Isolation and Genetic Characterization of a Bartonella Strain Closely Related to 
Bartonella tribocorum and Bartonella elizabethae in Israeli Commensal Rats

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Abstract. Ten Bartonella isolates were cultured from blood drawn from black rats (Rattus rattus) captured in the Tel 
Aviv area. Genetic characterization included amplification and sequencing of five gene fragments including the ribC, rpoB, 
16S, groEL, and gltA and the 16S-23S intergenic spacer region. Sequence comparisons showed that all 10 isolates were 
identical in all genes studied comprising a total of 3,873 bp analyzed. The sequences of each of the partial genes analyzed 
indicated a high sequence similarity (97–99.8%) to B. tribocorum or B. elizabethae. The gltA sequence was 100% homolo-
gous to a genotype identified in R. rattus in Dhaka, Bangladesh, suggesting the existence of a widespread Asian Bartonella 
strain infecting the black rats (R. rattus). The detection of a Bartonella genotype closely related to B. elizabethae in the 
biggest metropolitan center in Israel warrants further study of its zoonotic potential and pathogenic characteristics.

INTRODUCTION

Organisms of the genus Bartonella are vector-borne gram-
negative facultative intracellular bacteria, infecting endothelial 
cells and erythrocytes in mammalian reservoir hosts.1 Novel 
and re-emerging Bartonella spp. were recently reported, and > 20 Bartonella spp. have been recognized to date.2 Of the 
latter, at least 11 species or sub-species are known or suspected 
to infect humans.3,4

Several novel Bartonella spp. have been isolated from 
rodents in different parts of the world in recent years. These include B. tribocorum, B. elizabethae, B. taylorii, B. graha-
mite, B. doshiiae, B. birtlesii, B. rattimassiliensis, B. phoceensis, 
B. vinsonii subsp. arupensis, and B. washoensis.5–10 Because 
rats live in close proximity to humans, they might serve as res-
ervoirs for zoonotic Bartonella species and strains, yet to be 
determined.

The aim of this study was to isolate and molecularly charac-
terize Bartonella organisms from Israeli rats in a metropolitan 
center. We describe the isolation and genetic characterization 
of a genotype closely related to Bartonella tribocorum and to the 
zoonotic pathogen, Bartonella elizabethae, in Israeli black rats (Rattus rattus).

MATERIALS AND METHODS

Sample collection and Bartonella isolation. Blood was 
drawn and collected in EDTA tubes from 62 black rats 
(R. rattus) captured in north-central Tel Aviv. Frozen blood 
samples were thawed and cultured on chocolate blood agar 
plates (Hy Laboratories, Rehovot, Israel) and incubated 
aerobically at 35°C in 5% CO2 for up to 6 weeks. Plates 
were monitored for bacterial growth at least twice per 
week after initial plating. Bacterial material from colonies 
that morphologically were identified as Bartonella on 
original plates was picked onto a new agar plate. Only pure 
cultures that were not contaminated were further genetically 
characterized.

The study was approved by the Hebrew University’s 
Agricultural Faculty Institutional Animal Care and Use 
Committee (IACUC).

PCR and sequencing. DNA was extracted from bacterial 
isolates using the QiAmp DNA mini kit (Qiagen, Valencia, 
CA). For each sample, five partial genes including ribC, 
rpoB, 16S, groEL, and gltA and the intergenic spacer region 
(ITS) were amplified, using primer sets and protocols as 
previously described (Table 1).11–16 Five microliters of DNA 
was used per reaction. The double-stranded PCR products 
were run on 1.5% low melting agarose gels and visualized 
under an UV illuminator to determine whether there was 
a successful amplification in the PCR reaction. For all the 
PCR-positive reactions, DNA was purified using ExoSAP-IT 
(USB, Cleveland, OH). DNA sequencing was performed on 
an ABI3730 capillary sequencer using the BigDey reaction. 
Sequence comparisons between all 10 isolates were performed 
for all genes studied, a total of 3,873 bp.

The sequences obtained were analyzed initially by BLAST 
through the NCBI’s Mega-BLAST algorithm online to verify 
amplification of Bartonella genes.17 Subsequently, both sense 
and antisense sequences obtained from each sample were 
aligned together into a contig at high homology to confirm 
and resolve present ambiguities in both sequences using the 
GeneCodes Sequencher 4.8 alignment program. A final 
consensus sequence was generated from each alignment for 
 further analyses. The Tel Aviv isolate consensus sequence was 
aligned and compared with published Bartonella sequences,18 
using Clustal-X.19

Phylogenetic analysis. A phylogenetic analysis of the 
concatenation of all five genes (16S, GltA, RpoB, GroEL, and 
RibC) and the 16S-23S ITS together was preformed using 
General Time Reversible plus Invariant sites plus Gamma 
distributed model (GTR + G + I model). 

Phylogenetic analysis applied minimum evolution (ME) and 
maximum likelihood (ML) methods and was performed with 
PAUP* 4.0b10.20 Maximum likelihood analyses used heuristic 
searches using 10 random starting tree replicates and TBR 
branch swapping. Non-parametric ML bootstrap analyses 
were performed using 1,000 heuristic replicates. Settings for 
the model of DNA sequence evolution was estimated initially 
using Model-Test21 and optimized using multiple heuristic ML 
searches in PAUP* until parameter values stabilized.
Amino acid analysis. The three representative sequences of *B. tribocorum*, *B. elizabethae*, and the Tel Aviv isolate were translated into amino acid sequences and compared with each other. Each gene was analyzed separately.

RESULTS

*Bartonella* isolation and molecular characterization. *Bartonella* isolates were cultured from 10 (16%) different rats and underwent further genetic characterization. Five partial genes and the ITS of each of the 10 isolates were analyzed. The PCR and sequencing results of all isolates were identical in all genes studied, suggesting that all 10 isolates were of the same genotype. The sequences of each of the five partial genes analyzed and the ITS were deposited in GenBank (accession numbers: 16S, FJ577650; gltA, FJ577651; groEL, FJ577652; ribC, FJ577654; rpoB, FJ577655; ITS, FJ577653). The obtained sequences indicated a high sequence similarity (97–99.8%) to *B. tribocorum* or *B. elizabethae* (Table 2). A representative consensus sequence including all six partial loci was used for further analysis.

Phylogenetic analysis. A phylogenetic analysis containing a consensus sequence of all five genes (16S, gltA, rpoB, groEL, and ribC) and the ITS was performed. The phylogenetic analysis indicated a close relationship of the Tel Aviv isolate with *B. tribocorum* and *B. elizabethae* (Figure 1). The Tel Aviv isolate clustered together with *B. taylorii*, *B. grahamii*, *B. rattimassiliensis*, *B. tribocorum*, and *B. elizabethae* with a high bootstrap support (Figure 1). Among the latter five species, the Tel Aviv isolate was found to cluster together with sequences representing *B. tribocorum* and *B. elizabethae* with high bootstrap support of 85.

Comparison with strains from different parts of the world. Comparisons of the gltA of the Tel Aviv isolate with *Bartonella* genotypes from *R. rattus* from different geographical areas have shown that it was indistinguishable from one *Bartonella* isolate from a black rat from Porto Santo Island in Portugal and from four *Bartonella* isolates from black rats from Dhaka, Bangladesh (GenBank accession number AF086636).5,9

Amino acid analysis. The analyses indicated that most of the genomic substitutions between the sequences were proteometrically synonymous (S) with a few non-synonymous (NS) substitutions as indicated by the S/NS ratios, which were higher than 1 (Table 3). There were less non-synonymous substitutions between *B. tribocorum* and the Tel Aviv isolate compared with the number of non-synonymous substitutions between *B. elizabethae* and the Tel Aviv isolate (Table 3). The Tel Aviv isolate showed greater similarity to *B. tribocorum* in three genes, *rpoB*, *groEL*, and *gltA*, with only one non-synonymous substitution in the *rpoB* gene (Table 3). This non-synonymous substitution (S–A) was found also in the

<table>
<thead>
<tr>
<th>Gene</th>
<th>Segment amplified length (bp)</th>
<th>Reference numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ribC</td>
<td>540</td>
<td>11</td>
</tr>
<tr>
<td>rpoB</td>
<td>795</td>
<td>12</td>
</tr>
<tr>
<td>16S</td>
<td>890</td>
<td>13</td>
</tr>
<tr>
<td>ITS</td>
<td>602</td>
<td>14</td>
</tr>
<tr>
<td>groEL</td>
<td>721</td>
<td>15</td>
</tr>
<tr>
<td>gltA</td>
<td>325</td>
<td>16</td>
</tr>
</tbody>
</table>

### Table 1

Primers used to amplify five partial genes and the ITS of the Tel Aviv *Bartonella* genotype

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer name and sequence</th>
<th>Reverse primer name and sequence</th>
<th>Segment amplified length (bp)</th>
<th>Reference numbers</th>
</tr>
</thead>
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<tr>
<td>ribC</td>
<td>TAACCGATATTGGTTGTGGTGAAG</td>
<td>BARTON-2</td>
<td>540</td>
<td>11</td>
</tr>
<tr>
<td>rpoB</td>
<td>1400F</td>
<td>GCTAGAAAGTCTGGCAACATAACG</td>
<td>795</td>
<td>12</td>
</tr>
<tr>
<td>16S</td>
<td>P24-E</td>
<td>GGA ATT CCC TCC TTC AGT TAG GCT GG</td>
<td>890</td>
<td>13</td>
</tr>
<tr>
<td>ITS</td>
<td>H56s</td>
<td>CCG GAT CCC GAG ATG GCT TTT GGA GTA</td>
<td>602</td>
<td>14</td>
</tr>
<tr>
<td>groEL</td>
<td>Hs233</td>
<td>TGA ACC TCC GAC CTC AGC CTT ATC</td>
<td>721</td>
<td>15</td>
</tr>
<tr>
<td>gltA</td>
<td>Rp877-F</td>
<td>AAT CCA TTC CGC CCA TTC</td>
<td>325</td>
<td>16</td>
</tr>
</tbody>
</table>

Figure 1. Phylogenetic analysis of the Tel Aviv *Bartonella* strain sequences, total of 3,877 bp representing five genes (16S, gltA, rpoB, groEL, and ribC) and the ITS. The midpoint maximum likelihood tree under the GTR + G + I model of sequence evolution (−ln likelihood = 21,452.478). Numbers plotted along the branches indicate bootstrap values of maximum likelihood analysis. The sequences representing the different species are from Table 1 in La Scola and others.18
Bartonella are unavailable, it was proposed that a newly encountered as no clear criteria are available to date. 18 When these typic traits, but recently the former approach was commonly based on DNA–DNA hybridization and description of pheno-

gene homologies to known sequence for a Tel-Aviv 10 strains were identical facilitated the formation of a consensus analyzed. The fact that the partial gene sequences from all these (e.g., Thailand, Bangladesh, southern China), 9,22,23 which could environments. 5 They have been reported to serve as reservoir hosts for a variety of zoonotic pathogens such as Bartonella and Rickettsia spp. and strains/ genotypes have been frequently identified in recent years. The definition of a new Bartonella species was traditionally based on DNA–DNA hybridization and description of pheno-
typic traits, but recently the former approach was commonly replaced with multi-gene sequence analysis. 18,24 The analyses performed in this study indicated that the Tel Aviv isolate is a genotype closely related to both B. tribocorum and B. elizabethae but not identical to either.

The citrate synthase gene (gltA) is considered a relatively reliable gene for distinguishing between Bartonella genotypes. 18 Based on comparison of a segment of this gene (325 bp), the Israeli isolate was found identical (100% sequence similarity) to a genotype identified in R. rattus from Dhaka (Bangladesh) and from Portugal (accession number AF086636). 19 This finding suggests that there is a common Asian Bartonella genotype infecting the black rat (R. rattus), a genotype that is closely related to both B. tribocorum and B. elizabethae. It was suggested that this Bartonella genotype could have spread from Asia to other parts of the world through infected rats traveling by ship. 9

Bartonella tribocorum was first isolated from blood of two wild brown rats (Rattus norvegicus) in France. 4 The bacterium was also isolated from the blood of the yellow-necked field mouse (Apodemus flavicollis). 26 There is currently no evidence that B. tribocorum has a zoonotic potential. Conversely, B. elizabethae was isolated for the first time from the blood of an American patient with endocarditis and subsequently from rodents. 5,7 Its importance is considered higher in urban areas with poor hygiene conditions and in certain populations such as homeless people and drug users. One third of a cohort of 631 intravenous drug users in Baltimore, MD, had antibodies to B. elizabethae, 27 suggesting the high exposure to this pathogen among this population. B. elizabethae has not been detected in human patients or in other mammalian hosts in Israel to date. The detection of a genotype that is closely related to B. elizabethae in the biggest metropolitan center in Israel warrants further study of its zoonotic potential and pathogenic characteristics.

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### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species/percentage</th>
<th>Species/percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ribC</td>
<td>BE/97.8</td>
<td>BG/86.1</td>
</tr>
<tr>
<td>rpoB</td>
<td>BT/97</td>
<td>BTM/94.6</td>
</tr>
<tr>
<td>16S</td>
<td>BT/99.7</td>
<td>BE/99.5</td>
</tr>
<tr>
<td>ITS</td>
<td>BE/99.8</td>
<td>BT/91.1</td>
</tr>
<tr>
<td>groEL</td>
<td>BT/96.6</td>
<td>BE/93.1</td>
</tr>
<tr>
<td>gltA</td>
<td>BT/99.1</td>
<td>BG/94.9</td>
</tr>
</tbody>
</table>

Highest sequence similarity obtained (first match) for all five genes and the ITS was with B. tribocorum or B. elizabethae.

BE = B. elizabethae; BT = B. tribocorum; BG = B. grahamii; BH = B. henselae; BTM = B. tammiae.

### Table 3

Summary of the synonymous/non-synonymous substitutions among amino acid sequences of B. elizabethae, B. tribocorum, and the Tel Aviv isolate in four genes

<table>
<thead>
<tr>
<th>Sample/gene</th>
<th>rpoB ratio (SNS)</th>
<th>ribC ratio (SNS)</th>
<th>groEL ratio (SNS)</th>
<th>gltA ratio (SNS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. elizabethae-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel Aviv isolate</td>
<td>23 (46/2)</td>
<td>1.4 (12/9)</td>
<td>12.75 (51/4)</td>
<td>9.5 (19/2)</td>
</tr>
<tr>
<td>B. tribocorum-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel Aviv isolate</td>
<td>23 (23/1)</td>
<td>2.43 (34/14)</td>
<td>23 (23/0)</td>
<td>2 (2/0)</td>
</tr>
<tr>
<td>B. elizabethae-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. tribocorum-</td>
<td>15.7 (47/3)</td>
<td>2.6 (34/13)</td>
<td>14.25 (57/4)</td>
<td>6.5 (13/2)</td>
</tr>
</tbody>
</table>

S/SNS = synonymous/non-synonymous.

### REFERENCES


