

Antibiotics Susceptibility Reaction of *Pasteurella multocida* and *Mannheimia haemolytica* to Selected Eight Antibiotics

Sabiel, Y. A.¹; Musa, M. T.¹. and Hadya E Ahmed²

(1) Veterinary Research Institute, P.O. Box 8067 (Al-Amarat), Khartoum, Sudan. E. mail: sabiel-sd@hotmail.co.uk. (2) Animal Resources Research corporation, Khartoum,

Sudan. E. mail: musatibin@yahoo.com.

ملخص البحث

أجري اختبار حساسية 12 معزولة من المانهيمية الحالة للدم (*Mannhaemia haemolytica*) و8 معزولات من الباستيرية الفاتكة (*Pasteurella multocida*) لثمانية أنواع من المضادات الميكروبية بواسطة اختبار القرص المنتشر. هذه المعزولات تم عزلها من رئات ضان ملتية جمعت من سلخانة الدمازين بولاية النيل الأزرق - السودان. شملت هذه المضادات الميكروبية: سيفروفلوكساسين, سفترايكزون, سفبروكزيم, كلورامفينيكول, اميسلين, جنتاميسين, كوترايموكزازول وتتراسيكلين. كل المعزولات وجدت حساسه للسبروفلوكساسين, سفترايكزون, سفبروكزيم. ومعظمها مقاوم للجنتاميسين و كوترايموكزازول.

Summary

The antimicrobial susceptibility reactions of 12 *Mannheimia haemolytica* and 8 *Pasteurella multocida* isolates from pneumonic sheep, collected from Al-Damazin slaughterhouse, the Blue Nile State, Sudan, were evaluated by disk diffusion method. Each isolate was tested against ciprofloxacin, ceftriaxone, cefuroxime, chloramphenicol, ampicillin, gentamicin, co-trimoxazole and tetracycline. All isolates of the two species were sensitive to ciprofloxacin, ceftriaxone, cefuroxime and 90% of them were sensitive to chloramphenicol and ampicillin. Seventy - five per cent (9/12) of *M. haemolytica* isolates were resistant to gentamicin and co-trimoxazole. Fifty per cent (4/8) of *P. multocida* isolates were sensitive to tetracycline whereas 87.5% (7/8) of them were resistant to gentamicin and 62.5% (5/8) to co-trimoxazole.

Introduction

Respiratory diseases of sheep are spreading worldwide and in Sudan, incur significant economic losses. Bacterial and viral interactions with other predisposing factors play an important role in the occurrence of the diseases (Adlam, 1989). Members of the family *Pasteurellaceae* that are present in the mucous membrane of many animal species are opportunistic pathogens and some of them, including *P. multocida* and *M. haemolytica* cause diseases in animals and man (Zurlo, 2000).

Antimicrobial agents are widely used for the treatment of ovine respiratory infections. During acute infections and outbreaks of infectious diseases in groups or flocks, it is important to use effective antimicrobial agents as early as possible. In acute cases aspirin, flouxin, or ketoprofen may be used for short duration in conjunction with antibiotics therapy to avoid gastric ulceration or renal complications (Kahn and Lane, 2005). This empirical treatment is generally based on knowledge of the resistant pattern of the different bacterial pathogens to antimicrobial agents used in the particular animal species (Hendricksen *et al*, 2008). Resistance to some antibiotics is frequent in *P. multocida* and *M. Haemolytica*, although it is less common in other pasteurellae (Carter and Wise, 2004). Antimicrobial sensitivity tests are important; they are usually carried out to determine antibiotic sensitivity for effective treatment of bacterial infection *in vivo*. The effectiveness of antibiotic therapy is based on knowledge of the aetiological agent and its relevant antibiotic sensitivity (Wikipedia, 2010). The objectives of this study were to determine the antibiotic sensitivity of *P. Multocida* and *M. haemolytica* isolated from pneumonic lungs of sheep and to evaluate the efficacy of the present antibiotics used for the treatment of pneumonia especially pneumonic pasteurellosis in sheep.

Materials and Methods

The Kirby-Bauer agar diffusion method (Murray *et al*, 2003) was used to study antibiotic sensitivity reactions of twelve *M. haemolytica* and eight *P. multocida* isolates. Four morphologically similar discrete colonies of each isolate that were cultured onto Blood Agar (Oxoid, CM 271) were selected, picked up with a wire loop, transferred to a test tube

containing 4 ml normal saline (0.85%) and incubated for two hours at 37°C. Each suspension was adjusted with normal saline to 0.5 McFarland standards (Wikipedia, 2009). They were swabbed and streaked at three directions over the entire surface of the agar plate. Thereafter a disc (Multidisc G. X1-minus, Himedia laboratories, India) impregnated with the antibiotics was picked and placed onto the dried Muller-Hinton Agar plate with a sterile forceps and pressed down gently to ensure even contact with the medium. The plates were incubated in an inverted position for 18 h at 37°C. The sensitivity zones were measured; faint growth and tiny colonies were ignored. The results were recorded according to Patrick standards (Murray *et al*, 2003) as sensitive (S), intermediate (I) or resistance (R) (Table 1).

Table 1: Interpretive standards for disk diffusion method.

Antibiotic	Sensitive	Intermediate	Resistant	Unit in mm
Chloramphenicol	≥18	13-17	≤12	mm.
Ampicillin	≥17	14-16	≤13	mm.
Tetracycline	≥19	15-18	≤14	mm.
Gentamicin	≥15	13-14	≤12	mm.
Co-trimoxazole	≥16	11-15	≤10	mm.
Ceftriaxone	≥ 21	14-20	≤13	mm.
Cefuroxime	≥ 23	15-22	≥14	mm.
Ciprofloxacin	≥21	16-20	≤15	mm.

Results

All isolates of both bacterial species were sensitive to ciprofloxacin, ceftriaxone, cefuroxime and 90% of them were resistance to chloramphenicol and ampicillin. Seventy-five (9/12) of *M. haemolytica* isolates were resistant to gentamicin and co-trimoxazole. Fifty percent (4/8) of *P. multocida* isolates were sensitive to tetracycline and 87.5% (7/8) and 62.5% (5/8) were resistant to gentamicin and co-trimoxazole, respectively (Table 2).

Table 2: Antimicrobial drugs susceptibility of 12 *M. haemolytica* and 8 *P. multocida* isolates from pneumonic lungs of sheep.

Isolates No.	C	A	TE	G	CO	Ci	Cu	Cf
1	S	S	S	R	R	S	S	S
2	S	S	S	R	R	S	S	S
3	S	S	S	R	R	S	S	S
4	S	S	I	R	R	S	S	S
5	I	I	S	R	R	S	S	S
6	S	I	S	S	I	S	S	S
7	S	S	I	R	R	S	S	S
8	S	S	S	R	R	S	S	S
9	S	R	S	S	I	S	S	S
10	S	S	S	S	R	S	S	S
11	S	S	S	S	R	S	S	S
12	I	S	R	R	R	S	S	S

13	S	S	S	R	S	S	S	S
14	I	S	S	R	R	S	S	S
15	I	R	I	R	R	S	S	S
16	S	I	S	R	R	S	S	S
17	S	I	S	R	S	S	S	S
18	S	S	S	S	R	S	S	S
19	S	S	S	R	S	S	S	S
20	S	I	R	S	R	S	S	S

Key: Isolates from No. 1 to 12=*M. haemolytica*; from 13 to 20=*P. multocida*;

C=chloramphenicol; A= ampicillin; TE=tetracycline; G= gentamycine; Co=co-trimoxazole
 Ci=ceftriaxone; Cu= cefuroxime; Cf= ciprofloxacin; S= sensitive; R=resistant;

I=intermediate

Discussion

Antimicrobial sensitivity tests to *M. haemolytica* and *P. multocida* have been practised in many countries. Monitoring drug susceptibility of these bacterial species is essential to determine resistance development (Post *et al*, 1991; Watts *et al*, 1994). The antibiotic sensitivity profiles obtained are regionally different; therefore their continuous monitoring in each region is important to select an effective antibiotic drug for treatment of sheep pneumonia. An increase in the resistance against antibiotics in both organisms was reported in recent years (Welsh *et al*, 2004; Hendricksen *et al*, 2008). In the present study, ciprofloxacin, ceftriaxone, cefuroxime, chloramphenicol and ampicillin were found most effective antibiotics against both *P. multocida* and *M. haemolytica*. However, *P. multocida* and *M. haemolytica* showed minimal susceptibility to gentamycin and co-trimoxazole. These results are similar to those of Verna, or Vera *et al* (2006), Berge *et al* (2006) and Katsuda *et al*. (2009) who reported that all *M. haemolytica* and *P. multocida* strains were sensitive to ceftriaxone, cefuroxime and chloramphenicol.

Our findings are in agreement with Hendricksen *et al* (2008) who reported an increased resistance of *M. haemolytica* to ampicillin and tetracycline. Post *et al* (1991) reported that 90% of *P. multocida* isolates were moderately susceptible to ampicillin where *M. haemolytica* isolates were resistant to ampicillin. In a recent study, Milan *et al* (2009) have found that multi - resistance of *P. multocida* to multiple clinically used antibiotics is mediated by co-existence of small plasmids. In conclusion, the selection of antibiotic for effective therapy and prevention of increasing multi - drug resistance should be prudently performed on the basis of antimicrobial susceptibility tests, to treat cases of pneumonia in sheep with the suitable antibiotic effectively.

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