Antibiotic susceptibility profiles of Mycoplasma bovis strains isolated from cattle in Hungary, Central Europe

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Abstract

Background: Mycoplasma bovis is a worldwide pathogen, causative agent of pneumonia, mastitis, arthritis, and a variety of other symptoms in cattle. The economic losses due to mycoplasma pneumonia could be reduced by antibiotic treatment. The aim of the present study was to determine the in vitro susceptibility of M. bovis strains isolated from cattle in Hungary to eleven antibiotics.

Results: Minimal inhibitory concentration (MIC) values of 35 M. bovis strains collected from different parts of Hungary between 2010 and 2013 were determined by the microbroth dilution method. Strains with high MIC values were found in the case of all applied antibiotics. The most effective antibiotics tested in vitro were fluoroquinolones (MIC90 danofloxacin 0.312 µg/ml, enrofloxacin 0.312 µg/ml, marbofloxacin 0.625 µg/ml). Our results confirm the observations of increasing MIC values to antibiotics commonly used in the therapy of mycoplasma infections, primarily to tetracyclines; tetracycline (MIC90 16 µg/ml) and oxytetracycline (MIC90 ≥ 64 µg/ml) and macrolides; tylosin (MIC90 ≥ 128 µg/ml) and tilmicosin (MIC90 ≥ 128 µg/ml). The growth of many M. bovis strains was not inhibited by gentamicin (MIC90 8 µg/ml), spectinomycin (MIC90 ≥ 256 µg/ml), florfenicol (MIC90 8 µg/ml) or lincomycin (MIC90 ≥ 64 µg/ml).

Conclusions: Our results emphasize the necessity of periodic testing for antibiotic susceptibility in this geographic region. Based on our in vitro examinations, fluoroquinolones could be the most effective drugs for the therapy of M. bovis infections in Hungary. However, current antimicrobial use policies have to be taken into account to avoid further antibiotic resistance development and to reserve fluoroquinolones for the treatment of severe infections which have responded poorly to other classes of antimicrobials.

Keywords: Antibiotic resistance, MIC, Fluoroquinolones, Microbroth dilution, Mycoplasma bovis

Background

Mycoplasma bovis is a widely distributed pathogen, first isolated in the USA in 1961 from a case of severe mastitis in cattle [1]. It is associated with various diseases in cattle including calf pneumonia, mastitis, arthritis, otitis media and genital disorders [2]. M. bovis is considered responsible for a quarter to a third of economic losses in the cattle industry caused by respiratory diseases [3].

In Hungary, the average seropositivity rate of individual animals was found to be 11.3%, in certain herds it even exceeded 50.0%. Tested by enzyme-linked immunosorbent assay the overall rate of seropositive herds was 64.7% [4]. With the exception of seroprevalence on individual level, these values are relatively high in a European context [3,5].

Since no effective vaccine is available against M. bovis, adequate housing and appropriate antibiotic treatment are promoted in the control of the diseases caused by this agent. Antibiotic therapy of mastitis has often failed, but antimicrobial treatment of pneumonia has shown some success and it may help reduce economic losses [3,6]. Mycoplasmas are intrinsically resistant to β-lactam...
antimicrobials and sulphonamides, because they do not possess a cell wall and do not synthesize folic acid. Mycoplasmas are generally susceptible to antibiotics that affect protein (tetracyclines, macrolides, lincosamides, phenicols) or nucleic acid synthesis (fluoroquinolones) [2]. The decreased effectiveness of certain antimicrobial agents (spectinomycin, oxytetracycline and tilmicosin) traditionally used in the therapy of mycoplasma infections was reported in Europe [7].

The aim of this study was to determine the susceptibility of 35 Hungarian M. bovis isolates to eleven antibiotics using the microbroth dilution method.

Methods

Thirty-five M. bovis strains originating from dairy herds located in different parts of Hungary were tested in this study (Table 1, Figure 1). The samples were collected during routine diagnostic examinations or necropsies between 2010 and 2013. Ethical approval was not required for the study as all samples were collected during routine diagnostic examinations or necropsies. Nasal swabs, lung samples and a single lymph node were homogenized in 2 ml of Mycoplasma broth medium (pH 7.8) (Thermo Fisher Scientific Inc./Oxoid Inc., Waltham, MA) supplemented with 0.5% (w/v) sodium pyruvate, 0.5% (w/v) glucose and 0.005% (w/v) phenol red and cultured at 37°C in a 5% CO2 atmosphere. Following colour change (red to yellow shift) the cultures were inoculated onto solid Mycoplasma media (Thermo Fisher Scientific Inc./Oxoid Inc.) and were incubated at 37°C and 5% CO2 for 3 days, until visible colonies appeared. Mixed cultures were filter cloned only once to exclude contaminant Mycoplasma species and to minimize in vitro mutations of the isolates. DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen Inc., Hilden, Germany) according to the manufacturers’ instructions for Gram-negative bacteria. All isolates were identified by polymerase chain reaction (PCR) targeting the 16S rRNA gene of M. bovis [8]. The purity of the cultures (e.g. to exclude M. arginini or other Mycoplasma spp. contamination) was confirmed by a universal Mycoplasma PCR system targeting the 16S/23S rRNA intergenic spacer region in Mollicutes [9] followed by sequencing on an ABI Prism 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA), sequence analysis and BLAST search. The same once filter cloned passage of each M. bovis strain was submitted for a 4 gene based multi-locus sequence typing (MLST) and the sequencing data confirmed the purity of the isolates at strain level (i.e. not more than one M. bovis strain in the culture) [10]. Mixed primary cultures which failed to be purified by a single filter cloning were excluded from the study (data not shown). Aliquots of the third passage of purified cultures were stored frozen at −70°C until required. The number of colour changing units (CCU) was calculated by microplate dilution method, from the lowest dilution showing colour change after one week of incubation [11,12].

The following antimicrobial agents were examined during the microbroth dilution tests: three fluoroquinolones: danofloxacin (batch SZBA019XX), enrofloxacin (batch SZBA336XXV) and marbofloxacin (batch SZBC248XXV); two aminoglycosides: gentamicin (batch 051K17475V) and spectinomycin (batch SZBB166XXV); two tetracyclines: oxytetracycline (batch SZBC320XXV) and tetracycline (batch SZBA140XXV); two macrolides: tilmicosin (batch SZBC345XXV) and tylosin (batch SZBB160XXV); one phenicol: florfenicol (batch SZBC223XXV) and one lincosamide: lincomycin (batch SZBC340XXV); all products originated from VETRALAN, Sigma-Aldrich, Germany. They were diluted and stored according to the recommendations of Hannan [11]. Stock solutions of 1 mg/ml enrofloxacin, danofloxacin and marbofloxacin were prepared in 0.1 M NaOH; stock solution of 1 mg/ml florfenicol was prepared in 96% ethanol and in sterile distilled water; and the rest of the stock solutions of 1 mg/ml were prepared in sterile distilled water. All aliquots were stored at −70°C until needed, and dilutions were freshly prepared for each microtest. Twofold dilutions were prepared in the range 0.039-10 μg/ml for fluoroquinolones, 0.125-32 μg/ml for florfenicol, 0.25-64 μg/ml for gentamicin, tetracyclines and lincomycin, 0.5-128 μg/ml for macrolides and 1–256 μg/ml for spectinomycin.

The microbroth dilution test was performed as recommended by Hannan [11] using 104–105 CCU/ml of each strain. In brief, the 96-wells microtiter plates were designed to contain growth control (broth medium without antibiotic), sterility control (broth medium without antibiotic and Mycoplasma inoculum) and pH control (broth medium adjusted to pH 6.8) wells. Mycoplasma broth medium (pH 7.8) (Thermo Fisher Scientific Inc./Oxoid Inc.) supplemented with 0.5% (w/v) sodium pyruvate, 0.5% (w/v) glucose and 0.005% (w/v) phenol red was used as a culture medium. The duplicates of three clinical isolates and the duplicate of the type strain (M. bovis PG45, NCTC 10131) were tested on each 96-well microtiter plates. The MIC (minimal inhibitory concentration) value of each isolate was defined as the lowest concentration of the antibiotic that completely inhibits the growth in the broth (no pH and colour change) after a one week incubation period [12]. MIC50 and MIC90 values were defined as the lowest concentrations that inhibit 50% and 90% of bacterial isolates. The type strain (M. bovis PG45, NCTC 10131) was used for the quality control of MIC determination (Table 1).

Results

Our MIC values of M. bovis type strain PG45 were identical with values previously obtained for danofloxacin, enrofloxacin, marbofloxacin, spectinomycin, tilmicosin, oxytetracycline, tetracycline, gentamicin, florfenicol, lincomycin, spectinomycin, tetracycline, and enrofloxacin.
| Sample ID | Origin of herd | Date Sample source | MIC values (μg/ml) | Aminoglycosides | Tetracyclines | Macrolides | Phenicol | Lincosamide |
|-----------|----------------|--------------------|--------------------|-----------------|---------------|------------|----------|-------------|
|           |                |                    | Fluoroquinolones   |                 |               |            |          |             |
|           |                |                    | Danofloxacin      | Enrofloxacin    | Marbofloxacin | Gentamicin | Spectinomycin | Oxytetracycline | Tetracycline | Tilmicosin | Tylosin | Florfenicol | Lincomycin |
| PG45      | Connecticut    | 1961 Lung           | 0.156              | 0.156           | 0.625         | 4          | 4         | 2           | 2           | 0.25       | 0.5       | 0.5       | 1         |
| MYC2      | Püspökhatvan   | 2011 Lung           | 0.156              | 0.156           | 0.625         | 2          | 2         | 16          | 4           | 128        | 16        | 4         | 1         |
| MYC22     | Sümeg          | 2012 Lung           | 0.156              | 0.312           | 0.625         | 4          | 2         | 256         | 64          | 16         | 128        | 128       | 8         | 64        |
| MYC30     | Bugyi          | 2012 Lung           | 0.156              | 0.156           | 0.625         | 4          | 2         | 256         | 32          | 8          | 128        | 128       | 4         | 64        |
| MYC42     | Nemi           | 2012 Lung           | 0.156              | 0.156           | 0.625         | 8          | 4         | 4           | 64          | 8          | 128        | 32        | 8         | 1         |
| MYC43     | Zsana          | 2012 Lung           | 0.156              | 0.156           | 0.312         | 4          | 2         | 256         | 64          | 16         | 128        | 128       | 8         | 64        |
| MYC44     | Győrszentiván  | 2012 Lung           | 10                 | 10              | 10            | 2          | 2         | 256         | 64          | 8          | 128        | 128       | 8         | 64        |
| MYC45     | Budapest       | 2012 Lung           | 10                 | 10              | 10            | 2          | 2         | 256         | 64          | 8          | 128        | 128       | 4         | 64        |
| MYC46     | Budapest       | 2012 Lung           | 10                 | 10              | 10            | 4          | 2         | 256         | 64          | 8          | 128        | 128       | 8         | 64        |
| MYC47     | Dabas          | 2012 Lung           | 0.156              | 0.156           | 0.625         | 8          | 2         | 256         | 64          | 8          | 128        | 128       | 8         | 64        |
| MYC48     | Ósi            | 2012 Nasal swab     | 0.156              | 0.156           | 0.625         | 8          | 2         | 256         | 64          | 16         | 128        | 128       | 4         | 64        |
| MYC49     | Ósi            | 2012 Nasal swab     | 0.156              | 0.156           | 0.625         | 8          | 2         | 256         | 64          | 16         | 128        | 128       | 4         | 64        |
| MYC50     | Ósi            | 2012 Lung           | 0.156              | 0.156           | 0.625         | 4          | 2         | 256         | 64          | 8          | 128        | 128       | 4         | 64        |
| MYC51     | Ósi            | 2012 Nasal swab     | 0.156              | 0.08            | 0.312         | 4          | 2         | 256         | 64          | 8          | 128        | 128       | 4         | 64        |
| MYC52     | Solt           | 2012 Lung           | 0.156              | 0.156           | 0.312         | 8          | 4         | 2           | 2           | 0.25       | 0.5       | 0.5       | 4         | 0.5       |
| MYC53     | Solt           | 2012 Lung           | 0.156              | 0.156           | 0.625         | 16         | 4         | 2           | 2           | 0.25       | 0.5       | 0.5       | 4         | 1         |
| MYC65     | Csengersima    | 2012 Nasal swab     | 0.156              | 0.156           | 0.625         | 2          | 2         | 64          | 16          | 16         | 128        | 8         | 4         | 0.5       |
| MYC66     | Csengersima    | 2012 Nasal swab     | 0.156              | 0.156           | 0.625         | 8          | 4         | 64          | 8           | 128        | 16        | 8         | 1         |
| MYC67     | Csengersima    | 2012 Lung           | 0.08               | 0.08            | 0.312         | 4          | 4         | 16          | 4           | 128        | 16        | 8         | 2         |
| MYC68     | Csengersima    | 2012 Lung           | 0.156              | 0.156           | 0.625         | 4          | 4         | 32          | 4           | 128        | 16        | 4         | 0.5       |
| MYC69     | Komárom        | 2013 Nasal swab     | 0.156              | 0.156           | 0.625         | 2          | 4         | 32          | 8           | 128        | 32        | 8         | 1         |
| MYC70     | Komárom        | 2013 Nasal swab     | 0.156              | 0.156           | 0.625         | 4          | 2         | 32          | 4           | 128        | 32        | 4         | 1         |
| MYC71     | Komárom        | 2013 Nasal swab     | 0.156              | 0.156           | 0.625         | 4          | 2         | 32          | 4           | 128        | 32        | 4         | 1         |
| MYC72     | Komárom        | 2013 Nasal swab     | 0.156              | 0.156           | 0.625         | 4          | 4         | 32          | 4           | 128        | 32        | 4         | 1         |
| MYC73     | Komárom        | 2013 Nasal swab     | 0.156              | 0.156           | 0.625         | 4          | 4         | 32          | 8           | 128        | 32        | 4         | 1         |
| Isolate | Location | Year | Sample Type | MIC (μg/mL) | MIC (μg/mL) | MIC (μg/mL) | MIC (μg/mL) | MIC (μg/mL) | MIC (μg/mL) |
|---------|----------|------|-------------|------------|------------|------------|------------|------------|------------|
| MYC74   | Komárom  | 2013 | Nasal swab  | 0.156      | 0.156      | 0.625      | 4          | 4          | 32         | 8          | 128        | 16         | 4          | 1          |
| MYC75   | Komárom  | 2013 | Nasal swab  | 0.156      | 0.08       | 0.312      | 2          | 2          | 32         | 4          | 128        | 32         | 4          | 1          |
| MYC76   | Komárom  | 2013 | Nasal swab  | 0.156      | 0.156      | 0.625      | 4          | 4          | 64         | 8          | 128        | 16         | 8          | 2          |
| MYC77   | Kentészsi  | 2010 | Lung        | 0.312      | 0.156      | 0.625      | 2          | 2          | 256        | 64         | 8          | 128        | 128        | 4          | 64         |
| MYC78   | Hosszúpályi | 2011 | Lung        | 0.156      | 0.156      | 0.625      | 4          | 256        | 64         | 8          | 128        | 128        | 4          | 64         |
| MYC79   | Hosszúpályi | 2011 | Lung        | 0.156      | 0.156      | 0.625      | 8          | 256        | 64         | 16         | 128        | 128        | 8          | 64         |
| MYC80   | Ebes      | 2011 | Lymph node  | 0.156      | 0.156      | 0.625      | 4          | 256        | 32         | 4          | 128        | 128        | 4          | 64         |
| MYC81   | Felsőnyárád | 2013 | Lung        | 0.156      | 0.156      | 0.625      | 8          | 256        | 64         | 8          | 128        | 128        | 4          | 64         |
| MYC82   | Felsőnyárád | 2013 | Nasal swab  | 0.156      | 0.156      | 0.625      | 4          | 256        | 64         | 8          | 128        | 128        | 8          | 64         |
| MYC83   | Alsótold  | 2013 | Lung        | 0.312      | 0.156      | 0.625      | 4          | 256        | 64         | 8          | 128        | 128        | 8          | 64         |
| MYC84   | Felsőnyárád | 2013 | Nasal swab  | 0.156      | 0.156      | 0.625      | 4          | 256        | 64         | 8          | 128        | 128        | 4          | 64         |
and tylosin using the microbroth dilution method Table 1; [12,13]. The MIC value of PG45 (2 μg/ml) for oxytetracycline was within the range of previously published studies applying microbroth dilution test (0.1/0.125/0.16/4 μg/ml) [6,11-13]. The MIC value (1 μg/ml) of PG45 for lincomycin was higher than in a previous study (0.25 μg/ml) [13]. For gentamicin, tetracycline, and florfenicol data determined by microbroth dilution test were not available. Our results for type strain PG45 were consistent throughout the study.

The MIC values of the eleven antimicrobial agents obtained from the examinations of the Hungarian M. bovis isolates are shown in Figure 2 and listed in Tables 1 and 2. Strains with elevated MIC values were found in the case of all applied antibiotics. Fluoroquinolones were found to be the most active compounds in vitro. The antibiotic susceptibility profiles of the Hungarian strains were consistent within the tested group of fluoroquinolones (Figure 2A-C). Three isolates (MYC44, MYC45 and MYC46) had high MIC values (≥10 μg/ml) to danofloxacin, enrofloxacin and marbofloxacin, while the rest of the strains were inhibited by these antimicrobial agents with MICs ≤0.312 or 0.625 μg/ml. The MICs for gentamicin clustered steadily around the MIC50 value (4 μg/ml) (Figure 2D). MIC values of spectinomycin divided the strains into two distinct populations, with 48% of isolates yielding MICs of ≤4 μg/ml and the rest clustering with MICs ≥256 μg/ml (Figure 2E). Two M. bovis isolates (MYC52 and MYC53) originating from the same herd were inhibited by both tetracyclines and macrolides with low MIC values (Figure 2F-I). Among the macrolides, the MICs of tilmicosin showed bimodal distribution, as two strains yielded MICs ≤0.5 μg/ml, while the rest yielded MICs ≥128 μg/ml. The narrow range of MIC values (4–8 μg/ml) of florfenicol is demonstrated on Figure 2J. MICs for lincomycin also clustered the strains into a group with MICs ≤2 μg/ml and with MICs ≥64 μg/ml (Figure 2K).

Isolates originating from the same herd showed similar antibiotic susceptibility profiles (Table 1).

Discussion

Gerchman et al. [13] studied 11 M. bovis strains isolated from cattle imported from Hungary to Israel between 2005 and 2007. The most active compounds found during in vitro examinations were fluoroquinolones (danofloxacin, enrofloxacin and marbofloxacin), which is in accordance with our results, except that the MIC values described before were higher than the ones detected in this study (MIC90 1.25 μg/ml, 1.25 μg/ml, 5 μg/ml versus 0.312 μg/ml, 0.312 μg/ml, 0.625 μg/ml). Decreased spectinomycin susceptibility was detected in the strains from the imported
animals (MIC$_{90} > 1024 \, \mu g/ml$ obtained with E-test method), which is consistent with our results (MIC$_{90} \geq 256 \, \mu g/ml$). In contrast to the results obtained by Gerchman et al. [13] (4 \, \mu g/ml, 8 \, \mu g/ml, 1 \, \mu g/ml) the MIC$_{90}$ values of oxytetracycline (\geq 64 \, \mu g/ml), tilmicosin (\geq 128 \, \mu g/ml) and tylosin (\geq 128 \, \mu g/ml) yielded in the present study indicate limited susceptibility to these antibiotics. The comparison of the results of the previous publication and the present study emphasize the importance of the systematic monitoring of antibiotic susceptibility of M. bovis strains in the region [13,14].

Fluoroquinolones inhibited the growth of the majority of the Hungarian M. bovis strains at low MIC values (with only 3 exceptions), confirming previous observations that this group of antimicrobial agents is effective against M. bovis [6,7,13-18]. MIC values of marbofloxacin were remarkably higher than that of danofloxacin and enrofloxacin. The observed difference, first noted by Gerchman et al. [13] is probably due to the increased use of marbofloxacin during the past years [13]. Extremely high MIC values for fluoroquinolones (\geq 10 \, \mu g/ml) were found in strains MYC44-46. The similarity in the resistance profile of these three strains is consistent with the results of a previous genetic study in the country, where these strains clustered into a separate subclade by MLST [10].

Most Hungarian M. bovis strains included in the present examination showed moderate susceptibility to gentamicin, with similar or lower MIC values (MIC$_{90} 8 \, \mu g/ml$)

| Table 2 Summary of range, mode, MIC$_{50}$ and MIC$_{90}$ values of the 35 M. bovis strains isolated from cattle in Hungary |
|---------------------------------|---------------------------------|-----------------|-----------------|
| **Fluoroquinolones** | **Danofloxacin** | 0.078 to \geq 10 | 0.156 | 0.156 | 0.312 |
| **Enrofloxacin** | 0.078 to \geq 10 | 0.156 | 0.156 | 0.312 |
| **Marbofloxacin** | 0.312 to \geq 10 | 0.625 | 0.625 | 0.625 |
| **Aminoglycosides** | **Gentamicin** | 2 to 16 | 4 | 4 | 8 |
| **Spectinomycin** | 2 to \geq 256 | \geq 256 | \geq 256 | \geq 256 |
| **Tetracyclines** | **Oxytetracycline** | 2 to \geq 64 | \geq 64 | \geq 64 | \geq 64 |
| **Tetracycline** | \geq 0.25 to 16 | 8 | 8 | 16 |
| **Macrolides** | **Tylosin** | \geq 0.5 to \geq 128 | \geq 128 | \geq 128 | \geq 128 |
| **Tilmicosin** | \geq 0.5 to \geq 128 | \geq 128 | \geq 128 | \geq 128 |
| **Phencol** | **Florfenicol** | 4 to 8 | 4 | 4 | 8 |
| **Lincosamide** | **Lincomycin** | 0.5 to \geq 64 | \geq 64 | \geq 64 | \geq 64 |

All values are expressed as \mu g/ml.
than isolates from Belgium and Israel (MIC90 6 µg/ml, 32 µg/ml) [13,19]. Spectinomycin, another member of the aminoglycosides, was used traditionally as an active compound against M. bovis and it is still considered effective in Japan [6,12,14]. However, high MIC values of spectinomycin (≥256 µg/ml) were observed in more than half of the studied Hungarian isolates, which is in agreement with recent reports from other countries [7,13,15-17,19], confirming a globally emerging resistance to spectinomycin.

Heterogenic profiles of M. bovis susceptibility to tetracyclines are reported from all over the world [6,7,13,14,16,19]. Only two Hungarian isolates showed low MIC value to oxytetracycline and tetracycline, demonstrating the high level of resistance to tetracyclines among the strains. In accordance with our results, increasing resistance to oxytetracycline was reported previously in Britain, Belgium, Japan and France [7,14,16,19].

All but two of the Hungarian M. bovis strains showed high level of resistance to macrolides, with MIC90 values (≥128 µg/ml) consistent with previously published data, suggesting that macrolides are losing their efficacy on mycoplasmas [6,7,13,14,16]. For example an earlier clinical study demonstrated the effective use of tilmicosin [20] but another study [21] twelve years later already demonstrated the ineffectiveness of tilmicosin against M. bovis in vivo which also emphasizes the spread of antibiotic resistance due to the escalating use of antibiotics in veterinary practice. In the present study MICs of tilmicosin grouped around two distinct values, while the distribution of MICs of tylosin was gradually dispersed (Figure 2H-I). MIC values of tylosin were lower (8-128 ≤ µg/ml) or similar to MICs of tilmicosin (≥128 µg/ml). Similar observations were reported in the case of M. bovis strains by Gerchman et al. [13] and in the case of M. gallisepticum isolates by Jordan and Horrocks [22]. The slower development of tylosin resistance is supposed to be the cause of the difference between the MIC values of these antibiotics [23], and our results provide further evidence for this phenomenon.

Outstandingly low MIC values of all tetracyclines and macrolides were observed in two Hungarian isolates originating from the same herd (MYC52-53) and in the case of the reference PG45 strain. These three strains were closely related and they also formed a separate genetic clade in the MLST analysis performed previously [10].

The Hungarian isolates showed high MIC values to florfenicol. The MIC90 values (8 µg/ml) were similar to values obtained earlier in the United Kingdom (16 µg/ml), USA (4 µg/ml) and France (16 µg/ml) [6,7,16].

MIC90 values of lincomycin (≥64 µg/ml) were higher than the ones (1 µg/ml, 8 µg/ml, 64 µg/ml) described elsewhere [12,14,19]; and more than half of the strains isolated from cattle in Hungary demonstrated high MIC values to this member of lincomamides.

The results of in vitro antibiotic susceptibility tests can only predict the expected in vivo efficacy of the antibiotics, thus they only indicate the potential usefulness of a certain antimicrobial agent in the therapy. Standard breakpoints (susceptible, intermediary, resistant categories) have not yet been defined for the interpretation of M. bovis susceptibility to antibiotics [24], but several authors derived breakpoints for mycoplasmas from breakpoints of other bovine pathogens, and in some cases values were adopted from other host species [6,13-17]. Taking into account all these criteria, fluoroquinolones seem to be the most active compounds in vivo against the M. bovis strains existing in Hungary. Although the in vitro antibiotic susceptibility tests are promising, the use of fluoroquinolones against M. bovis could be controversial in vivo. In the United Kingdom Nicholas and Ayling [3] reported on a study where the monthly fluoroquinolone treatment repeated over three months did not prevent the development of respiratory disease caused by M. bovis.

Conclusions
The present study determined the antibiotic susceptibility profiles of 35 M. bovis strains isolated from cattle in Hungary and it highlighted the importance of regular testing of antibiotic susceptibility in the region. Our results confirmed the increasing resistance to antibiotics commonly used for the treatment of mycoplasma infections, primarily to tetracyclines and macrolides. Based on the presented in vitro examinations, fluoroquinolones could be the most effective in the therapy of M. bovis infections in Hungary. However, the identification of three fluoroquinolone resistant isolates lends support for the EU recommendation that prudent antimicrobial use policies have to be strictly observed when members of this antibiotic group are applied [25]. In order to avoid the development of resistance fluoroquinolones should only be used based on the results of susceptibility testing and in cases of severe infections when treatment failed with other classes of antimicrobials.

Abbreviations
MIC: Minimal inhibitory concentration; MLST: Multi-locus sequence typing.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
KM and ZK performed the microbroth dilution tests, analysed the data and wrote the manuscript. LF and VH performed the microbroth dilution tests. TM and KE analysed the data and wrote the manuscript. SJ, NS, IT and LM collected the samples and isolated the strains. MG designed the study, presented in vitro examinations, fluoroquinolones could be the most effective in the therapy of M. bovis infections in Hungary. However, the identification of three fluoroquinolone resistant isolates lends support for the EU recommendation that prudent antimicrobial use policies have to be strictly observed when members of this antibiotic group are applied [25]. In order to avoid the development of resistance fluoroquinolones should only be used based on the results of susceptibility testing and in cases of severe infections when treatment failed with other classes of antimicrobials.

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