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Short communication

ANTIMICROBIAL SUSCEPTIBILITY SCREENING TEST OF *MANNHEIMIA HAEMOLYTICA* AND *PASTEURELLA MULTOCIDA* (SEROGROUP A) MOROCCAN STRAINS ISOLATED FROM RUMINANTS

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Summary

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The present study is the first report aimed to determine the antibiotic susceptibility profiles of *Mannheimia haemolytica* and *Pasteurella multocida* serogroup A Moroccan isolates. Each isolate was tested for sensitivity to amoxicillin (A), amoxicillin/clavulanic acid (AMC), gentamicin (CN), streptomycin (STR), florfenicol (FFC), doxycycline (DO), erythromycin (E), spiramycin (SP), nalidixic acid (NA), flumequine (UB), enrofloxacin (ENF) and sulfamethoxazole (SXT). All isolates showed resistance to the antibiotics tested at a rate greater than 14%, except for one *P. multocida* isolate which had no resistance profile against AMC. The highest level of resistance was found against NA for *P. multocida* (100%) and against UB (82.4%) for *M. haemolytica*. The sensitivity rates for *P. multocida* were between 0 (against NA) and 85.7% (against STR, AMC, FFC), whereas sensitivity of *M. haemolytica* isolates ranged from 17.6% against UB and 79.4% against AMC. For both *Pasteurellaceae* species, the AMC was the most effective antimicrobial agent, however multi-drug resistance was observed in all isolates raising the interest to monitor the antimicrobial susceptibility of *Pasteurellaceae* species to determine appropriate antibiotic for treatment of pasteurellosis.

Key words: antibiotic susceptibility, *Mannheimia haemolytica*, Moroccan isolates, *Pasteurella multocida*

Pasteurella multocida and *Mannheimia haemolytica* both belonging to the family *Pasteurellaceae,* are known as opportunistic pathogens causing pasteurellosis (Fulton *et al.*, 2009). Because resistance to antibiotics is frequent in *Pasteurella-*

ceae species (Portis *et al.*, 2012; Cameron & McAllister, 2016), therefore antibiotic susceptibility tests are important. The medical and economic importance of "respiratory pasteurellosis" in intensively reared ruminants is at the background of extensive use of anti-infective drugs in veterinary medicine. The progress of antibiotic reinstatement is a constant threat requiring permanent surveillance (Martel *et al.*, 2017). Based on importations and sales of veterinary drugs, Moroccan veterinary competent authorities (ONSSA) started collecting data on the use of antibiotics since 2015. The monitoring of using this type of products showed that the family of tetracyclines, especially oxytetracycline, is the most commonly used antibiotic family in ruminants, followed by polypeptides, especially colistin (Kawtar, 2018; Soukaina, 2018).

The aim of the present study was to determine for the first time the antibiotic susceptibility profiles of forty-one Moroccan isolates, identified *as P. multocida* and *M. haemolytica* from animals with signs of respiratory distress and dead animals.

Forty-one *M. haemolytica* and *P. multocida* strains (Table 1) were isolated from nasal swabs and tissues taken from sheep, goat and cattle. A total of 41 lung and 121 nasal swabs samples were collected from 162 animals with respiratory diseases during 2015 to 2017 in six different regions in Morocco (Sebbar *et al.*, 2018).

The antimicrobial susceptibility test on the isolates was performed using Kirby-Bauer disc diffusion method (Hudzicki, 2009). The bacterial suspensions were prepared, left to react for a few minutes and poured on Mueller-Hinton agar (Oxoid, UK) plate. After removal of the excess volume, the plates were allowed to dry for five minutes and the antimicrobial discs were placed on the surface approximately 2 cm apart. Then they were incubated at 37 °C overnight. The inhibition zones were measured with a ruler to the nearest millimetre (Hudzicki, 2009). *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as quality control strains (Bauer *et al.*, 1966). Each of the above isolates were subjected to a panel of twelve antibiotics recommended by OIE manual (OIE, 2018): amoxicillin (A, 25 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), gentamicin (CN, 15 µg), streptomycin (STR, 10 UI), florfenicol (FFC, 30 µg), doxycycline (DO, 30 UI), erythromycin (E, 15 UI), spiramycin (SP, 100 µg), nalidixic acid (NA, 30 µg), flumequine (UB, 30 µg), enrofloxacin (ENF, 5 µg) and sulfamethoxazole (SXT, 23.75 µg).

The strains were classified as sensitive, intermediate or resistant, using the zone diameter (Wikins *et al.*, 1972). The ranges for each drug used in this study are

Table 1. *Mannheimia haemolytica* and *Pasteurella multocida* isolates from clinical nasal swabs and lung samples from ruminants in Morocco.

	Pasteurellaceae species		Total
	Mannheimia haemolytica	*Pasteurella multocida*	
Swabs	28	5	33
Lung samples	6	2	8
Number of isolates	34	7	41
Percentage	82.9%	17.1%	100%

Table 2. Inhibition zone diameter (mm) interpretation chart for the determination of antibiotic sensitivity and resistance

Antimicrobial agent	Disc potency	Resistant	Intermediate	Sensitive
Amoxicillin	25 µg	<14	15–20	≥21
Amoxicillin/clavulanic Acid	20/10 µg	<14	15–20	≥21
Gentamicin	15 µg (10 UI)	<14	15	≥16
Streptomycin	10 UI	<13	14	≥15
Florfenicol	30 µg	<15	16–18	≥19
Doxycycline	30 UI	<17	18	≥19
Erythromycin	15 UI	≤13	14–22	≥23
Spiramycin	100 µg	<16	17–21	≥22
Nalidixic acid	30 µg	<15	16–19	≥20
Flumequine	30 µg	<21	22–24	≥25
Enrofloxacin	5 µg	<17	18–21	≥22
Sulfamethoxazole	23.75 µg	<10	11–15	≥16

given in Table 2 according to the Antibiogram Committee of the French Society of Microbiology (AC-FSM) veterinary recommendations (EUCAST, 2019).

A Chi-square test and Fisher exact test were performed to determine the significance of the resistance rates regarding antibiotic families. A P value < 0.05 was considered statistically significant. Statistical analyses were performed using the IBM SPSS (version 25) package.

According to antimicrobial results, antimicrobial resistance rates of isolates from both *Pasteurellaceae* species were over 14% in all antibiotic drug classes. This has been reported in recent years (Hendriksen *et al.*, 2008; Jamali *et al.*, 2014). An exception was one strain of *P. multocida* serogroup A which had no resistance to AMC (beta-lactams family), comparably to rates reported previously (Yoshimura *et al.*, 2001; Welsh *et al.*, 2004). The highest level of resistance was found against quinolones – NA for *P. multocida* (100%) and against UB (82.4%) for *M. haemolytica* in contrast to the work carried out by Sellyei *et al.* (2009). So, the demonstrated resistance suggests that these antimicrobials should not be repeatedly used for both control and treatment of pasteurellosis.

The sensitivity rates for *P. multocida* were between 0 (against NA) and 85.7% (against STR, AMC, FFC), whereas sensitivity of *M. haemolytica* isolates ranges from 17.6% against UB and 79.4% against AMC. Similar results have been reported by Onat *et al.* (2010). All results of antimicrobial susceptibility screening test are summarised in Table 3.

The monitoring of the antimicrobial susceptibility of *Pasteurellaceae* species is essential to determine resistance development. Increases in resistance against antibiotics in both *Pasteurellaceae* species have been reported in the last years (Welsh *et al.*, 2004; Catry *et al.*, 2006; Hendriksen *et al.*, 2008) and confirmed in this current study that showed multi-drug resistance, as resistance profiles were established to at least three classes of antimicrobial agents.

Substantial resistance with respect to antibiotic families was obtained for beta-lactams, especially AMC (P=0.009) and macrolides families (P<0.001) which is very close to the results reported previously by Onat *et al.* (2010).

Table 3. Number of isolates tested and sensitivity to tested antibiotics

Antibiotic families	Code	*Mannheimia haemolytica (n=34)*			*Pasteurella multocida (n=7)*		
		S (%)	Int (%)	R (%)	S (%)	Int (%)	R (%)
Beta-lactams	A	17 (50)	4 (11.8)	13 (38.2)	2 (28.6)	2 (28.6)	3 (42.9)
	AMC	27 (79.4)	0	7 (20.6)	6 (85.7)	1 (14.3)	0
Aminoglycosides	STR	25 (73.5)	1 (2.9)	8 (23.5)	6 (85.7)	0	1 (14.3)
	CN	24 (70.6)	3 (8.8)	7 (20.6)	5 (71.4)	0	2 (28.6)
Macrolides	E	10 (29.4)	3 (8.8)	21 (61.8)	2 (28.6)	0	5 (71.4)
	SP	10 (29.4)	3 (8.8)	21 (61.8)	2 (28.6)	0	5 (71.4)
Sulfamides	SXT	23 (67.6)	0	11 (32.4)	5 (71.4)	0	2 (28.6)
Quinolones	NA	9 (26.5)	0	25 (73.5)	0	0	7 (100)
	UB	6 (17.6)	0	28 (82.4)	2 (28.6)	0	5 (71.4)
Tetracyclines	DO	14 (41.2)	0	20 (58.8)	3 (42.9)	0	4 (57.1)
Fluoroquinolo-nes	ENF	15 (44.1)	4 (11.8)	15 (44.1)	2 (28.6)	1 (14.3)	4 (57.1)
	FFC	24 (70.6)	5 (14.7)	5 (14.7)	6 (85.7)	0	1 (14.3)

S: Sensitive, Int: Intermediate, R: Resistant; A: Amoxicillin, AMC: Amoxicillin/clavulanic acid, CN: Gentamicin, STR: Streptomycin, FFC: Florfenicol, DO: Doxycycline, E: Erythromycin, SP: Spiramycin, NA: Nalidixic acid, UB: Flumequine, ENF: Enrofloxacin, SXT: Sulfamethoxazole.

Finally, the present study has shown that multi-drug resistance was found in all isolates. Amoxicillin/clavulanic acid was the most effective antimicrobial agent for both *Pasteurellaceae* species.

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