

HOKKAIDO UNIVERSITY

Title	Antimicrobial susceptibility of Mycoplasma bovis isolates from Bosnia and Herzegovina
Author(s)	Rifatbegovi , Maid; Ba i , Azra; Paši , Šemso; Maksimovi , Zinka
Citation	Japanese Journal of Veterinary Research, 69(1), 43-49
Issue Date	2021-02
DOI	10.14943/jjvr.69.1.43
Doc URL	http://hdl.handle.net/2115/80619
Туре	bulletin (article)
File Information	JJVR69-1_43-49_MaidRifatbegovic.pdf



Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP



Regional Study

Antimicrobial susceptibility of *Mycoplasma bovis* isolates from Bosnia and Herzegovina

Maid Rifatbegović¹⁾, Azra Bačić²⁾, Šemso Pašić¹⁾ and Zinka Maksimović^{1,*)}

¹⁾ Department of Microbiology and Infectious diseases, University of Sarajevo, Veterinary faculty, Zmaja od Bosne 90. Sarajevo 71000, Bosnia and Herzegovina

²⁾Institute for Biomedical Diagnostics and Research "GENOM", Slavka Gavrancica 17c. Travnik 72270, Bosnia and Herzegovina

Received for publication, July 5, 2020; accepted, October 21, 2020

Abstract

The minimum inhibitory concentrations (MICs) of enrofloxacin, ciprofloxacin, norfloxacin, gentamicin, spectinomycin, oxytetracycline, tylosin, florfenicol and tiamulin were determined against 24 *Mycoplasma bovis* isolates from cattle in Bosnia and Herzegovina using the microbroth dilution test. The lowest MIC values ($\leq 0.03 \mu$ g/ml) were obtained for tiamulin, gentamicin and oxytetracycline. Spectinomycin and tylosin had the highest MIC values ($\geq 128 \mu$ g/ml). Tiamulin had the lowest MIC₅₀ value ($< 0.03 \mu$ g/ml) and MIC₉₀ value (0.5μ g/ml). Among the fluoroquinolones, enrofloxacin had the lowest MIC₅₀ value (0.25μ g/ml) and MIC₉₀ value (1μ g/ml), followed by ciprofloxacin (MIC₅₀ of 0.5 μ g/ml; MIC₉₀ of 2 μ g/ml). The highest MIC₅₀ value (4μ g/ml) was found for norfloxacin, oxytetracycline and tylosin, while spectinomycin and tylosin yielded the highest MIC₉₀ values (>128 μ g/ml).

Key Words: Antimicrobial resistance, Minimum inhibitory concentration, Mycoplasma bovis

Mycoplasma bovis is considered one of the most pathogenic bovine mycoplasmas and has caused large financial losses worldwide²⁰⁾. It is a major cause of pneumonia, responsible for at least a quarter of all calf pneumonias in Europe²¹⁾. In addition to causing mastitis and arthritis, *M. bovis* is associated with other diseases in cattle including reproductive disorders, keratoconjunctivitis and otitis media²⁰⁾. Respiratory disease is one of the most common illnesses affecting cattle in Bosnia and Herzegovina (B&H). Among mycoplasmas, *M. bovis* is the species most frequently isolated from pneumonic cattle (57.4%) in B&H¹⁹⁾. Since vaccines are not yet available, sanitary control measures and appropriate antimicrobial treatment are required to control M. bovis infections¹⁷⁾. Antimicrobials traditionally used for control of M. bovis infections are tetracycline, tilmicosin and spectinomycin²¹⁾. Increases in antimicrobial resistance in mycoplasmas has become a major concern⁴. Reported variabilities in strain susceptibility to antimicrobials may be related to geographical origin, year of isolation, type of livestock production system, clinical presentation, or site of isolation¹⁷⁾. Antimicrobial

^{*} Corresponding author: Zinka Maksimović; Department of Microbiology and Infectious diseases, University of Sarajevo, Veterinary faculty, Zmaja od Bosne 90. Sarajevo 71000, Bosnia and Herzegovina Telephone/fax: ++387 33 617 370. E-mail: zinka.maksimovic@vfs.unsa.ba

resistance in *M. bovis* to tetracyclines, macrolides, lincosamides, aminoglycosides, chloramphenicols, and fluoroquinolones appears to be rising¹⁷⁾. Thus, antimicrobial susceptibility testing of *M. bovis* isolates is crucial for prompt and appropriate antimicrobial treatment, to evaluate changes in antimicrobial susceptibility and to reduce the development of antimicrobial resistance. The objective of this study was to determine the minimum inhibitory concentration (MIC) of nine antimicrobials against 24 *M. bovis* isolates from cattle in B&H. This is the first study to report the antimicrobial profiles of *M. bovis* in B&H.

The minimum inhibitory concentrations (MICs) of nine antimicrobials were determined against 24 field isolates of *M. bovis*, as well as against the reference strain PG45. All isolates were obtained from respiratory cases from dairy and fattening beef herds located in several regions of B&H. Prior to testing, all isolates were cloned and identified as *M. bovis* by previously described polymerase chain reaction (PCR) protocols^{22,25,27)}. Modified Hayflick's medium³⁰⁾ was used for the isolation of mycoplasmas. The same medium without thallium acetate and antibiotics was used in susceptibility testing. The microbroth dilution test was performed by previously described recommendations¹²⁾. The stock solutions of the antimicrobials were prepared following the guidelines of the Clinical and Laboratory Standards Institute (CLSI)^{5,6)}. Most of the antimicrobials (enrofloxacin, spectinomycin sulphate tetrahydrate, oxytetracycline hydrochloride, tylosin tartrate, florfenicol, and tiamulin hydrogen fumarate) were purchased from FM Pharm Subotica, Serbia. Ciprofloxacin, norfloxacin and gentamicin were provided by Bosnalijek, Sarajevo, B&H. The final concentration ranges were from 0.03 to 128 µg/ ml. Viable counts were made to establish the dilutions required to achieve a final concentration of 105 colour-changing units/ml (CCU/ml) for each isolate. The incubation periods and dilution procedures were performed in the same manner to produce inoculum for MIC tests¹²⁾. Each

plate contained an uninoculated medium as a negative control and growth controls lacking an antimicrobial. The plates were incubated at 37°C for 48 hours. The MIC of each antimicrobial was determined as the lowest concentration of the antimicrobial agent that inhibited the growth of $M. \ bovis^{11,12}$.

The MIC range, distribution, mode, mean, MIC₅₀ and MIC₉₀ values are presented in Table 1. The lowest MIC values ($\leq 0.03 \ \mu g/ml$) were obtained for tiamulin, gentamicin and oxytetracycline. Spectinomycin and tylosin showed the highest MIC values ($\geq 128 \ \mu g/ml$). The MIC₅₀ values for all antimicrobials ranged from <0.03 to 4 $\mu g/ml$, while the MIC₉₀ values ranged from 0.5 to greater than 128 $\mu g/ml$. Tiamulin had the lowest MIC₅₀ value (<0.03 $\mu g/ml$) and the lowest MIC₅₀ value (0.5 $\mu g/ml$).

Among the fluoroquinolones, enrofloxacin showed the lowest MIC_{50} value (0.25 µg/ml) and MIC_{90} value (1 µg/ml), followed by ciprofloxacin (MIC_{50} of 0.5 µg/ml and MIC_{90} of 2 µg/ml). Norfloxacin yielded the highest MIC_{50} (4 µg/ ml) and MIC_{90} values (8 µg/ml). Apart from norfloxacin, the highest MIC_{50} value of 4 µg/ml was found with oxytetracycline and tylosin, while spectinomycin and tylosin yielded the highest MIC_{90} value (>128 µg/ml).

Control of *M. bovis* infection depends on early identification and treatment with appropriate antimicrobials. Antimicrobials commonly used for therapy include the tetracyclines, macrolides and some fluoroquinolones¹⁷⁾. However, observed MIC trends indicate the development of resistance to these antimicrobials in *M.* $bovis^{3,8,17)}$. Due to the absence of antimicrobial resistance surveillance and the lack of monitoring for antimicrobial usage, there is inadequate information on antimicrobial agents used in animals in B&H. In addition, there are no local guidelines for antibiotic use, which effects therapeutic approaches for bovine respiratory disease most commonly associated with M. $bovis^{19,23)}$. Thus, the data on the antimicrobials used in cattle in B&H were provided by personal communication

Antimicro agent	Number of isolates with MIC (μ g/ml) of												$\frac{\mathrm{MIC}_{50}{}^{a}}{(\mu g/ml)}$	MIC ₉₀ ^b (µg/ml)	Mode	Mean		
		≤0.03	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128				
Fluoroqui- nolones	Enro- floxacin			7	7	6	2	1			1				0.25	1	0.125	1.07
	Cipro- floxacin		1	3	7	3	5	4				1			0.5	2	0.25	2.03
	Nor- floxacin					4	4	1	7	6	1	1			4	8	4	5.5
Aminogly- cosides	Genta- micin	4	1	1	5	6	3	3	1						0.5	2	0.5	0.73
	Specti- nomycin			2	3	4	6	1			1	1		6	1	>128	1	34.46
Tetracycline	Oxytetra- cycline	1				3	2	4	5	3	2	3	1		4	32	4	10.31
Macrolide	Tylosin					4	3	3	2	6	1	2	•	3	4	>128	8	22.13
Phenicol	Florfe- nicol				2	7	6	5	4						1	4	0.5	1.5
Pleuromutilin	Tiamu- lin	14	1	4	3	1	1								< 0.03	0.5	< 0.03	0.16

Table 1. MIC distribution for nine antimicrobial agents against 24 Mycoplasma bovis isolates from cattle in Bosnia and Herzegovina

 $^{a}\mathrm{MIC}_{50}$, the lowest concentration of antimicrobial that inhibit 50% of the isolates;

 ${}^{\mathrm{b}}\mathrm{MIC}_{90}$, the lowest concentration of antimicrobial that inhibit 90% of the isolates.

with field veterinarians. In the present study, the lowest MIC values of $<0.03 \mu g/ml$ were obtained for tiamulin, gentamicin and oxytetracycline. Tiamulin was also the most active antimicrobial used against M. bovis isolates obtained from the cattle affected with respiratory diseases in the Netherlands in 1993²⁹⁾, in Japan between 1996 and 1997¹⁴⁾ and in Belgium between 1997 and 2000³¹⁾. So far, there have been no reported pleuromutilins resistance mechanisms for M. *bovis*¹⁷⁾. This could be explained by the fact that some pleuromutilins, such as tiamulin, are not available for use in cattle³³⁾. Considering that only a few studies^{11,14,29,31)} included testing of tiamulin, the effects of this antimicrobial on *M. bovis* should be further investigated. In our study, gentamicin yielded lower MIC values (<0.03-4 µg/ml) compared to those seen in previous studies^{10,28,31)}.

The differences in MIC values for gentamicin obtained in the present and other studies could be due to rare use of this antimicrobial agent in cattle in B&H. In the present study, spectinomycin and tylosin exhibited MIC₉₀ values of >128 µg/ml, indicating potential antimicrobial resistance in the Bosnian isolates. However, these antimicrobials are scarcely used for treatment of bovine respiratory disease in B&H. Significant differences between the MIC_{50} (1 µg/ml) and the MIC_{90} (>128 µg/ml) values detected for spectinomycin are in accordance with the results obtained for the isolates in Britain (4 and >128 μ g/ml, respectively)¹⁾ and Israel (2 and >1024 μ g/ ml, respectively)¹⁰. In France, the MIC₅₀ values for this antimicrobial increased from 4 to > 64 μ g/ml⁸⁾ over a three decade period. In contrast, low MIC values for spectinomycin were observed in *M. bovis* in the United States by Rosenbusch et al^{24} , in Canada by Cai et al^{3} and in Japan by Uemura et al^{32} . Macrolides are often the firstline treatment for respiratory infection in cattle³⁴⁾. Resistance to macrolides is widely distributed in *M. bovis* isolates¹⁷⁾. Tylosin was among the first macrolides introduced for animal use⁷⁾. An increase in MIC₅₀ values for tylosin was recorded in France (from 2 to >64 μ g/ml)⁸⁾ and in Canada (from 0.5 to 16 μ g/ml) over a 30 year period³⁾. Our

study detected MIC_{90} values of >128 µg/ml for tylosin, which is in agreement with the studies in Hungary²⁸⁾, Canada¹⁵⁾ and Israel¹⁰⁾. Low MIC values for this macrolide were identified in the Netherlands (MIC range 0.06-4 µg/ml) in 1993²⁹⁾ and in Japan (MIC range 0.78-12.5 µg/ml) in 2003¹⁴⁾. There was a significant overall increase of MIC values for tylosin in isolates obtained before 2000 (0.025–>100 μ g/ml) compared to those obtained from isolates between 2000 and 2016 (0.125->256 µg/ml)⁹⁾. These observations indicate acquired resistance to tylosin in *M. bovis* isolates from various countries worldwide. In a study conducted on *M. ovipneumoniae* isolates in B&H, tylosin was one of the antimicrobials yielding the highest MIC₉₀ values¹⁸⁾. In our study MIC₅₀ value of 4 µg/ml for oxytetracycline corresponds well to values determined from respiratory cases in European isolates^{13,16)}. Oxytetracycline was among the antimicrobials that exhibited the highest MIC_{90} values for *M. ovipneumoniae* isolates in B&H¹⁸⁾. This antimicrobial is one of the most commonly used drugs in B&H for therapy of respiratory disease. Nevertheless, changes in M. bovis susceptibility against this antimicrobial were previously demonstrated. The MIC₅₀ values increased in UK cattle between 2004 and 2009, from 1 to 32 µg/ml²⁾. In Canada, the MIC₅₀ values increased between 1978 and 1991, from 2 to 4 µg/ ml, and remained at this level from 1991 until 2009^{3} . The MIC₅₀ value of 1 µg/ml derived in our study for florfenicol is lower than those values reported in most European countries^{2,16)}, which may be due to the limited application of this antimicrobial in cattle in B&H. Florfenicol was found to be one of the most effective antimicrobials against *M. bovis* (MIC range of 2-32 µg/ml) in the United States²⁶⁾. In the present study, enrofloxacin had an MIC₅₀ value of 0.25 µg/ml, similar to the results from previous investigations in Europe^{13,16)}, Canada¹⁵⁾, the United States²⁴⁾ and Japan³²⁾. An increase in MIC₅₀ values from 0.25 to 0.5 µg/ml was reported for enrofloxacin in UK isolates from 2004 and 2009, and between 1978–1979 and 2010–2012 for French isolates^{2,8,17)}.

Although enrofloxacin is frequently used for treatment of affected cattle in B&H, it showed low MIC_{50} and MIC_{90} values. This could be explained by the fact that this antimicrobial has only in the last few years been used routinely for the therapy of infectious respiratory diseases in cattle in B&H. Ciprofloxacin had an MIC₅₀ of 0.5 µg/ml and MIC_{90} of 2 µg/ml, similar to previous studies (0.5 and 1 μ g/ml, respectively)¹⁷⁾. This antimicrobial is rarely administered in affected cattle in B&H. In addition, enrofloxacin and ciprofloxacin had the lowest MIC_{50} and MIC_{90} values for *M*. ovipneumoniae isolates from sheep and goats in B&H¹⁸⁾. On the other hand, norfloxacin yielded the highest MIC_{50} (4 µg/ml) and MIC_{90} values (8 µg/ml). This fluoroquinolone is not available for use in B&H, and thus its administration is most likely limited, which raises the question of natural resistance in *M. bovis* isolates.

However, fluoroquinolones should not be used as a first line treatment and without prior antimicrobial susceptibility testing³³⁾. Variations in antimicrobial susceptibility of M. bovis observed in different studies worldwide could be related to the genetic heterogeneity of the isolates. However, in some studies, the acquisition in M. bovis of resistance to antimicrobials was attributed to the emergence and spread of a single clone¹⁷⁾. In addition, differences in antimicrobial susceptibilities of M. bovis isolates could be associated with differing usage practices for antimicrobials, the year of isolation, clinical presentations, or the sites of isolation $^{9,17)}$. Although conducted on relatively small numbers of isolates, the present study revealed differences in the antimicrobial profiles of M. bovis from cattle with respiratory disease in B&H. The MIC₅₀ and MIC₉₀ values suggest tiamulin, followed by enrofloxacin as the most effective in vitro antimicrobials for M. bovis isolates. Gentamicin and ciprofloxacin, followed by florfenicol were also efficient in vitro antimicrobial agents against M. bovis. High MIC values were observed for several antimicrobials, particularly for spectinomycin, tylosin and oxytetracycline. These findings imply a need to monitor antimicrobial susceptibility patterns in M. bovis in order to ensure appropriate antimicrobial treatment, assess changes in antimicrobial susceptibility and to prevent antimicrobial resistance. In view of the growing ineffectiveness of antibiotics in treating M. bovis infections and due to a lack of effective vaccines, other control measures should be applied to prevent M. bovis infections: screening animals for M. bovis prior their introduction in herds, early detection of infected animals, separation of calves from the adults, culling chronically infected animals, reducing stocking densities, improving ventilation, and other actions that support good farming practices^{17,20,21}.

Acknowledgements

The authors are grateful to M. Scott Cornwell, DVM, Dipl. ACVPM for English language revision of this article.

References

- Ayling RD, Baker SE, Peek ML, Simon AJ, Nicholas RA. Comparison of *in vitro* activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against recent field isolates of *Mycoplasma bovis*. Vet Rec 146, 745–747, 2000.
- Ayling RD, Rosales RS, Barden G, Gosney FL. Changes in antimicrobial susceptibility of *Mycoplasma bovis* isolates from Great Britain. Vet Rec 175, 486, 2014.
- Cai HY, McDowall R, Parker L, Kaufman EI, Caswell JL. Changes in antimicrobial susceptibility profiles of *Mycoplasma bovis* over time. Can J Vet Res 83, 34–41, 2019.
- 4) Chernova OA, Medvedeva ES, Mouzykantov AA, Baranova NB, Chernov VM. Mycoplasmas and their antibiotic resistance: The problems and prospects in controlling infections. Acta Naturae 8, 24–34, 2016.

- CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 4th ed., Clinical and Laboratory Standards Institute, 2018.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 28th ed., Clinical and Laboratory Standards Institute, 2018.
- European Medicines Agency. Reflection paper on the use of macrolides, lincosamides and streptogramins (MLS) in food-producing animals in the European Union: development of resistance and impact on human and animal health, 2010. www.ema.europa.eu/ (accessed 19 May 2020)
- 8) Gautier-Bouchardon AV, Ferré S, Le Grand D, Paoli A, Gay E, Poumarat F. Overall decrease in the susceptibility of *Mycoplasma bovis* to antimicrobials over the past 30 years in France. PLoS One 9, e87672, 2014.
- Gautier-Bouchardon AV. Antimicrobial resistance in *Mycoplasma* spp. Microbiol Spectr 6, 1–21, 2018.
- Gerchman I, Levisohn S, Mikula I, Lysnyansky I. In vitro antimicrobial susceptibility of Mycoplasma bovis isolated in Israel from local and imported cattle. Vet Microbiol 137, 268– 275, 2009.
- Hannan PC, Windsor GD, de Jong A, Schmeer N, Stegemann M. Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. Antimicrob Agents Chemother 41, 2037-2040, 1997.
- Hannan PCT. Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. Vet Res 31, 373-395, 2000.
- Heuvelink A, Reugebrink C, Mars J. Antimicrobial susceptibility of *Mycoplasma bovis* isolates from veal calves and dairy cattle in the Netherlands. Vet Microbiol 189, 1-7, 2016.
- 14) Hirose K, Kobayashi H, Ito N, Kawasaki Y, Zako M, Kotani K, Ogawa H, Sato H.

Isolation of mycoplasmas from nasal swabs of calves affected with respiratory diseases and antimicrobial susceptibility of their isolates. J Vet Med Ser B Infect Dis Vet Public Heal 50, 347–351, 2003.

- 15) Jelinski M, Kinnear A, Gesy K, Andrés-Lasheras S, Zaheer R, Weese S, McAllister TA Antimicrobial sensitivity testing of *Mycoplasma bovis* isolates derived from western Canadian feedlot cattle. Microorganisms 8, 124, 2020.
- 16) Klein U, de Jong A, Moyaert H, Garch FE, Leon R, Richard-Mazet A, Rose M, Maes D, Pridmore A, Thomson JR, Ayling RD. Antimicrobial susceptibility monitoring of *Mycoplasma hyopneumoniae* and *Mycoplasma bovis* isolated in Europe. Vet Microbiol 204, 188–193, 2017.
- Lysnyansky I, Ayling RD. Mycoplasma bovis: Mechanisms of resistance and trends in antimicrobial susceptibility. Front Microbiol 7, 1-7, 2016.
- 18) Maksimović Z, Bačić A, Rifatbegović M. Antimicrobial susceptibility of caprine and ovine *Mycoplasma ovipneumoniae* isolates. Microb Drug Resist 26, 1271-1274, 2020.
- Maksimović Z, Bačić A, Rifatbegović M. Mycoplasmas isolated from ruminants in Bosnia and Herzegovina between 1995 and 2015. Veterinaria 65, 27-31, 2016.
- Nicholas R, Ayling R, Mcauliffe L. Mycoplasma Diseases of Ruminants. Wallingford, CABI. 2008.
- Nicholas RAJ, Ayling RD. Mycoplasma bovis: Disease, diagnosis, and control. Res Vet Sci 74, 105–112, 2003.
- 22) Pinnow CC, Butler JA, Sachse K, Hotzel H, Timms LL, Rosenbusch RF. Detection of *Mycoplasma bovis* in preservative-treated field milk samples. J Dairy Sci 84, 1640–1645, 2001.
- 23) Rifatbegović M, Assunção P, Poveda JB, Pasić S. Isolation of *Mycoplasma bovis* from the respiratory tract of cattle in Bosnia and Herzegovina. Vet Rec 160, 484–485, 2007.
- 24) Rosenbusch RF, Kinyon JM, Apley M, Funk ND, Smith S, Hoffman LJ. In vitro antimicrobial inhibition profiles of Mycoplasma

bovis isolates recovered from various regions of the United States from 2002 to 2003. J Vet Diagnostic Investig 17, 436–441, 2005.

- 25) Sachse K, Salam HS, Diller R, Schubert E, Hoffmann B, Hotzel H. Use of a novel realtime PCR technique to monitor and quantitate *Mycoplasma bovis* infection in cattle herds with mastitis and respiratory disease. Vet J 186, 299–303, 2010.
- 26) Soehnlen MK, Kunze ME, Karunathilake KE, Henwood BM, Kariyawasam S, Wolfgang DR, Jayarao BM. In vitro antimicrobial inhibition of Mycoplasma bovis isolates submitted to the Pennsylvania Animal Diagnostic Laboratory using flow cytometry and a broth microdilution method. J Vet Diagnostic Investig 23, 547–551, 2011.
- 27) Subramaniam S, Bergonier D, Poumarat F, Capaul S, Schlatter Y, Nicolet J, Frey J. Species identification of *Mycoplasma bovis* and *Mycoplasma agalactiae* based on the uvrC genes by PCR. Mol Cell Probes 12, 161–169, 1998.
- 28) Sulyok KM, Kreizinger Z, Fekete L, Hrivnák V, Magyar T, Jánosi S, Schweitzer N, Turcsányi I, Makrai L, Erdélyi K, Gyuranecz M. Antibiotic susceptibility profiles of *Mycoplasma bovis* strains isolated from cattle in Hungary, Central Europe. BMC Vet Res 10, 2014.
- 29) Ter Laak EA, Noordergraaf JH, Verschure MH. Susceptibilities of Mycoplasma bovis, Mycoplasma dispar, and Ureaplasma diversum strains to antimicrobial agents in vitro. Antimicrob Agents Chemother 37, 317-321, 1993.
- 30) Thiaucourt F, Bölske G, Leneguersh B, Smith D, Wesonga H. Diagnosis and control of contagious caprine pleuropneumonia. Rev Sci Tech 15, 1415–1429, 1996.
- 31) Thomas A, Nicolas C, Dizier I, Mainil J, Linden A. Antibiotic susceptibilities of recent isolates of *Mycoplasma bovis* in Belgium. Vet Rec 153, 428–431, 2003.
- 32) Uemura R, Sueyoshi M, Nagatomo H. Antimicrobial susceptibilities of four species

of *Mycoplasma* isolated in 2008 and 2009 from cattle in Japan. J Vet Med Sci 72, 1661–1663, 2010.

- World Organization for Animal Health. OIE list of antimicrobials of veterinary importance, 2019. www.oie.int/ (accessed 19 May 2020).
- 34) Zaheer R, Cook SR, Klima CL, Stanford K, Alexander T, Topp E, Read RR, McAllister TA. Effect of subtherapeutic vs. therapeutic administration of macrolides on antimicrobial resistance in *Mannheimia haemolytica* and enterococci isolated from beef cattle. Front Microbiol 4, 1-14, 2013.