Antimicrobial susceptibility of *Francisella tularensis* subsp. *holarctica* strains from Hungary, Central Europe

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**Objectives:** Determining the *in vitro* susceptibility to 11 antibiotics of *Francisella tularensis* subsp. *holarctica* strains belonging to the phylogenetic group B.13, from different areas of Hungary.

**Methods:** Twenty-nine *F. tularensis* strains isolated between 2003 and 2010 from free-ranging European brown hares (*Lepus europaeus*) and a captive patas monkey (*Erythrocebus patas*) were collected from different parts of Hungary and examined for antibiotic susceptibility with commercially available MIC test strips on modified Francis agar plates; values were interpreted according to CLSI breakpoints.

**Results:** The strains were susceptible to aminoglycosides (MIC90 values: gentamicin, 0.75 mg/L; and streptomycin, 6.0 mg/L), tetracyclines (MIC90 values: tetracycline, 0.5 mg/L; and doxycycline, 1.0 mg/L), quinolones (MIC90 values: ciprofloxacin, 0.047 mg/L; and levofloxacin, 0.023 mg/L) and chloramphenicol (MIC90 value: 1.5 mg/L), i.e. antibiotics commonly used in therapy. Tigecycline (MIC90 value: 0.19 mg/L) and rifampicin (MIC90 value: 1.0 mg/L) were also active against *F. tularensis* strains, while resistance to erythromycin (MIC90 value: >256 mg/L) and linezolid (MIC90 value: 32 mg/L) was observed in all strains.

**Conclusions:** Based on the results, quinolones are recommended as first choice therapy for *F. tularensis* infection. The *in vitro* susceptibility of the strains to tigecycline may encourage the application of this antibiotic as well. The similar antibiotic susceptibilities of the Hungarian strains belonging to different subclades of phylogenetic group B.13 indicates that strains from other Central and Eastern European countries belonging to this group might also have the same susceptibility profile.

**Keywords:** antibiotics, MICs, tularaemia, zoonosis

**Introduction**

*Francisella tularensis*, a fastidious, Gram-negative bacterium, is the causative agent of the highly contagious zoonotic disease, tularaemia, which has gained increased attention during the last decade due to the possibility of its use in bioterrorism events.1 As *F. tularensis* subsp. *holarctica* is prevalent all over the Northern hemisphere, it is endemic in Europe, including Hungary, where reported human cases have numbered between 5 and 200 annually for the last decade.2 Close contact with European brown hares (*Lepus europaeus*) or tick bite were most frequently reported in the anamneses of these cases.2 Tularaemia may occur in six well-recognized clinical forms in humans: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic and typhoid (or septicaemic) tularaemia.1 The antibiotics of choice in the treatment of tularaemia are aminoglycosides, quinolones, chloramphenicol or tetracyclines, bearing in mind the side effects and probability of relapse.1 A general picture of the antibiotic susceptibility of European *F. tularensis* subsp. *holarctica* strains can be obtained from several current and previous studies.3–9

The aim of this study was to characterize the *in vitro* antimicrobial susceptibility profile of 29 selected Hungarian *F. tularensis* subsp. *holarctica* strains to 11 antibiotics (erythromycin, streptomycin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, doxycycline, tigecycline, rifampicin, linezolid and chloramphenicol) that could potentially be used in clinical therapy.
Materials and methods

**F. tularensis isolation and characterization**

Samples were collected between 2003 and 2010 for *F. tularensis* isolation from seropositive European brown hares shot during hunting and from zoo monkeys, originating from different parts of Hungary. *F. tularensis* strains were isolated from tissue samples on modified Francis agar (chocolate agar plate containing 1% glucose and 0.1% cysteine) after mouse passage. In brief, the homogenized tissue samples were injected subcutaneously into NMRI mouse and at 7–10 days post-infection, heart blood and bone marrow samples were inoculated on to culture media. Strains were identified with the MicroLog MicroStation System, GN2 MicroPlate (both from Biolog Inc., Hayward, CA, USA) and with a 16S rRNA gene-based PCR method, as described previously. The strains were also phylogenetically characterized using canonical single nucleotide polymorphism assays as described previously, placing the strains into a global phylogeographical framework.

**Antibiotic susceptibility tests**

The susceptibility of *F. tularensis* strains to 11 antibiotics (erythromycin, streptomycin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, doxycycline, tigecycline, rifampicin, linezolid and chloramphenicol) was determined with MIC test strips (Liofilchem s.r.l., Roseto degli Abruzzi, Italy) on 5 mm-thick modified Francis agar plates. The strains were cultured for 48 h on modified Francis agar at 37°C in a 5% CO2 atmosphere. Three to four colonies were suspended in 3 mL of physiological saline, with the turbidity adjusted to be equivalent to that of a 0.5 McFarland standard. The plates were inoculated using sterile cotton swabs and one MIC test strip was placed on each plate within 15 min. After 48 h of incubation at 37°C in a 5% CO2 atmosphere, the MIC results were read according to the manufacturer’s instructions. The *F. tularensis* subsp. holarctica live vaccine strain (LVS (NCTC 10857)) was included as a quality control. The breakpoints were interpreted according to CLSI standards for *F. tularensis*, where available, and to CLSI standards for Enterobacteriaceae, staphylococci or *Streptococcus pneumoniae* where specific standards were unavailable.

**Results**

A collection of 69 *F. tularensis* subsp. holarctica strains was established and 29 of these strains were systemically chosen for antibiotic susceptibility profile characterization considering their geographical origin, host species and genetic characteristics. The selected strains originated from European brown hares (28 strains) and a patas monkey (*Erythrocebus patas*) from different parts of Hungary. Previous phylogenetic analyses demonstrated that 15 strains belonged to the subclades B.23/14/25, B.20/21/33, B.33/34/B.37/38 and B.Tul07/2007 of group B.13, the most prevalent *F. tularensis* subsp. holarctica group in Central and Eastern Europe, and further examinations based on this study classified the rest of the strains into group B.13 as well. According to the MIC values that inhibited the growth of 90% of the strains (MIC90), resistance to erythromycin (>256 mg/L) and linezolid (32 mg/L) and susceptibility to aminoglycosides (gentamicin, 0.75 mg/L; and streptomycin, 6.0 mg/L), quinolones (ciprofloxacin, 0.047 mg/L; and levofloxacin, 0.023 mg/L), tetracyclines (tetracycline, 0.5 mg/L; and doxycycline, 1.0 mg/L), rifampicin (1.0 mg/L), tigecycline (0.19 mg/L) and chloramphenicol (1.5 mg/L) were observed in all 29 *F. tularensis* subsp. holarctica strains (Table 1 and Table S1 (available as Supplementary data at JAC Online)).

**Discussion**

The main aim of this study was to examine the susceptibility of the Hungarian *F. tularensis* strains to antibiotics with a potential to be used in clinical therapy. As the resistance of *F. tularensis* subsp. holarctica to β-lactam antibiotics and cephalosporins (with few exceptions) has already been confirmed in several studies, these antibiotics were excluded from the present study.

Categorization of *F. tularensis* subsp. holarctica strains based on erythromycin resistance produces two biosvars, where biosvar I is erythromycin sensitive (present in Western Europe: France, Germany, Spain and Switzerland) while biosvar II is resistant (present in Northern and Eastern Europe: Austria, Germany, Sweden and Turkey). All Hungarian strains proved to be consistently resistant to erythromycin, thus confirming their classification into biosvar II.

Although linezolid is used in the treatment of infections caused by Gram-positive bacteria, and it is especially active against vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*, the *in vitro* susceptibility of *F. tularensis* to linezolid was also demonstrated in a recent Turkish study. Contrary to the results of the Turkish study, all Hungarian *F. tularensis* strains were resistant to linezolid, similarly to North American *F. tularensis* subspp. *holarctica* strains, with MIC values generally at the breakpoint of resistance.

In the treatment of human tularemia infections, the aminoglycosides gentamicin and streptomycin are the antibiotics of choice in Hungary. All strains were susceptible *in vitro* to both antibiotics, but it should be noted that in one case (the strain from the patas monkey) the MIC value for streptomycin reached the limit of intermediate susceptibility (8 mg/L).

In 2011, the National Centre of Epidemiology (Budapest, Hungary) recommended ciprofloxacin and chloramphenicol for post-exposure prophylaxis of tularemia. The examined *F. tularensis* strains showed high susceptibility to quinolones (ciprofloxacin and levofloxacin) and chloramphenicol as well, although the latter has serious side effects; thus, its use in therapy is limited to exceptional cases (e.g. tularemia with meningitis).

The WHO’s guidelines on tularemia also recommend tetracyclines and especially doxycycline for the therapy of tularemia. The examined strains showed good *in vitro* susceptibility to both tetracycline and doxycycline; however, the risk of relapse should be considered during the clinical use of these antibiotics.

*F. tularensis* susceptibility to tigecycline was detected for the first time in the above-mentioned research in Turkey. Tigecycline is a member of the glycyclines, a new class of antibiotics that achieves high intracellular concentrations; hence, its use in the treatment of tularemia has also been recommended. Examining the Hungarian strains’ susceptibility to tigecycline, the results were consistent with the susceptibility reported in the publication of Yesilyurt et al. Due to the low *in vitro* MIC values of tigecycline, this antibiotic may have potential in the clinical therapy of tularemia in Hungary as well.

Rifampicin was also effective *in vitro* against the *F. tularensis* strains, but due to its tendency for emerging resistance in monotherapy, its use is only recommended in combination with other antibiotics (e.g. tetracyclines).

In conclusion, on the basis of *in vitro* examinations, quinolones are recommended as first choice in the therapy of...
Table 1. In vitro activity of 11 antibiotics against 29 Hungarian \textit{F. tularensis} subsp. \textit{holarctica} clinical strains and \textit{F. tularensis} reference strains (LVS and Schu S4), and the CLSI susceptibility breakpoints

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MICs (mg/L) for clinical strains</th>
<th>MICs (mg/L) for LVS (NCTC 10857)</th>
<th>MICs (mg/L) for Schu S4$^a$</th>
<th>CLSI susceptibility breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range</td>
<td>MIC$_{50}$</td>
<td>MIC$_{90}$</td>
<td>0.38</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>3.0–8.0</td>
<td>4.0</td>
<td>6.0</td>
<td>0.094</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.38–1.0</td>
<td>0.5</td>
<td>0.75</td>
<td>0.008</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.012–0.047</td>
<td>0.032</td>
<td>0.047</td>
<td>0.006</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.004–0.023</td>
<td>0.016</td>
<td>0.023</td>
<td>0.19</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.19–0.72</td>
<td>0.38</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.125–1.5</td>
<td>0.75</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.5–1.5</td>
<td>1.0</td>
<td>1.5</td>
<td>0.094</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.5–2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.19</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.094–0.19</td>
<td>0.125</td>
<td>0.19</td>
<td>0.064</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&gt;256.0</td>
<td>&gt;256.0</td>
<td>&gt;256.0</td>
<td>&gt;256.0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>12.0–48.0</td>
<td>24.0</td>
<td>32.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

NA, data not available.
$^a$Values originate from the study of Johansson et al.$^{14}$
$^b$CLSI standard breakpoints for \textit{F. tularensis}.
$^c$CLSI standard breakpoints for staphylococci.
$^d$CLSI standard breakpoints for Enterobacteriaceae.
$^e$CLSI standard breakpoint for \textit{S. pneumoniae}.

Tularaemia, but aminoglycosides, tetracyclines and chloramphenicol could also be safely used against \textit{F. tularensis}. The in vitro effectiveness of tigecycline against \textit{F. tularensis} subsp. \textit{holarctica} suggests the applicability of this antibiotic in tularaemia treatment as well, but further in vivo examinations are required for confirmation. The use of macrolides (e.g. erythromycin) and linezolid in the treatment of tularaemia should be avoided in Hungary.

Since \textit{F. tularensis} is a highly clonal bacterium, it inherits DNA in a vertical manner and does not transfer DNA laterally between cells.$^{10,11}$ The coherent antibiotic susceptibilities of the examined strains affiliated to different subclades of one phylogenetic group (B.13) suggests that other strains of the same group from Central and Eastern Europe may react similarly to these antibiotics.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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