.03 mg L^{-1}. The vast majority of the P. multocida strains were also susceptible to Enrofloxacin followed by Ceftriazone, Flumequine, Carbenicileen and Neurofl Roxacin. A nationwide survey reported in Japanese Journal reported that 40% of Pasteurella were resistant to Penicillin G and 53.7% of the same bacteria were resistant to Erythromycin (Okamoto et al., 2002).
The correlation between the E test and disk diffusion method, as compared in this study, varied depending on the antimicrobial agent tested. The E test and disk diffusion were the most practical and preferable method for susceptibility testing of Pasteurella and the overall exact agreement was 82.9%.

In conclusion, these data indicated that sheep and goats are reservoirs of P. multocida those are resistant to antibiotics. Multi drug resistance in food animal pathogens is certainly a public health concern and reinforces the need for more prudent use of antibiotics by farmers, veterinarians and physicians. Therefore, a continued development of methods to reduce the risk of pathogens in food producing animals is critical.

In summary, only the use of a standardized methodology in combination with veterinary specific breakpoints as recommended in the NCCLS document allow a reliable assessment of the number of antimicrobial resistant strains among P. multocida strains in Iran. Penicillin was used as an effective choice for P. multocida in Iran and this is the first report of Penicillin resistance.

ACKNOWLEDGMENTS
We are indebted to Dr Mohammed H. Hosseini for his antibiogram assistance. This study was supported by the Project Fund (Grant No. 89069-18-14-2).

REFERENCES
FDA, 2010. The judicious use of medically important antimicrobial drugs in food-producing animals. 
U.S. Department of Health and Human Services, Food and Drug Administration Center for 
Veterinary Medicine, No. 209, April 13, 2012.

resistance among Pasteurella spp. recovered from Missouri and Iowa cattle with bovine 

Gottlieb, T. and G.R. Nimmo, 2011. Antibiotic resistance is an emerging threat to public health: An 

Graham, J.P., J.J. Boland and E. Silberfeld, 2007. Growth promoting antibiotics in food animal 

Ismail, S., 2004. Antibiotic resistance pattern of Pasteurella multocida isolates from pasteurellosis 


Antimicrobial resistance in Pasteurella and Mannheimia: Epidemiology and genetic basis. Vet. 

Kehrenberg, C., S.A. Salmon, J.L. Watts and S. Schwarz, 2001b. Tetracycline resistance genes in 
isolates of Pasteurella multocida, Mannheimia haemolytica, Mannheimia glucosidial and 
Mannheimia varigena from bovine and swine respiratory disease: Intergeneric spread of the 

of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion 


Aerobically: Approved Standard. 5th Edn., National Committee for Clinical Laboratory 

Okamoto, H., K. Tateda, Y. Ishii, T. Matsumoto, T. Kobayashi, S. Miyazaki and K. Yamaguchi, 
2002. High frequency of Erythromycin resistance and distribution of mefE and ermF genes in 


dilution and NCCLS broth microdilution MIC methods for in vitro susceptibility testing of 
Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis: The BSAC 

of rapid PCR method for simultaneous identification of species, specific capsular type and 