AKADÉMIAI KIADÓ

Antimicrobial susceptibility of *Pasteurella multocida* strains isolated from different host species

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RESEARCH ARTICLE

ABSTRACT

The spread of antibiotic resistance is one of the biggest challenges of our time, making it difficult to treat bacterial diseases. *Pasteurella multocida* is a widespread facultative pathogenic bacterium, which causes a wide range of diseases in both mammals and birds. In the present study, antibiotic susceptibility of 155 *P. multocida* strains were tested using the broth microdilution method to obtain the minimum inhibitory concentration (MIC) values for 15 antibiotics. The most effective antibiotics against pasteurellosis were ceftiofur, tetracycline, doxycycline, florfenicol and tilmicosin. Of the strains, 12 proved to be multi-drug resistant (MDR). To combat antibiotic resistance, it is important to establish a pre-treatment antibiotic susceptibility profile. A well-chosen antibiotic would not only make the treatment more successful but may also slow down the spread of resistance and the evolution of MDR strains.

KEYWORDS

antimicrobial resistance, MIC, *Pasteurella multocida*

INTRODUCTION

Pasteurella multocida is a widespread Gram-negative bacterium causing diseases in a wide range of animal species. As a primary pathogen, it is involved in the aetiology of haemorrhagic septicaemia in cattle and buffalo (De Alwis, 1992), atrophic rhinitis in pigs (Magyar and Lax, 2002) and fowl cholera (Rhoades and Rimler, 1989). As a secondary invader, it is associated with pneumonia in swine and ruminants, as well as various respiratory tract diseases in rodents (Boyce et al., 2010). Furthermore, it has zoonotic potential, infecting humans usually through dog or cat bites or scratches (Freshwater, 2008).

Antibiotics are widely used to treat diseases caused by *P. multocida*. Over the last decades, several publications have reported the emergence of multidrug-resistant (MDR) bacterial strains. Inappropriate antibiotic treatments enable the resistant strain to survive and transfer its resistance genes to other bacteria. As a consequence, the treatment of bacterial diseases is more and more challenging. It is important to be well informed about the current resistance profile of infectious agents, since using the right antibiotic will make the treatment more successful. This can be achieved through on-going monitoring programmes (White et al., 2002; Bello et al., 2019; Bourély et al., 2019; Vilaró et al., 2020).

This article aims to extend this database by comparing the antibiotic resistance profiles of *P. multocida* strains isolated from different host species. In addition to the Hungarian isolates, French isolates were also included in the study, and thus the antibiotic sensitivity pattern between two different countries was also compared.

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MATERIALS AND METHODS

Bacterial strains

A total of 155 *P. multocida* strains were used in the study. The strains were isolated from different animal species: 117 from birds, 38 from mammals. Most mammalian isolates came from cattle (25), three strains each were from pigs, sheep and goats, while two strains each were isolated from cats and fallow deer. In birds, most strains came from geese (43) and turkeys (27), 18 strains were isolated from Mullard ducks, 12 from ducks, 9 from pheasants, 6 from albatrosses and 2 from chickens. Out of the strains, 98 (63.2%) were collected from various Hungarian localities, while the remaining strains were obtained mainly from France (57). The strains were isolated between 2004 and 2022.

Antibiotic susceptibility testing

The MIC was determined using the broth microdilution method with 96-well microtiter plates (Sarstedt Inc, Germany) according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2002). *Escherichia coli* ATCC 25922 was included as quality control. The following antibiotics were tested: penicillin (0.015–16 µgmL^{-1}), amoxicillin (0.06–64 µgmL^{-1}), ampicillin (0.06–64 µgmL^{-1}) apramycin (0.06–64 µgmL^{-1}), ceftiofur (0.015–16 µgmL^{-1}), clindamycin (0.125–128 µgmL^{-1}), colistin (0.06–64 µgmL^{-1}), doxycycline (0.06–64 µgmL^{-1}), enrofloxacin (0.015–16 µgmL^{-1}), erythromycin (0.06–64 µgmL^{-1}), florfenicol (0.06–64 µgmL^{-1}), flumequine (0.06–64 µgmL^{-1}), sulfamethoxazole (0.25–256 µgmL-1), tetracycline (0.03–32 µgmL^{-1}) and tilmicosin (0.25–256 µgmL^{-1}). Cation-adjusted Mueller Hinton Broth was used. After incubation at 37 °C for 24 h, the turbidity (quantified) of the suspensions were read at 450 nm using an ELISA reader. The lowest concentration of antibiotic that inhibited the growth of the tested bacterial strain, i.e. the MIC was determined by comparing these numbers. The MIC values were classified into sensitive, intermediate or resistant categories according to the CLSI criteria (CLSI, 2002, 2015). For amoxicillin, we used the values set for ampicillin, while for doxycycline we used the values set for tetracycline. For sulfamethoxazole, flumequine, colistin and apramycin, no CLSI recommendation for *P. multocida* was found, so in their case, only the obtained values were recorded.

Determination of MIC$_{50}$, MIC$_{90}$ values

The MIC values obtained were also used to determine the MIC$_{50}$ and MIC$_{90}$ values of the antibiotics, i.e. the concentration of antibiotics that inhibited 50% (78 of 155 strains) and 90% (140 strains) of the tested bacterial strains. The most frequent antibiotic concentration (mode) was also determined.

Identification of MDR strains

Multidrug-resistance was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial classes (Magiorakos et al., 2012).

RESULTS

The antibiotic resistance profiles of *P. multocida* are summarised in Table 1. A 100% resistance rate was observed for clindamycin. For erythromycin, 81.3% of strains showed reduced sensitivity while 9.7% were resistant. For amoxicillin, more than half of the strains (50.3%) were in the intermediate category, while 35.48 % were susceptible. When ampicillin was tested, a high percentage of strains (68.4) were sensitive, while moderate sensitivity was observed in 23.2% and resistance in 14.2% of the cases. A total of 131 (84.5%) strains were susceptible to penicillin, and the same number of strains proved to be susceptible to enrofloxacin. The proportion of intermediate strains was higher for the latter antibiotic (12.9%) than for the former one (8.4%).

Table 1. Percentages of antibiotic resistance of *Pasteurella multocida* strains by origin and host species. Abbreviations: S: susceptible, I: intermediate, R: resistant, PEN: penicillin, AMO: amoxicillin, AMP: ampicillin, CEFT: ceftiofur, TTC: tetracycline, CLINDA: clindamycin, DOX: doxycycline, ERY: erythromycin, ENRO: enrofloxacin, TILM: tilmicosin, FFC: florfenicol

	Mammalian strains (*n* = 38)			Avian strains (*n* = 117)			Hungarian strains (*n* = 98)			French strains (*n* = 57)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
PEN	55	26	18	94	3	3	77	13	10	98	0	2
AMO	39	29	32	34	57	9	41	41	18	26	67	7
AMP	47	34	18	75	20	5	65	21	13	74	26	0
CEFT	95	3	3	97	1	2	96	1	3	98	2	0
TTC	92	3	5	93	0	7	94	1	5	91	0	9
CLINDA	0	0	100	0	0	100	0	0	100	0	0	100
DOX	97	3	0	95	2	3	97	2	1	93	2	5
ERY	13	58	29	8	89	3	7	78	15	12	88	0
ENRO	89	3	8	83	16	1	77	19	4	98	2	0
TILM	79	5	16	98	1	1	91	3	6	98	0	2
FFC	95	5	0	97	0	3	95	2	3	100	0	0

High sensitivity was observed for ceftiofur (96.8%), tetracycline (92.9%), doxycycline (95.5%), florfenicol (96.8%) and tilmicosin (93.5%) (Fig. 1).

The French strains showed a high sensitivity to penicillin (98%), while this value was lower (77%) for the Hungarian strains. A higher percentage of the Hungarian strains were sensitive to amoxicillin (41%) than French strains (26%), but in both cases most strains had moderate sensitivity. No ampicillin-resistant French strains were isolated, whereas 13% of the Hungarian strains were resistant to this antibiotic. Most strains from the two countries showed moderate susceptibility to erythromycin, but while no resistant cases were found among the French strains, 15% of the Hungarian strains proved to be resistant to this antibiotic. Both Hungarian and French strains had high susceptibility to ceftiofur, tetracycline, doxycycline, tilmicosin and florfenicol. No French strains were found to be resistant to florfenicol and only 3% of the Hungarian strains were resistant to this drug. 98% of the French strains were sensitive to enrofloxacin and none showed resistance. In contrast, only 77% of the Hungarian strains were sensitive to this antibiotic, the remaining strains showed intermediate sensitivity or resistance (Table 1).

Strains resistant for penicillin were from cattle (4), goats and geese (2–2), duck, Mullard duck and pig (1–1). Most intermediate strains (69.2%) were of bovine origin. The strains isolated from cats, sheep, pheasants, albatrosses and chickens were all sensitive to penicillin.

Testing amoxicillin identified 22 resistant strains, including six from cattle, one from pig, fallow deer, albatross, pheasant, duck and Mullard duck, two each from cats and goats, three from turkeys and four from geese.

In the case of ampicillin, resistant strains were isolated from pheasant, duck, Turkey, pig (1–1), goats (2), geese (3) and cattle (4). The intermediate strains were detected from albatross, sheep, pigs (1–1), ducks, cats (2–2), Mullard ducks (3), turkeys (8), geese and cattle (9). All strains from fallow deer and chickens were found to be susceptible.

Analysis of the ceftiofur revealed 2 intermediate strains, isolated from cattle and Mullard duck. The three resistant strains were from cattle, goose and duck. The strains isolated from albatross, fallow deer, pheasants, sheep, goats, cats, turkeys, pigs and chickens were all susceptible.

For doxycycline, resistant strains were isolated from geese (1) and ducks (3). The intermediate strains were isolated from cattle (1), goose (1) and duck (1). From chickens, pigs, Mullard ducks, turkeys, cats, goats, sheep, pheasants, fallow deer and albatrosses only susceptible strains were isolated.

Strains resistant to tetracycline were isolated from ducks (4), geese (3), Mullard duck (1) and cattle (2). One intermediate strain was detected in cattle. All the strains isolated from albatrosses, fallow deer, pheasants, sheep, goats, cats, turkeys, pigs and chickens were susceptible.

All strains from fallow deer, pheasants, sheep, ducks, cats and Mullard ducks showed moderate sensitivity to erythromycin. Three strains from albatrosses, one strain from goose, pig and chicken, 4 strains from turkeys and cattle were sensitive. Two goats, four geese, one pig and eight cattle strains were resistant.

Testing florfenicol revealed 2 intermediate strains, isolated from a pig and a goat. The three resistant strains were from geese. From chickens, cattle, turkeys, Mullard ducks, cats, ducks, sheep, pheasants, fallow deer and albatrosses only susceptible strains were isolated.

In the analysis of enrofloxacin, resistant strains were primarily found in cattle (3) and geese (1). All strains isolated from pheasants (9), plus one cattle, duck, albatross and eight geese strains had moderate susceptibility. All strains isolated from chickens, pigs, turkeys, Mullard ducks, cats, goats, sheep and fallow deer were found to be susceptible.

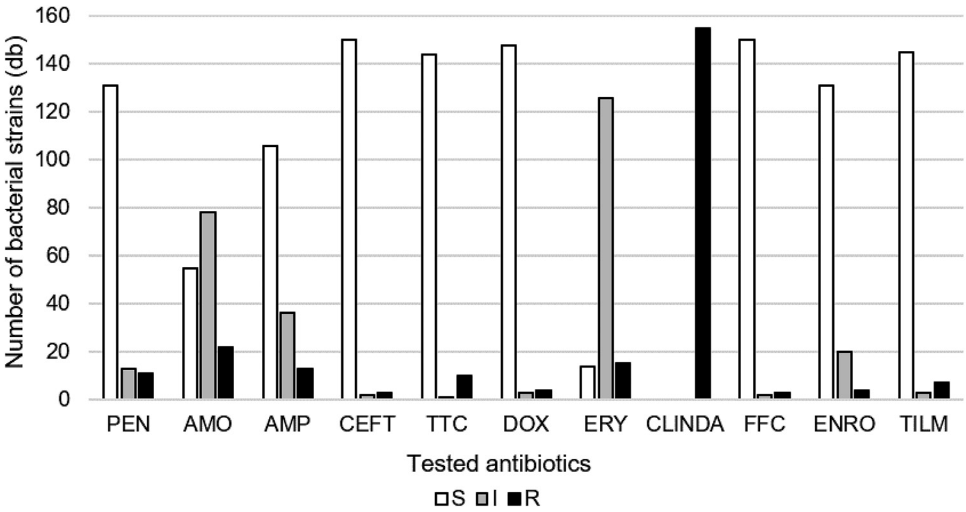

Fig. 1. Pasteurella multocida strains ($n = 155$) classified into antibiotic sensitivity categories based on their MIC values according to the CLSI criteria (CLSI, 2002, 2015). Abbreviations: S: susceptible, I: intermediate, R: resistant, PEN: penicillin, AMO: amoxicillin, AMP: ampicillin, CEFT: ceftiofur, TTC: tetracycline, DOX: doxycycline, ERY: erythromycin, CLINDA: clindamycin, FFC: florfenicol, ENRO: enrofloxacin, TILM: tilmicosin

Strains resistant to tilmicosin were isolated mainly from cattle (5), pig and Turkey (1 from either). Intermediate susceptible strains were from sheep, geese and cattle (1–1). All strains from albatrosses, fallow deer, pheasants, ducks, goats, cats, Mullard ducks and chickens were susceptible.

Of the strains isolated from avian species, 70.5% were sensitive to the selected antibiotics, while this proportion was slightly lower in mammals (64.0%). There were no notable differences in antibiotic susceptibility according to the host species. The highest resistance rates were found in strains from goats (33.3%), pigs (24.2%) and cattle (21.1%), and the lowest in strains from chickens and sheep (9.1–9.1%). Strains from chickens were found to be most susceptible (81.8%) (Fig. 2).

MIC$_{50}$, MIC$_{90}$

MIC$_{50}$ and MIC$_{90}$ values of the antibiotics and the mode of these values are shown in Table 2. Eight of the eleven antibiotics (penicillin, ampicillin, ceftiofur, tetracycline, doxycycline, enrofloxacin, tilmicosin, florfenicol) had MIC$_{50}$ values falling within the sensitive range, while this number was five (ceftiofur, tetracycline, doxycycline, tilmicosin, florfenicol) for MIC$_{90}$ values. The MIC$_{90}$ values for penicillin, ampicillin and enrofloxacin were moved into the intermediate range. Both the MIC$_{50}$ and MIC$_{90}$ values for clindamycin fell into the resistant category, while the same values for erythromycin fell into the intermediate category. The MIC$_{50}$ value for amoxicillin is in the intermediate category while the MIC$_{90}$ value is in the resistant category. No CLSI recommendation for *P. multocida* was found for sulfamethoxazole, flumequine, colistin and apramycin, so in these cases only the obtained values were recorded. The distribution of each value per antibiotic is shown in Fig. 3. Concerning the MIC values for apramycin, most of the strains were inhibited by the antibiotic with the higher concentration (64–32–16–8 μgmL^{-1}). For colistin, the majority of strains were inhibited by the middle of the antibiotic concentration range (4–2–1–0.5 μgmL^{-1}). Strains that reacted with sulfamethoxazole were often uninhibited by even the highest drug concentrations. Some of the strains were inhibited by flumequine at lower concentrations, while some of the strains were inhibited by higher antibiotic concentrations.

Identification of MDR strains

Of the 155 isolates included in the study, 12 (7.74%) MDR strains were identified. Out of the 12 strains, 8 were from mammalian and 4 were from avian species. MDR strains represented 3.4% of the avian strains and 21.1% of the mammalian strains, respectively. 66.7 % of the strains isolated from goats and 33.3 % of the strains isolated from pigs were MDR. This percentage was 20% in cattle, 8.3% in ducks and 7.0% in geese. All MDR strains were isolated from Hungary. Out of all the tested strains, 12.8% of the isolates showed multi-drug resistance. One of the MDR strains showed resistance to three, two to four, four to five, four to six and one to seven antibiotics, while half of the strains were resistant to three classes of antibiotics and half to four (Table 3). 100% of MDR strains were resistant to clindamycin and more than half of the strains were resistant to penicillin, amoxicillin, ampicillin and erythromycin. All MDR strains were sensitive to florfenicol (Fig. 4). Multi-drug resistant strains were isolated in 2007 (1), 2008 (2), 2013 (3), 2014 (1), 2015 (1), 2017 (2), 2019 (1), 2020 (1).

DISCUSSION

Depending on the geographical origin, the antibiotic susceptibility profile of strains to certain antibiotics was somewhat variable. The reason for this is probably the different medication protocols, as the antibiotic used for

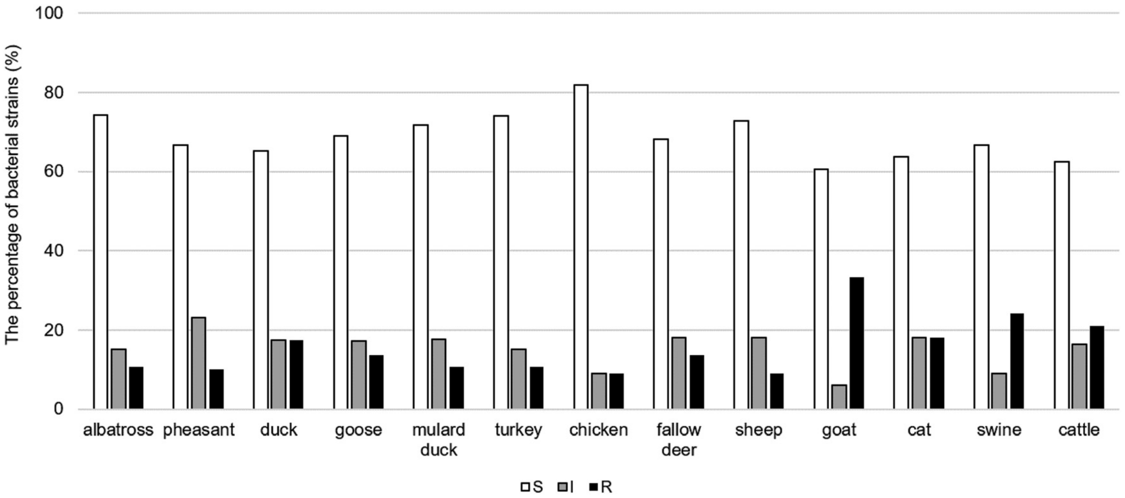

Fig. 2. Percentage of antibiotic susceptibility of *Pasteurella multocida* strains isolated from various animal species, based on data obtained with 11 antibiotics (penicillin, amoxicillin, ampicillin, ceftiofur, tetracycline, doxycycline, erythromycin, clindamycin, florfenicol, enrofloxacin, tilmicosin). Abbreviations used: S: susceptible, I: intermediate, R: resistant

Table 2. Distribution of MIC values (in units) per antibiotic. The MIC_{50} and MIC_{90} values of the antibiotics are also indicated, as well as the mode, i.e. the most frequent concentration. Vertical lines indicate breakpoints for resistance. Abbreviations: S: susceptible, I: intermediate, R: resistant, PEN: penicillin, AMO: amoxicillin, AMP: ampicillin, CEFT: ceftiofur, TTC: tetracycline, CLINDA: clindamycin, DOX: doxycycline, ERY: erythromycin, ENRO: enrofloxacin, TILM: tilmicosin, FFC: florfenicol, APRA: apramycin, COL: colistin, SX: sulfamethoxazole, FLU: flumequine

	Minimum inhibitory concentration (μgmL-1)																									
	0.015 >	0.015	0.03 >	0.03	0.06 >	0.06	0.125	0.25 >	0.25	0.5	1	2	4	8	16	16 <	32	32 <	64	64 <	128	128 <	256	256 <	MIC_{50}	MIC_{90}	Mode
PEN	1	0		2		13	67		47	14	1	0	0	1	0	9									0.125^{S}	0.5^{I}	0.125^{S}
AMO						0	1		9	44	78	13	5	1	4		0		0						1^{I}	2^{R}	1^{I}
AMP					2	0	4		23	78	35	2	4	2	1		0		2	2					0.5^{S}	1^{I}	0.5^{S}
CEFT	29	1		11		5	13		34	39	14	5	1	2	0	1									0.25^{S}	1^{S}	0.5^{S}
TTC			2	4		15	16		69	31	5	2	1	3	1		5	1							0.25^{S}	1^{S}	0.25^{S}
CLINDA							0		0	0	0	0	0	2	28		97		20		1	7			32^{R}	64^{R}	32^{R}
DOX					39	1	67		29	7	3	2	3	4	0		0		0	0					0.125^{S}	0.5^{S}	0.125^{S}
ERY					0	0	1		5	8	47	61	18	5	3		2		3	2					2^{I}	4^{I}	2^{I}
ENRO	94	2		16		8	6		5	9	11	4	0	0	0	0									$0.015 >^{S}$	0.5^{I}	$0.015 >^{S}$
TILM								4	0	7	20	58	42	13	4		2		2		0		1	2	2^{S}	8^{S}	2^{S}
FFC					1	0	3		41	97	4	4	2	0	2		1		0	0					0.5^{S}	0.5^{S}	0.5^{S}
APRA					0	0	0		0	0	0	0	1	0	14	39	79		21	1					32	64	32
COL						0	8		12	25	38	40	21	5	4		1		1	0					1	4	2
SX								0	0	0	0	0	0	1	2		3		1		4		21	123	256 <	256 <	256 <
FLU					53	1	36		17	4	7	5	5	1	2		5		17	2					0.125	64	0.06 >

treatment may facilitate the development of resistance (Pereira et al., 2014; Catry et al., 2016; Timsit et al., 2017). The sensitivity of the French strains to penicillin was very high (98%), while that of the Hungarian strains was only 77%. To amoxicillin, more than half of the French strains (67%) showed moderate sensitivity, while this value was 41% among the Hungarian strains. This may be due to the fact that amoxicillin is frequently used in France to treat bacterial diseases (Dheilly et al., 2011). Most strains (90% <) were sensitive to tetracycline, doxycycline and tilmicosin, regardless of origin. In the case of tetracycline and doxycycline, these sensitivity values were much higher than those reported in previous French and European studies El Garch et al. (2016), Bourély et al. (2019), El Garch et al. (2016) revealed that *P. multocida* strains from cattle and pigs had high susceptibility (96% <) to ceftiofur, florfenicol and enrofloxacin. The Hungarian strains used in this study were also sensitive to these antibiotics, but their sensitivity to enrofloxacin was lower.

Independently from geographical location and host species, most publications reported the efficacy of florfenicol, ceftiofur and enrofloxacin (Berge et al., 2006; Mohamed et al., 2012; Oh et al., 2018; Hayer et al., 2020; Schönecker et al., 2020; Vilaró et al., 2020). In this study, these antibiotics were also found to be very effective against *P. multocida*. On the other hand, recent studies reported an increased number of florfenicol-resistant strains (Jeong et al., 2021; Sabsabi et al., 2021). Resistance has also been reported against clindamycin, various sulphonamides, erythromycin and different tetracyclines (Diallo et al., 1995; Oh et al., 2018; Cid et al., 2019; Hayer et al., 2020; Elalamy, 2020; Vilaró et al., 2020). In contrast, in the present study, the strains showed high sensitivity to tetracycline as well as to doxycycline. Although a CLSI value for sulfamethoxazole was not available, the highest concentration of antibiotic used in the study was not able to kill 90% of the strains. These data suggest that a high percentage of *P. multocida* strains are resistant to sulfamethoxazole. Consequently, the number of MDR strains would also be increased. During the survey, most strains showed moderate sensitivity to erythromycin and amoxicillin. High proportion of intermediate sensitivity (Post et al., 1991) and resistance to erythromycin was also reported in previous publications (Elalamy, 2020). In our study, reduced sensitivity was observed for enrofloxacin, penicillin and ampicillin, which is in harmony with previous observations (Post et al., 1991; Watts et al., 1994). The present study found 100% resistance to clindamycin. This high rate was described in earlier publications as well (Cid et al., 2019; Jones et al., 2013).

In a former study with strains isolated from pigs, the MIC_{50} and MIC_{90} values of erythromycin and the MIC_{50} value of penicillin were the same as the results obtained in the present study (Fales et al., 1990). Apart from these values, the present study determined higher concentrations, reflecting the fact that the antibiotic susceptibility of strains decreases over time. In Australia (Dayao et al., 2014), low resistance rates were observed for florfenicol (2%), penicillin and ampicillin (4-4%). In contrast, no Hungarian strain

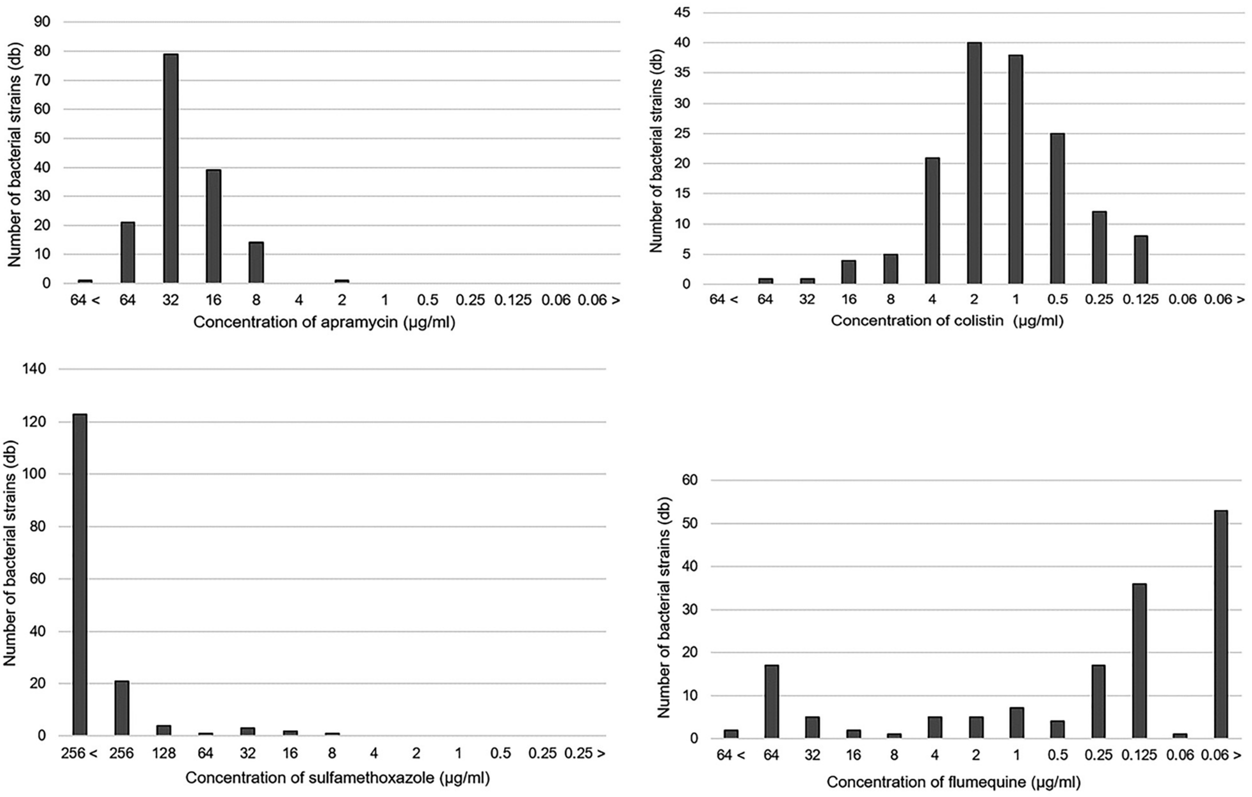

Fig. 3. Frequency of antibiotic concentrations obtained for sulfamethoxazole, colistin, flumequine and apramycin (antibiotics without a CLSI recommendation)

Table 3. Antibiotic resistance profile of multidrug-resistant bacteria. Abbreviations: PEN: penicillin, AMP: ampicillin, TTC: tetracycline, ERY: erythromycin, CLINDA: clindamycin, TILM: tilmicosin, DOX: doxycycline, CEFT: ceftiofur, ENRO: enrofloxacin

Strain ID	Host species	Antibiogram (resistant drugs)	Total number of resisted drugs	Total number of resisted antibiotic classes
4221	cattle	PEN, AMO, AMP, TTC, ERY, CLINDA, TILM	7	4
3036	goose	PEN, AMO, TTC, DOX, ERY, CLINDA	6	4
4149	cattle	PEN, AMO, AMP, CEFT, CLINDA, ENRO	6	4
4231	cattle	TTC, ERY, CLINDA, ENRO, TILM	5	4
3029	goose	AMP, TTC, ERY, CLINDA	4	4
4539	goose	CEFT, TTC, ERY, CLINDA	4	4
3509	cattle	PEN, AMO, AMP, ERY, CLINDA, TILM	6	3
4112	swine	PEN, AMO, AMP, ERY, CLINDA, TILM	6	3
3699	goat	PEN, AMO, AMP, ERY, CLINDA	5	3
3700	goat	PEN, AMO, AMP, ERY, CLINDA	5	3
4576	duck	PEN, AMO, AMP, CEFT, CLINDA	5	3
4147	cattle	ERY, CLINDA, ENRO	3	3

showed resistance to florfenicol. On the other hand, Hungarian strains exhibited a higher percentage of resistance when penicillin and ampicillin were studied. Finally, the Hungarian strains were more sensitive to tetracycline than the Australian strains (Dayao et al., 2014). In Korea, a higher (18.5%) resistance rate to florfenicol was reported in a 2010–2016 survey, and similar percentages (4%) were found for penicillin and ampicillin as in the Australian study (Dayao et al., 2014). Both Hungarian and Korean strains had high susceptibility to ceftiofur and enrofloxacin. The Korean strains were more sensitive to tilmicosin (Oh et al., 2018). High (15–50%) resistance levels to tilmicosin have been previously documented in Spain (Cid et al., 2019).

In a study in Spain, MIC values of *P. multocida* strains isolated from Spanish and Portuguese sheep were determined to tetracycline, doxycycline and enrofloxacin. Most strains were sensitive to these antibiotics, however, tetracycline and doxycycline were more effective than enrofloxacin. In this study, ovine strains were all susceptible to these antibiotics but doxycycline had a lower MIC value when

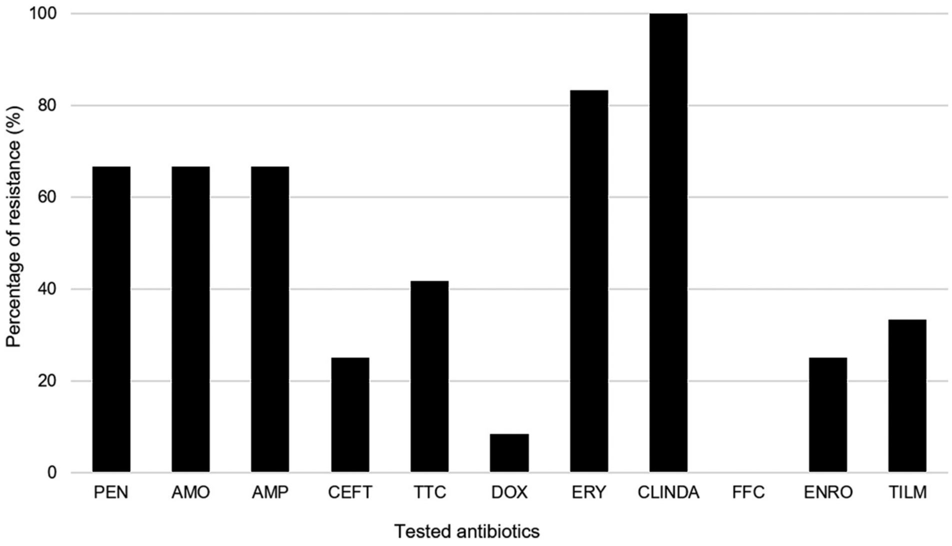

Fig. 4. Percentage occurrence of resistance per antibiotic in the case of MDR strains, $n = 12$. Abbreviations: MDR: multidrug resistance, PEN: penicillin, AMO: amoxicillin, AMP: ampicillin, CEFT: ceftiofur, TTC: tetracycline, DOX: doxycycline, ERY: erythromycin, CLI: clindamycin, FFC: florfenicol, ENRO: enrofloxacin, TILM: tilmicosin

compared to tetracycline (Bello et al., 2019). Also, the effectiveness of tetracycline, florfenicol and ceftiofur was reported in a previous publication that studied strains from goat and sheep (Berge et al., 2006). In this study, the strains from goats showed higher resistance rate. Most of the strains detected from small ruminants were resistant to penicillin, ampicillin, amoxicillin and erythromycin. Based on a Spanish observation, however, strains from sheep showed resistance to tilmicosin and clindamycin (Cid et al., 2019).

Strains of bovine origin of the present study were highly resistant to clindamycin, erythromycin, amoxicillin and tilmicosin, but were sensitive to other antibiotics. In North America, high resistance rates were also found for erythromycin, while reduced susceptibility was observed for ampicillin (Watts et al., 1994). In Canada, more than half of the strains from cattle were resistant to penicillin and tetracycline, but were highly sensitive to ceftiofur, enrofloxacin and florfenicol (Timsit et al., 2017). Similar observations were made for strains isolated in Switzerland (Schönecker et al., 2020).

Most of the strains of poultry origin in the present study were sensitive to the majority of the tested antibiotics, while all strains were resistant to clindamycin. For erythromycin and amoxicillin, a high proportion of strains were in the intermediate category. More than 15% of strains had intermediate sensitivity to amoxicillin and enrofloxacin. In a survey in Mississippi, a prominent percentage of strains were sensitive to doxycycline, which was also observed in the present study. We observed high sensitivity to florfenicol, ceftiofur and enrofloxacin. We also observed strains susceptible to tetracycline, but at a lower rate than with doxycycline. Similar results to the present study have been obtained with erythromycin, penicillin and clindamycin (Jones et al., 2013). Resistance to florfenicol has also been reported in Korea and China (Chen et al., 2020; Jeong et al., 2021).

In general, strains isolated from birds proved to be slightly more susceptible than those isolated from mammals. The most notable differences were observed for penicillin and ampicillin: 94% and 74% of avian strains were susceptible, compared to 55% and 47% of mammalian strains, respectively. A high proportion of strains from both groups showed moderate susceptibility to erythromycin, but a higher percentage of mammalian strains were resistant to this agent than avian strains (28% versus 3%). Both groups were highly (80% <) sensitive to enrofloxacin, but the rate of moderate sensitivity was also considerable for strains of bird origin. This is probably due to the fact that enrofloxacin is often used to treat poultry diseases (Dheilly et al., 2011). A high proportion (98%) of avian strains was sensitive to tilmicosin. In mammals, the susceptibility rate was also high (79%), but 16% of the strains were resistant in contrast to the <1% among the avian strains. A similar difference in the susceptibility of strains from different animal species was found in a French study, where 99% of duck strains were susceptible to tilmicosin, compared to 81% of strains from cattle (Bourély et al., 2019). Tilmicosin is mainly used to treat respiratory disease in mammals, as the drug accumulates in the lungs, thus increasing the effectiveness of the treatment (Scorneaux and Shryock, 1999).

Every year, more and more publications report multidrug resistant strains and their proportion is progressively increasing. In Australia in 2014, 4.8% of *P. multocida* strains were found to be MDR (Dayao et al., 2014), which is substantially lower than the currently reported 12.2% Hungarian values. Values similar to our strains were reported in Korea (17.1%) and Spain (18.1%) (Lizarazo et al., 2006; Oh et al., 2018).

This study also proves the need to determine the antibiotic susceptibility profile of bacteria before starting treatment, which can be influenced by several factors. By using an antibiotic to which the given strain is susceptible, not only will the treatment be more successful, but also the development of resistant strains can be reduced. However, with regular monitoring of susceptibility, MDR strains can also be identified and eliminated with the appropriate drugs. Further investigation of MDR strains also enables further research into the development of resistance.

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