Acta Veterinaria Hungarica 67 (4), pp. 489–498 (2019)
DOI: 10.1556/004.2019.048

ANTIMICROBIAL SUSCEPTIBILITY OF *PASTEURELLA MULTOCIDA* ISOLATED FROM SHEEP AND PIGS IN SPAIN – SHORT COMMUNICATION

Dolores CID[1], José Francisco FERNÁNDEZ-GARAYZÁBAL[1,2], Chris PINTO[1,3], Lucas DOMÍNGUEZ[1,2] and Ana Isabel VELA[1,2*]

[1]Animal Health Department, Veterinary School, Universidad Complutense de Madrid, Av. Puerta de Hierro, s/n, 28040 Madrid, Spain; [2]Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense, Madrid, Spain; [3]London School of Hygiene and Tropical Medicine/ Royal Veterinary College, University of London, London, United Kingdom

(Received 20 May 2019; accepted 25 September 2019)

Pasteurella multocida is responsible for economically important diseases in sheep and pigs. Antimicrobial susceptibility studies are essential for initiating rational and effective empirical therapy of *P. multocida* infections. In this study we investigated the antimicrobial susceptibility to 18 antimicrobial agents of 156 clinical isolates of *P. multocida* from sheep (n = 87) and pigs (n = 69) using the microdilution method. Both sheep and pig isolates exhibited low levels of resistance (≤ 15%) to ceftiofur, gentamicin, neomycin, spectinomycin, chlortetracycline, tulathromycin, florfenicol, danofloxacin, and enrofloxacin and trimethoprim/sulphamethoxazole, high resistance rates (> 15% up to 50%) to oxytetracycline, tilmicosin, and tiamulin, and very high resistance rates (> 50%) to tylosin tartrate, clindamycin, and sulphadimethoxine. However, sheep isolates exhibited significantly lower percentages of resistance and lower MIC_{90} values ($P < 0.05$) than pig isolates for most of the antimicrobials tested. In addition, sheep isolates exhibited also significantly lower phenotypic antimicrobial resistance diversity (8 resistotypes vs. 30 resistotypes). LAC-LIN-SUL-MAC was the resistotype most frequently detected in sheep (39.1%) and LIN-SUL-MAC in pig isolates (26.1%). The differences in susceptibility patterns could be influenced by the lower use of antimicrobials in the small ruminant industry compared with the pig farming industry.

Key words: Antimicrobial resistance, *Pasteurella multocida*, pigs, sheep, production system

Pasteurella multocida is an important pathogen responsible for a diversity of diseases with an economic impact in different livestock species, including sheep and pigs. (Wilson and Ho, 2013). Although this pathogen is usually susceptible to several antimicrobials, resistance in different animals has been report-

*Corresponding author; E-mail address: avela@ucm.es; Phone: 0034 (913) 943-3709

ed (San Millan et al., 2009; Tang et al., 2009), which represents a threat regarding treatment options. Therefore, monitoring antimicrobial susceptibility is essential to acquire regional information and help veterinarians to initiate rational and effective empirical therapy in acute situations. Antimicrobial susceptibility studies in *P. multocida* have mainly been performed in isolates from cattle, poultry, or pigs (Post et al., 1991; Lizarazo et al., 2006; Kumar et al., 2009; Furian et al., 2016). However, similar studies including isolates from sheep are scarce (Berge et al., 2006; Sarangi et al., 2015; Cucco et al., 2017). In Spain, information on the antimicrobial susceptibility of porcine *P. multocida* isolates has been reported (Lizarazo et al., 2006), but no similar studies have been conducted in sheep. Thus, the aim of this study was to assess the antimicrobial susceptibility of clinical *P. multocida* isolates from sheep and pigs, providing information on their antimicrobial resistance patterns. This study provides the first data about the antimicrobial susceptibility of ovine *P. multocida* isolates in Spain.

One hundred and fifty-six *P. multocida* isolates obtained between 2001 and 2009 from sheep (n = 87) and pigs (n = 69) were included in this study. All porcine isolates were recovered from clinical cases of pneumonia, septicaemia, and arthritis, and most of the ovine isolates from cases of pneumonia (García-Alvarez et al., 2017). Bacteria were isolated from samples on Columbia blood agar plates (bioMérieux) incubated at 37 °C for 24 h. Isolates were biochemically identified by the commercial identification system API 20E strips (bioMérieux, S.A.) and further confirmed by a species-specific PCR assay (Townsend et al., 1998). Capsular types and sequence types (STs) were determined previously (García-Alvarez et al., 2017).

Antimicrobial susceptibility was determined by the microdilution method using a commercially prepared, dehydrated 96-well microtitre MIC panel (BOPO6F, Sensititre; Trek Diagnostic Systems Inc., UK). The antimicrobial agents used and their respective dilution ranges are indicated in Tables 1 and 2. Inocula were prepared from a 24-h Columbia blood agar plate by suspending four colonies in 5 mL of sterile distilled water. The inoculum was adjusted to 0.5 McFarland standard and further diluted 1/100 in 10 mL of Muller-Hinton broth. Fifty microlitres of the adjusted inoculum was deposited in each well of the microplate panel. Microdilution panels were sealed and further incubated at 37 °C for 24 h. The breakpoints used are shown in Tables 1 and 2. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were included as quality controls with each batch of organisms tested. In this study, resistance rates were classified into three categories: low rates, percentage of resistant strains < 15%; high rates, 15–50%; and very high rates, > 50%. Multidrug-resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. For the purpose of this study, a bacterial isolate was considered resistant to an antimicrobial category when it was resistant to at least one agent in that category. The association between the host origin, clinical

origin, capsular type or sequence type of *P. multocida* isolates and antimicrobial susceptibility was determined using the Chi-square test, with $P < 0.05$ considered significant. Data were analysed using the Epi InfoTM 7 program of the Centers for Disease Control and Prevention (CDC).

Antimicrobial resistance data of *P. multocida* have been commonly generated using different methodologies and different antimicrobials as well as using different breakpoints, which can hamper comparison of our results. Despite these drawbacks and using the breakpoints indicated in Tables 1 and 2, we detected in both sheep and pig isolates low levels of resistance to CEF, GEN, NEO, SPE, CTET, TUL, FFN, DANO, ENRO and SxT (≤ 15%; Tables 1 and 2, Fig. 1), which agrees with most previous reports both for pig (Yoshimura et al., 2001; Tang et al., 2009; Sellyei et al., 2009; Nedbalcová and Kučerová, 2013; Dayao et al., 2014; de Jong et al., 2014; El Garch et al., 2016; Cucco et al., 2017) and sheep isolates (Sarangi et al., 2015; Cucco et al., 2017). On the other hand, high resistance rates (> 15–50%) to OXY, TIL and TIA and very high resistance rates (> 50%) to TYLT, CLI, and SDM were identified (Tables 1 and 2, Fig. 1). Overall, similar high or very high levels of resistance to these antimicrobials have also been reported previously (Lizarazo et al., 2006; Tang et al., 2009; Nedbalcová and Kučerová, 2013; de Jong et al., 2014; Tahamtan and Hayati, 2014; El Garch et al., 2016; Cucco et al., 2017). Based on these high levels in the resistance *in vitro*, these antimicrobials should not be used empirically to treat *P. multocida* infections or be used with caution under field conditions. Of special concern are the very high levels of resistance to TYLT and SDM found in this study both in pig and sheep isolates, as these antimicrobials are considered critically important and highly important, respectively, for human medicine (World Health Organization, 2019).

It was unexpected that all strains of *P. multocida* from sheep were resistant to ampicillin (Table 1), compared with the low level of resistance (1.2%) detected for penicillin. As no CLSI-defined breakpoints for ampicillin are available for ovine *P. multocida* isolates, in this study we used the breakpoint recommended in the Clinical and Laboratory Standard Institute Vet08 guideline for cattle isolates (CLSI, 2018; ≥ 0.25 μg/ml) which is much higher than that recommended for swine isolates (≥ 2 μg/ml). Similarly, using the breakpoint recommended by the European Committee on Antimicrobial Susceptibility Testing for *P. multocida* (EUCAST, 2019; ≥ 1 μg/ml), all sheep isolates would have been considered susceptible. Therefore, the high percentage of resistance to ampicillin among sheep isolates is likely biased and overestimated by the breakpoint used in this study and point out the necessity for establishing specific breakpoints for ovine isolates.

Table 1

Minimum inhibitory concentrations (MICs) for 18 antimicrobial agents of ovine *Pasteurella multocida* isolates (n = 87)

Anti-microbial	Number of isolates with MIC of (µg/ml)													Breakpoint (µg/ml)	MIC_{90}	Resistance (%)
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
PEN	< 81	5		1			>							≥ 1[b]	0.12	**1.2**
AMP		< 87						>						≥ 0.25[b]	0.25	**100**
CEF		< 86		1			>							≥ 8[b]	0.25	**0**
GEN				< 66	19	2		>						> 4[a]	2	**0**
NEO						< 84	3		>					> 16[a]	4	**0**
SPE							< 5	72	10	>				≥ 128[b]	32	**0**
CTET			< 64	6	2	14	1							> 8[a]	4	**0**
OXY			< 55	13	5			14						> 8[a]	16	**16.1**
TYLT			<	1	2		8	50	24 >	2				≥ 8[e]	32	96.6
TUL				< 65	10	4	5	1	1	1 >				≥ 64[b]	4	**1.2**
TIL						< 54	5	2	19	7 >				≥ 32[d]	32	29.9
TIA				<		2	12	46	27 >					≥ 32[c]	32	31.0
FFN		< 45	41	1			>							≥ 8[b]	0.5	**0**
DANO	< 87			>										≥ 1[b]	0.12	**0**
ENRO	< 86	1			>									≥ 2[b]	0.12	0
CLI		<				2	8	70	7 >					≥ 4[c]	16	**100**
SDM												< 23 >	64	> 256[a]	> 256	**73.6**
	2/38	4/76														
SXT	< 87 >													> 8/152[a]	2/38	0

[a]Breakpoints recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie for *Pasteurellaceae* (CASFM-VET, 2019). [b]Breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) for *P. multocida* (2018-VET08); when available, specific breakpoints for cattle and swine were applied to sheep and pig isolates, respectively. [c]Breakpoints recommended by the CLSI for bacteria isolated from animals (2002; M31-A2). [d]Breakpoints recommended by the CLSI for *Pasteurella* spp. other than *P. multocida* (2017; VET06). [e]For TYLT no breakpoint is available. For this antimicrobial, the considered breakpoint was established based on the distribution of MIC values of the tested strains. Abbreviations: PEN, penicillin; AMP, ampicillin; CEF, ceftiofur; GEN, gentamicin; NEO, neomycin; SPE, spectinomycin; CTET, chlortetracycline; OXY, oxytetracycline; TYLT, tylosin tartrate; TUL, tulathromycin; TIL, tilmicosin; TIA, tiamulin; FFN, florfenicol; DANO, danofloxacin; ENRO, enrofloxacin; CLI, clindamycin; SDM, sulphadimethoxine; SXT; Trimethoprim/Sulphamethoxazole. (<): minimum value of concentration used; (>): maximum value of concentration used. Resistance percentages that were statistically significant ($P < 0.05$) with respect to pig isolates are shown in bold

Table 2

Minimum inhibitory concentrations (MICs) for 18 antimicrobial agents of porcine *Pasteurella multocida* isolates (n = 69)

Anti-microbial	Number of isolates with MIC of (μg/ml)													Breakpoint (μg/ml)	MIC_{90}	Resistance (%)
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
PEN	< 35	4	7	5	2	2	14 >							≥ 1[b]	8	**33.3**
AMP		< 39	1	6	4	4	1	4 >	10					≥ 2[b]	> 32	**33.3**
CEF		< 55	1	5	2		6 >							≥ 8[b]	2	**8.7**
GEN				< 23	30	11	1	4 >						> 4 [a]	4	**7.2**
NEO						< 43	12	6	8 >					> 16[a]	32	**11.6**
SPE							< 5	36	18	1 >	9			≥ 128[d]	> 128	**13.0**
CTET			< 18	14	13	8	9 >	7						> 8[a]	8	**10.1**
OXY			< 17	10	10	3	4 >	25						> 8[a]	> 16	**36.2**
TYLT			<	3			6	28	32 >					≥ 8[e]	32	95.7
TUL				< 43	12		4	1	1	8 >				≥ 64[b]	64	**11.6**
TIL						< 39	15	2	2	11 >				≥ 32[b]	64	18.8
TIA				< 7	3	5	8	33	13 >					≥ 32[c]	32	18.8
FFN		< 12	37	9	6		5 >							≥ 8[b]	2	**7.2**
DANO	< 49	6	9	1 >	4									≥ 2[d]	0.5	**5.8**
ENRO	< 60	3	3	2	1 >									≥ 1[b]	0.25	4.3
CLI		< 1		3	9	8	16	32 >						≥ 4[c]	16	**81.2**
SDM												< 7 >	62	> 256[a]	> 256	**89.9**
	2/38	4/76														
SXT	< 55 >	14												> 8/152[a]	4/76	0

[a]Breakpoints recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie for *Pasteurellaceae* (CASFM-VET, 2019). [b]Breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) for *P. multocida* (2018-VET08); when available, specific breakpoints for cattle and swine were applied to sheep and pig isolates, respectively. [c]Breakpoints recommended by the CLSI for bacteria isolated from animals (2002; M31-A2). [d]Breakpoints recommended by the CLSI for *Pasteurella* spp. other than *P. multocida* (2017; VET06). [e]For TYLT no breakpoint is available. For this antimicrobial, the considered breakpoint was established based on the distribution of MIC values of the tested strains. Abbreviations: PEN, penicillin; AMP, ampicillin; CEF, ceftiofur; GEN, gentamicin; NEO, neomycin; SPE, spectinomycin; CTET, chlortetracycline; OXY, oxytetracycline; TYLT, tylosin tartrate; TUL, tulathromycin; TIL, tilmicosin; TIA, tiamulin; FFN, florfenicol; DANO, danofloxacin; ENRO, enrofloxacin; CLI, clindamycin; SDM, sulphadimethoxine; SXT, Trimethoprim/Sulphamethoxazole. (<): minimum value of concentration used; (>): maximum value of concentration used. Resistance percentages that were statistically significant ($P < 0.05$) with respect to pig isolates are shown in bold

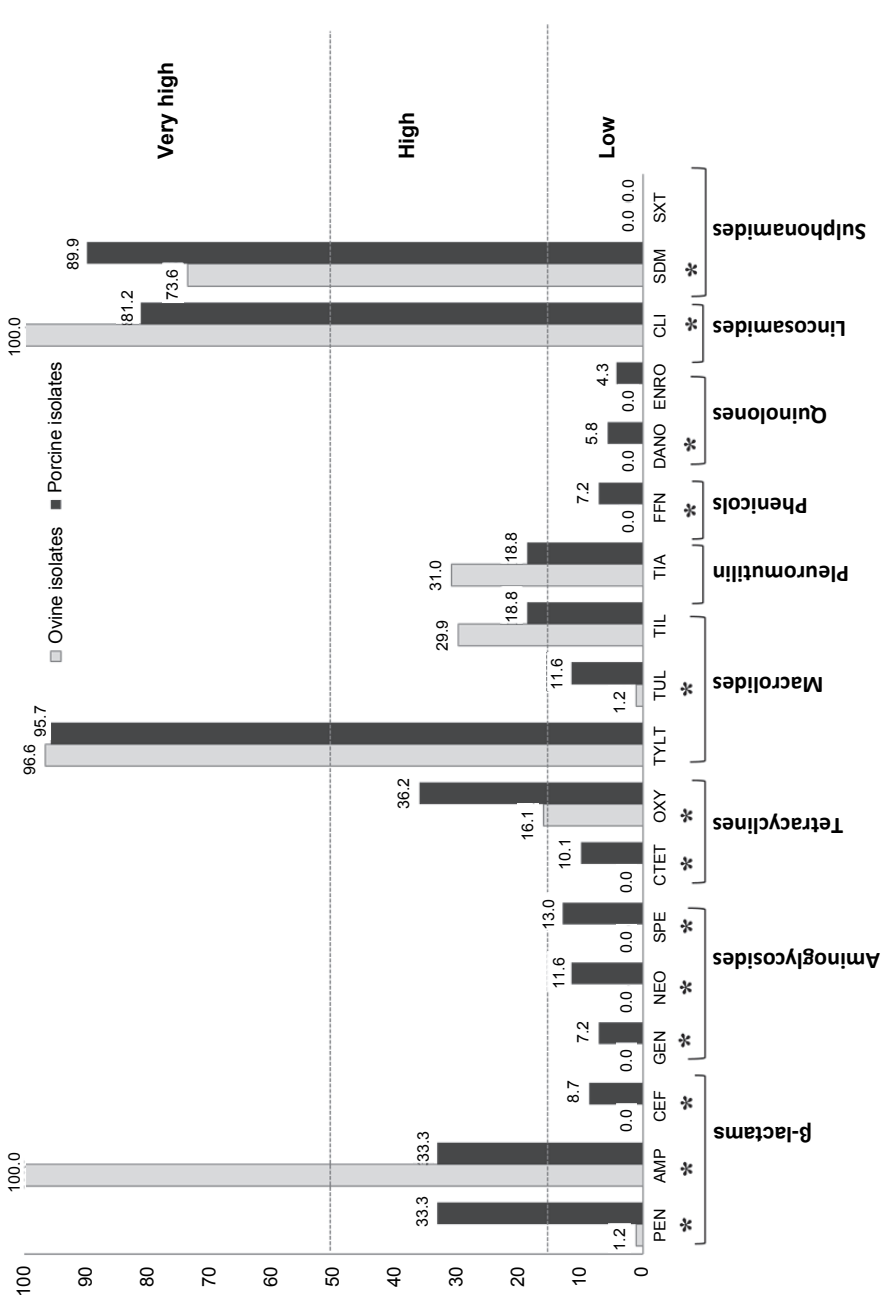

Fig. 1. Antimicrobial resistance to 18 antimicrobials tested of *P. multocida* isolates obtained from sheep and pigs. *Statistically significant differences ($P < 0.05$) between sheep and pig isolates

The resistotypes identified in this study and their distribution among sheep and pig isolates are shown in Table 3. None of the ovine or porcine *P. multocida* isolates was susceptible to the nine antimicrobial categories tested, with the resistotype LAC-LIN-SUL-MAC being the most frequently detected among sheep isolates (39.1%) and the resistotype LIN-SUL-MAC among pig isolates (26.1%; Table 3). No statistically significant differences were detected between percentages of sheep and pig MDR isolates (97.7% vs. 92.8%) but sheep isolates exhibited lower phenotypic antimicrobial resistance diversity (8 resistotypes vs. 30 resistotypes) than pig isolates (Table 3). More than half (55.8%) of the *P. multocida* isolates of this study were genetically characterised by MLST by García-Alvarez et al. (2017), with most of the ovine and porcine isolates belonging to a limited number of sequence types (ST50 and ST19 among ovine isolates and ST3, ST11 and ST62 among porcine isolates). A comparison of the antimicrobial resistance patterns and STs of the isolates included in both studies did not detect any association between resistance patterns and prevalent genotypes ($P > 0.05$; data not shown). Therefore, unlike in other pathogens (Klugman, 2003; Durante-Mangoni and Zarrilli, 2011; Edelstein et al., 2013), the differences in the antimicrobial resistance between sheep and pig isolates should not be related to the presence of particular resistant genotypes within the population of *P. multocida.* Moreover, capsular types A and D were the most frequent in *P. multocida* isolates in both animal species as determined previously (García-Alvarez et al., 2017). No associations were identified between resistance patterns of *P. multocida* with capsular types or with the clinical origin of the isolates (data not shown).

Sheep isolates exhibited significantly lower percentages of resistance ($P < 0.05$) than pig isolates for 12 antimicrobials (Tables 1 and 2, Fig. 1). Moreover, the MIC_{90} values for most antimicrobials were also lower in sheep than in pig isolates (Tables 1 and 2). The differences in the level of antimicrobial susceptibility between sheep and pig isolates could be associated with the different amount of antimicrobials used in the two farming sectors. In fact, pig farming is one of the livestock activities with the highest antimicrobial use (Moreno, 2014), while the use of antimicrobials is minimal in the small ruminant industry (Santman-Berends et al., 2014). Therefore, the higher resistance rates observed among pig isolates might reflect the selective pressure related to the higher use of antimicrobials in pig farming, at least for some antimicrobials.

Furthermore, the resistance rates and MIC_{90} values observed in this study among porcine *P. multocida* isolates for most antimicrobials were higher than those observed in a similar study carried out in Spain (Lizarazo et al., 2006), suggesting a shift in the resistance to these antimicrobials in Spanish *P. multocida* isolates from pigs. These results point out the need for active surveillance programmes to monitor the antimicrobial resistance patterns of *P. multocida*.

Table 3

Resistance phenotypes (resistotypes) of *Pasteurella multocida* isolates according to host species

Resistance to	Ovine		Porcine		Total	
	n	%	n	%	n	%
LAC-LIN	2	2.3			2	1.3
LAC-SUL			3	4.3	3	1.9
LIN-MAC			2	2.9	2	1.3
LAC-LIN-MAC	15	17.2	1	1.4	16	10.3
LAC-LIN-PLE			1	1.4	1	0.6
LAC-SUL-MAC	1	1.1			1	0.6
LIN-SUL-MAC			18	26.1	18	11.5
LIN-MAC-TET			2	2.9	2	1.3
SUL-MAC-ANF			1	1.4	1	0.6
SUL-MAC-AMI			1	1.4	1	0.6
MAC-PLE-TET			1	1.4	1	0.6
LAC-LIN-MAC-TET			1	1.4	1	0.6
LAC-LIN-MAC-PLE	5	5.7			5	3.2
LAC-LIN-SUL-MAC	34	39.1	6	8.7	40	25.6
LAC-SUL-MAC-TET			3	4.3	3	1.9
LIN-SUL-MAC-PLE			4	5.8	4	2.6
LIN-SUL-MAC-TET			3	4.3	3	1.9
SUL-MAC-TET-AMI			1	1.4	1	0.6
SUL-MAC-TET-QUIN			1	1.4	1	0.6
LIN-MAC-SUL-TET			2	2.9	2	1.3
LIN-SUL-MAC-AMI			4	5.8	4	2.6
LAC-LIN-SUL-MAC-TET	11	12.6	2	2.9	13	8.3
LAC-LIN-SUL-MAC-AMI			1	1.4	1	0.6
LAC-LIN-SUL-MAC-PLE	16	18.4	1	1.4	17	10.9
LIN-MAC-PLE-TET-AMI			1	1.4	1	0.6
LIN-MAC-SUL-PLE-TET			1	1.4	1	0.6
LAC-LIN-SUL-MAC-AMI-QUIN			1	1.4	1	0.6
LAC-LIN-SUL-MAC-TET-AMI			1	1.4	1	0.6
LAC-LIN-SUL-MAC-PLE-TET	3	3.4	1	1.4	4	2.6
LIN-SUL-MAC-TET-AMI-QUIN			1	1.4	1	0.6
LAC-SUL-MAC-PLE-TET-ANF-AMI			1	1.4	1	0.6
LAC-LIN-SUL-MAC-PLE-TET-ANF-AMI			1	1.4	1	0.6
LAC-LIN-SUL-MAC-PLE-TET-ANF-AMI-QUIN			2	2.9	2	1.3
Total	87	100.0	69	100.0	156	100.0

Abbreviations: LIN: lincosamides; SUL: sulphonamides; MAC: macrolides; TET: tetracyclines; LAC: β-Lactams; QUIN: quinolones; AMI: aminoglycosides; ANF: phenicols; PLE: pleuromutilins

Acknowledgements

The authors thank Almudena Casamayor and Sherezade Leao for their invaluable technical assistance. This study was supported by GR/SAL/0580/2004 (Community of

Madrid, Spain) and AGL2009-10136 (Ministerio de Ciencia e Innovación, Spain). Chris Pinto was supported by the Programme Alban, the European Union Programme of High Level Scholarships for Latin America, scholarship No. E07D404011PE.

References

Berge, A. C., Sischo, W. M. and Craigmill, A. L. (2006): Antimicrobial susceptibility patterns of respiratory tract pathogens from sheep and goats. J. Am. Vet. Med. Assoc. **229,** 1279–1281.

Clinical and Laboratory Standards Institute (2002): Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals – Approved Standard, 2nd edition. Document M31-A2, CLSI/NCCLS, Wayne, Pennsylvania, USA.

Clinical and Laboratory Standards Institute (2017): Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated from Animals. Document VET06. 1st edition. Wayne, Pennsylvania, USA.

Clinical and Laboratory Standard Institute (2018): Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Document VET08, Approved Standard, 4th edition. Wayne, Pennsylvania, USA.

Comité de l'Antibiogramme de la Société Française de Microbiologie. Recommandations Vétérinaires (2019): Paris, France: Société Française de Microbiologie. https://www.sfm-microbiologie.org/2019/07/09/casfm-veterinaire-2019/.

Cucco, L., Massacci, F. R., Sebastiani, C., Mangili, P., Bano, L., Cocchi, M., Luppi, A., Ortenzi, R., Pezzotti, G. and Magistrali, C. F. (2017): Molecular characterization and antimicrobial susceptibility of *Pasteurella multocida* strains isolated from hosts affected by various diseases in Italy. Vet. Ital. **57,** 21–17.

Dayao, D. A. E., Gibson, J. S., Blackall, P. J. and Turni, C. (2014): Antimicrobial resistance in bacteria associated with porcine respiratory disease in Australia. Vet. Microbiol. **171,** 232–235.

de Jong, A., Thomas, V., Simjee, S., Moyaert, H., El Garch, F., Maher, K., Morrissey, I., Butty, P., Klein, U., Marion, H., Rigaut, D. and Vallé, M. (2014): Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased cattle and pigs across Europe: The VetPath study. Vet. Microbiol. **172,** 202–215.

Durante-Mangoni, E. and Zarrilli, R. (2011): Global spread of drug-resistant *Acinetobacter baumannii*: molecular epidemiology and management of antimicrobial resistance. Future Microbiol. **6,** 407–422.

Edelstein, M. V., Skleenova, E. N., Shevchenko, O. V., D'Souza, J. W., Tapalski, D. V., Azizov, I. S., Sukhorukova, M. V., Pavlukov, R. A., Kozlov, R. S., Toleman, M. A. and Walsh, T. R. (2013): Spread of extensively resistant VIM-2-positive ST235 *Pseudomonas aeruginosa* in Belarus, Kazakhstan, and Russia: a longitudinal epidemiological and clinical study. Lancet Infect. Dis. **13,** 867–876.

El Garch, F., de Jong, A., Simjee, S., Moyaert, H., Klein, U., Ludwig, C., Marion, H., Haag-Diergarten, S., Richard-Mazet, A., Thomas, V. and Siegwart, E. (2016): Monitoring of antimicrobial susceptibility of respiratory tract pathogens isolated from diseased cattle and pigs across Europe, 2009–2012: VetPath results. Vet. Microbiol. **194,** 11–22.

European Committee on Antimicrobial Susceptibility Testing (2019): Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 9.0, http://www.eucast.org.

Furian, T. Q., Borges, K. A., Laviniki, V., Rocha, S. L., de Almeida, C. N., do Nascimento, V. P., Salle, C. T. and Morales, H. L. (2016): Virulence genes and antimicrobial resistance of *Pasteurella multocida* isolated from poultry and swine. Braz. J. Microbiol. **47,** 210–216.

García-Alvarez, A., Vela, A. I., San Martín, E., Chaves, F., Fernández-Garayzábal, J. F., Lucas, D. and Cid, D. (2017): Characterization of *Pasteurella multocida* associated with ovine pneumonia using multi-locus sequence typing (MLST) and virulence-associated gene profile analysis and comparison with porcine isolates. Vet. Microbiol. **204,** 180–187.

Klugman, K. P. (2003): The role of clonality in the global spread of fluoroquinolone-resistant bacteria. Clin. Infect. Dis. **36,** 783–785.

Kumar, P., Singh, V. P., Agrawal, R. K. and Singh, S. (2009): Identification of *Pasteurella multocida* isolates of ruminant origin using polymerase chain reaction and their antibiogram study. Trop. Anim. Health Prod. **41,** 573–578.

Lizarazo, Y. A. V., Ferri, E. F. R., de la Fuente, A. J. M. and Martin, C. B. G. (2006): Evaluation of changes in antimicrobial susceptibility patterns of *Pasteurella multocida* subsp. *multocida* isolates from pigs in Spain in 1987–1988 and 2003–2004. Am. J. Vet. Res. **67,** 663–668.

Moreno, M. A. (2014): Survey of quantitative antimicrobial consumption per production stage in farrow-to-finish pig farms in Spain. Vet. Rec. Open **13,** e000002.

Nedbalcová, K. and Kučerová, Z. (2013): Antimicrobial susceptibility of *Pasteurella multocida* and *Haemophilus parasuis* isolates associated with porcine pneumonia. Acta Vet. Brno **82,** 3–7.

Post, K. W., Cole, N. A. and Raleigh, R. H. (1991): *In vitro* antimicrobial susceptibility of *Pasteurella haemolytica* and *Pasteurella multocida* recovered from cattle with bovine respiratory disease complex. J. Vet. Diagn. Invest. **3,** 124–126.

San Millan, A., Escudero, J. A., Gutierrez, B., Hidalgo, L., Garcia, N., Llagostera, M., Dominguez, L. and Gonzalez-Zorn, B. (2009): Multiresistance in *Pasteurella multocida* is mediated by coexistence of small plasmids. Antimicrob. Agents Chemother. **53,** 3399–3404.

Santman-Berends, I., Luttikholt, S., Van den Brom, R., Van Schaik, G., Gonggrijp M., Hage, H. and Vellema, P. (2014): Estimation of the use of antibiotics in the small ruminant industry in The Netherlands in 2011 and 2012. PLoS One **9,** e105052.

Sarangi, L. N., Thomas, P., Gupta, S. K., Priyadarshini, A., Kumar, S., Nagaleekar, V. K., Kumar, A. and Singh, V. P. (2015): Virulence gene profiling and antibiotic resistance pattern of Indian isolates of *Pasteurella multocida* of small ruminant origin. Comp. Immunol. Microbiol. Infect. Dis. **38,** 33–39.

Sellyei, B., Varga, Z., Szentesi-Samu, K., Kaszanyitzky, E. and Magyar, T. (2009): Antimicrobial susceptibility of *Pasteurella multocida* isolated from swine and poultry. Acta Vet. Hung. **57,** 357–367.

Tang, X., Zhao, Z., Hu, J., Wu, B., Cai, X., He, Q. and Chen, H. (2009): Isolation, antimicrobial resistance, and virulence genes of *Pasteurella multocida* strains from swine in China. J. Clin. Microbiol. **47,** 951–958.

Tahamtan, Y. and Hayati, M. (2014): Multi drug resistance of *Pasteurella* spp. isolated from sheep and goats in Iran. Res. J. Microbiol. **9,** 51–58.

Townsend, K. M., Frost, A. J., Lee, C. W., Papadimitriou, J. M. and Dawkins, H. J. (1998): Development of PCR assays for species- and type-specific identification of *Pasteurella multocida* isolates. J. Clin. Microbiol. **36,** 1096–1100.

Wilson, B. A. and Ho, M. (2013): *Pasteurella multocida*: from zoonosis to cellular microbiology. Clin. Microbiol. Rev. **26,** 631–655.

World Health Organization (2019): Critically Important Antimicrobials for Human Medicine. 6th rev. ed., Geneva. Licence: CC BY-NC-SA 3.0 IGO; https://creativecommons.org/licenses/by-nc-sa/3.0/igo.

Yoshimura, H., Ishimaru, M., Endoh, Y. S. and Kojima, A. (2001): Antimicrobial susceptibility of *Pasteurella multocida* isolated from cattle and pigs. J. Vet. Med. B: Infect. Dis. Vet. Public Health **48,** 555–560.