Brevundimonas diminuta infections and its resistance to fluoroquinolones

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Objectives: To report infections caused by Brevundimonas diminuta and antibiotic studies of this Gram-negative bacterium.

Patients and methods: Seven patients with infection and eight bacterial strains were studied. Tests included antibiotic susceptibility and analysis of the DNA gyrase and topoisomerase genes and the effect of efflux pump inhibitor Phe-Arg-β-naphthylamide (PANA).

Results: The patients all had underlying disease of cancer. The infections involved bloodstream (one case), intravascular catheter (four cases), urinary tract (one case) and pleural space (one case of empyema). Fever up to 39.2°C characterized these infections, which resolved upon treatment by combination antibiotics. Microbiologically, all organisms were resistant to multiple fluoroquinolones and cefepime, but were susceptible to amikacin, imipenem and ticarcillin/clavulanate. These quinolone-resistant B. diminuta strains were probably selected out by the prophylactic use of a quinolone in six of these patients. Additionally, the B. diminuta type strain ATCC 11568T that was isolated before the quinolone era from water was also resistant to ciprofloxacin and intermediate to levofloxacin, suggesting intrinsic quinolone resistance. The DNA gyrase and topoisomerase of six analysed strains all contained GyrA Ala-83 and Met-87, GyrB Leu-466 or Thr-466, and ParC Gln-57, Val-66 and Ala-80 that were probably the cause of fluoroquinolone resistance. PANA had nearly negligible effect.

Conclusions: B. diminuta is intrinsically resistant to fluoroquinolones and can be selected out to cause infections.

Keywords: B. diminuta, fluoroquinolone resistance, quinolones

Introduction

Brevundimonas diminuta and Brevundimonas vesicularis are non-lactose-fermenting environmental Gram-negative bacilli previously assigned to the genus Pseudomonas. These organisms are infrequently isolated in clinical microbiology laboratories. B. vesicularis has been implicated in rare cases of human infections; however, to our knowledge, B. diminuta infection has not been reported although implied. Here we report seven cases of B. diminuta infections that occurred in patients with cancer. The bacterial strains were analysed for antibiotic susceptibility and the mechanisms underlying their fluoroquinolone resistance, i.e. GyrA, GyrB, ParC and efflux pump.

Materials and methods

Study setting and bacterial strains

The cases occurred sporadically during 1998–2004 at The University of Texas M.D. Anderson Cancer Center in Houston, a 500 bed tertiary care cancer centre. All clinical data were obtained through review of the medical records. The bacteria were isolated from blood cultures in five cases, from urine culture in one case and from pleural catheter discharge in one case. Approximately 30 000 blood cultures were performed yearly at this institution using the Bectec 9240 automated culturing system (BD Diagnostic Systems, Sparks, MD, USA) and Isolator tubes (Wampole Laboratories, Princeton, NJ, USA). The Isolator tube, when positive, allowed quantification of bacterial colonies from 10 mL of blood cultured. In addition to

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Table 1. Clinical features of patients with *Brevundimonas diminuta* infections

<table>
<thead>
<tr>
<th>Case number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, sex</td>
<td>26, M</td>
<td>69, M</td>
<td>74, M</td>
<td>37, F</td>
<td>54, M</td>
<td>55, M</td>
<td>41, M</td>
</tr>
<tr>
<td>Primary disease</td>
<td>leukaemia</td>
<td>lymphoma</td>
<td>leukaemia</td>
<td>lymphoma, BMT</td>
<td>leukaemia, BMT, skin GVHD</td>
<td>sarcoma</td>
<td>sarcoma, chest catheter</td>
</tr>
<tr>
<td>WBC, % neutrophils</td>
<td>6.9×10^9/L, 37% from baseline</td>
<td>0.2×10^9/L, 0%</td>
<td>0.8×10^9/L, 4%</td>
<td>6.7×10^9/L, 80%</td>
<td>13.3×10^9/L, 69% from baseline</td>
<td>6.1×10^9/L, 62%</td>
<td>23.6×10^9/L, 88% from baseline</td>
</tr>
<tr>
<td>Antibiotic prophylaxis</td>
<td>levofloxacin</td>
<td>levofloxacin</td>
<td>levofloxacin</td>
<td>ciprofloxacin, SXT</td>
<td>SXT</td>
<td>levofloxacin</td>
<td>none</td>
</tr>
<tr>
<td>Source of <em>B. diminuta</em></td>
<td>CVC blood and peripheral blood</td>
<td>CVC blood</td>
<td>CVC blood</td>
<td>CVC blood</td>
<td>CVC blood</td>
<td>urine, 10,000 to 50,000 cfu/mL</td>
<td>empyema</td>
</tr>
<tr>
<td>Co-isolated organism</td>
<td>coagulase-negative <em>Staphylococcus</em></td>
<td>none</td>
<td>none</td>
<td>none</td>
<td><em>Moraxella osloensis</em></td>
<td><em>Enterococcus</em> sp.</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Signs and symptoms</td>
<td>fever to 39°C</td>
<td>fever to 38.3°C</td>
<td>fever</td>
<td>fever to 39.2°C</td>
<td>leucocytosis</td>
<td>possible UTI</td>
<td>fever to 39°C, pus from catheter</td>
</tr>
<tr>
<td>Antibiotic therapy</td>
<td>vancomycin, cefepime</td>
<td>imipenem, nafcillin, tobramycin, levofloxacin</td>
<td>meropenem, ticarcillin/clavulanate, nafcillin</td>
<td>ticarcillin/clavulanate, levofloxacin</td>
<td>levofloxacin</td>
<td>levofloxacin</td>
<td>ticarcillin/clavulanate</td>
</tr>
<tr>
<td>Outcome</td>
<td>resolved</td>
<td>resolved</td>
<td>resolved</td>
<td>resolved</td>
<td>resolved</td>
<td>resolved</td>
<td>resolved</td>
</tr>
</tbody>
</table>

BMT, bone marrow transplantation; CVC, central venous catheter; GVHD, graft versus host disease; SXT, trimethoprim–sulfamethoxazole; UTI, urinary tract infection; WBC, white blood cells.
seven clinical strains, the B. diminuta type strain ATCC 11568T was also included in the study.

Bacterial identification

Two of the seven B. diminuta strains were identified by biochemical tests performed in our laboratory and a reference laboratory (Houston City Health Laboratory, Houston, TX, USA). The other five strains, being recent isolates, were identified presumptively as B. diminuta by biochemical tests and definitively by sequencing analysis of the 16S ribosomal RNA gene. Briefly, genomic DNA from pure culture colonies was extracted and subjected to amplification by a polymerase chain reaction (PCR) for a 593 bp fragment of the 16S RNA gene. A set of universal bacterial primers was used for the amplification: 5'-TGCCAGACCGCCGCTACATAC-3' and 5'-CGCTCGTGTCGAGGACCTAACC-3' (positions 515–1107 of GenBank accession J01859 for Escherichia coli). The amplicon was sequenced by the dye-terminator method in an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA), and sequence analysis was performed through a query to the GenBank Basic Local Alignment Search Tool (BLAST).

Antibiotic susceptibility test and efflux pump inhibitor

The antibiotic susceptibility test was performed using Etest (AB Biodisk, Solna, Sweden) and interpreted according to the breakpoints set for Pseudomonas aeruginosa and non-Enterobacteriaceae by the NCCLS. The efflux pump inhibitor Phe-Arg-β-naphthylamide dihydrochloride (PANA) (Sigma, St Louis, MO, USA) was used to assess the role of efflux pumps. A concentration of 20 mg/L PANA was incorporated into the agar for routine Etest.

Analysis of gyrA, gyrB and parC genes

Three sets of PCR primers were used to amplify the genes for DNA gyrase and topoisomerase IV. The primers for gyrA were: 5'-TACGCCATGAGCGTGATCGTC-3' and 5'-GTTGTGCGGCCGCGGATGT-TGGT-3' (positions 334–822 of Pseudomonas aeruginosa GI 459928). The primers for gyrB were: 5'-GAACGACAGCTACCA-CGAGAC-3' and 5'-TGCGCTATCACAAGATCATCCT-3' (positions 480 to 1196 of Brevundimonas vesicularis GI 19909566) (T. Hamada, 2002, unpublished data). The primers for parC were: 5'-GCGGCCAGGCGGCGGATGT-TGGT-3' (forward) and 5'-ATCCGCGGTGCCCCTGCAAC-3' (reverse) or 5'-CCCGCGGTGCCCCTGCAAC-3' (reverse) (based on a few consensus sequences). Amplification of parE was attempted using the primers 5'-CGGCTGTCGAC-GGC-3' and 5'-CGGCTGTCGAC-GGC-3', which correspond to 618 and 1783 of Caulobacter crescentus AE005832.

GenBank accessions

The partial gyrA and gyrB sequences of ATCC 11568T and the parC sequences of clinical strain MDA0824 were deposited as GenBank accessions AY654591, AY654592 and AY971356, respectively.

Results

Clinical features of infections

The clinical features of the seven patients are summarized in Table 1. The patients were six men and one woman and ranged in age from 26 to 74 years (median 54). Five of them had haematological malignancy. The first patient, on the day of...
chemotherapy, exhibited a fever to 39°C and relative leukocytosis (6.9 × 10⁹/L) from his baseline of 2.7 × 10⁹/L despite prophylaxis with levofloxacin. From the cultures of the central venous catheter (CVC) and peripheral blood, B. diminuta and coagulase-negative Staphylococcus were isolated. The Isolator tubes were also positive, and from the 10 mL of blood cultured, 21–30 and 13–15 colonies of B. diminuta were obtained from the CVC and peripheral blood, respectively. The bacteremia was further treated with vancomycin and cefepime (before the culture results) and the patient defervesced 3 days later without CVC removal.

For patients 2 to 5, all had B. diminuta isolated from the CVC blood, thus representing catheter infections. All patients had a prophylactic fluoroquinolone or trimethoprim/sulfamethoxazole. Patients 2, 3 and 4 spiked a fever (to 39.2°C) and the infections resolved on further treatment with combination antibiotics. Patient 5, though afebrile, had a significant leucocytosis (13.3 × 10⁹/L with 69% neutrophils) from his baseline of 7.8 × 10⁹/L with 48% neutrophils despite trimethoprim/sulfamethoxazole prophylaxis. This patient suffered from chronic graft versus host disease with bilateral leg wounds, for which he had been treated with tacrolimus, an immunosuppressive drug. Patient 5 with complication of chronic pleural effusion and placement of a chest catheter presented with fever, shortness of breath, and purulent discharge from the catheter. Both B. diminuta and *Moraxella osloensis*, another Gram-negative bacterium that occasionally causes CVC-related blood infec-
sions in cancer patients, were isolated from discharge. The *Staphylococcus aureus* and *P. mirabilis* were also positive, and from the 10 mL of blood cultured, 21–30 and 13–15 colonies of *B. diminuta* were isolated from the urine with 10 000–50 000 cfu/mL for each organism. The possible urinary tract infection (UTI) was further treated with levofloxacin (before the culture results) and the patient defervesced 3 days later without CVC removal.

The sixth patient who suffered from prostatic sarcoma had an episode of severe obstructive uropathy with significant urethral discharge. In spite of the levofloxacin use, *B. diminuta* and an *Enterococcus* sp. were isolated from the urine with 10 000–50 000 cfu/mL for each organism. The possible urinary tract infection (UTI) was further treated with levofloxacin (before availability of the culture results) and the patient was discharged.

The last patient who suffered from angiosarcoma of the heart with complication of chronic pleural effusion and placement of a chest catheter presented with fever, shortness of breath, and purulent discharge from the catheter. Both *B. diminuta* and *Staphylococcus aureus* were isolated from discharge. The catheter was replaced and empyema drained with concurrent antibiotic therapy. The patient made a full recovery.

**Antibiotic susceptibility**

The susceptibility test results of the seven clinical strains and the type strain ATCC 11568T are summarized in Table 2. All eight strains were found to be susceptible to amikacin, imipenem and ticarcillin/clavulanate. They were infrequently susceptible to trimethoprim/sulfamethoxazole (three of eight strains tested), ceftriaxone (one of six) and ceftazidime (one of eight). They were all resistant to cefepime and ciprofloxacin and either resistant or intermediate to ampicillin. All five available clinical strains that were tested were also resistant to gatifloxacin and levofloxacin. The ATCC strain was intermediate to levofloxacin and susceptible to gatifloxacin.

**Analysis of gyrA, gyrB and parC**

A portion of the gyrA gene encompassing the potential quinolone-resistance-determining region (QRDR) in *B. diminuta* was successfully amplified and sequenced for the six available strains, and up to 479 nucleotides (157 amino acids) were obtained and analysed. The region shared 65% identical residues with that of *E. coli*,13 and 79% with that of *Caulobacter crescentus*,14 a non-pathogenic free-living water bacterium (no antibiotic susceptibility data available) that is closest to *B. diminuta* in phylogeny.16 Among the six strains, the amino acid residues were conserved to 99–100%. A limited alignment of the QRDR is shown in Figure 1. The region is conserved for members of the family Enterobacteriaceae and *P. aeruginosa*, and the *B. diminuta* strains contained six significantly different residues (underlined). Among them, Ala-83 and Met-87 were candidate residues responsible for or contributing to the quinolone resistance because substitutions at these positions are known to confer resistance in Enterobacteriaceae and *P. aeruginosa* (Table 3) and organisms in other genera.

The 40 residues from the *B. diminuta* QRDR (Figure 1) were queried to the GenBank through protein–protein BLAST (blastp) to assess the occurrence frequency of Ala-83 and Met-87. Among the 505 best-matched sequences, there were 37 (7.3%) GyrA proteins containing Ala-83, but none contained Met-87. Thus, Met-87 was rare and probably unique to *B. diminuta*.

A portion of the gyrB gene was amplified successfully and sequenced for ATCC 11568T and four clinical strains. The region contained 220 amino acid residues and was more conserved than GyrA across species, from 68% identical with *E. coli* to 85% identical with *C. crescentus*. For the five *B. diminuta* strains, all residues were conserved to 99–100%. In comparison with known quinolone-resistant residues for *E. coli* and *P. mirabilis*, one residue, Leu-466 or Thr-466 was found to be potentially important for resistance (Table 3).

The analysis of the parC gene is shown in Table 3. Of the 138 amino acids analysed, the strains were conserved to 99–100%.

![Figure 1. Alignment of the GyrA QRDR residues. Position numbers refer to the E. coli residues. Dots indicate identical residues. Significantly different residues are underlined. Data sources for E. coli, P. aeruginosa, P. mirabilis and C. crescentus are Refs 13, 10, 15 and 11, respectively.](https://academic.oup.com/jac/article-abstract/55/6/853/725607/856)
Table 3. Representative quinolone-resistant residues of GyrA, GyrB and ParC and their counterparts in *Brevundimonas diminuta*

<table>
<thead>
<tr>
<th>Organism, category, references</th>
<th>GyrA positions</th>
<th>GyrB positions</th>
<th>ParC positions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacteriaceae (eight species)</strong>, S to R, Ref. 15</td>
<td>83 87</td>
<td>426 447 464 466</td>
<td>57 66 80 84</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em>, S to R, Ref. 16</td>
<td>Thr or Ser to Ile, Leu, Phe, Tyr, Arg</td>
<td>Asp or Glu to Gly, Tyr, Asn</td>
<td>Ser to Tyr, Glu to Asp</td>
</tr>
<tr>
<td><em>Escherichia coli</em>, S to R, Ref. 17</td>
<td>Asp to Asn</td>
<td>Lys to Glu</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em>, S to R, Refs 10, 18, 19</td>
<td>Thr to Ile</td>
<td>Asp to Gly, Ile, Asn, Tyr, Ser</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enterica</em>, S to R, Ref. 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em>, S to R, Ref. 21</td>
<td>Ser to Ala</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em>, S to R, Ref. 22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. diminuta</em>, this study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 11568 T</td>
<td>Ala</td>
<td>Met</td>
<td>Asp</td>
</tr>
<tr>
<td>2192 (case 1)</td>
<td>Ala</td>
<td>Met</td>
<td></td>
</tr>
<tr>
<td>1007 (case 2)</td>
<td>Ala</td>
<td>Met</td>
<td></td>
</tr>
<tr>
<td>0824 (case 5)</td>
<td>Ala</td>
<td>Met</td>
<td></td>
</tr>
<tr>
<td>2271 (case 6)</td>
<td>Ala</td>
<td>Met</td>
<td></td>
</tr>
<tr>
<td>1126 (case 7)</td>
<td>Ala</td>
<td>Met</td>
<td></td>
</tr>
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</table>

S, susceptible; R, resistant. Bold type represents amino acids of potential significance for quinolone resistance in *B. diminuta*. Blank spaces indicate lack of data or not done.
and they shared 53% identical residues with that of *E. coli* and 86% with that of *C. crescentus*. Three residues, Gln-57, Val-66 and Ala-80, all identical among the six strains, were significantly different from usual quinolone-sensitive residues in other organisms.

Amplification of the *parE* gene was attempted, which yielded the same *gyrB* gene for two strains (0824 and 2271) while other strains were not amplified.

**Effect of PANA**

The potential effect of efflux pump inhibitor PANA was assessed (Table 4). The chemical showed a consistent, albeit small, reduction in MIC for ceftriaxone (1.5- to 2.7-fold) and ampicillin (2- to 6-fold). It had no effect on cefepime. The effects on ciprofloxacin, levofloxacin and gatifloxacin were similar to that of ciprofloxacin, suggesting its intrinsic resistance to quinolones. Intrinsic resistance to quinolones is rare but has been reported for aquatic *Aeromonas* spp., i.e. *A. caviae*, *A. hydrophila* and *A. sobria*, in which the QRDR residue GyrA Ser-83 is mutated to Ile or Thr.

The quinolone resistance of our *B. diminuta* strains, these residues are Ala-83 and Met-87. The analogy for Ala-83 is apparent and the Met-87 is structurally conserved to the resistance residue Ile-87; thus, these residues are the likely cause of *B. diminuta*’s quinolone resistance.

The GyrB subunit is less commonly associated with quinolone resistance; however, mutations of four residues are found causing resistance: Asp-426 to Asn, Lys-447 to Glu, Ser-464 to Tyr or Phe, and Glu-466 to Asp (Table 3). In our *B. diminuta* strains, Asp-426, Arg-447 and Ser-464 were identical or similar to those quinolone-sensitive sequences, and the only difference was Leu-466 or Thr-466. Thus, Leu-466 or Thr-466 might have further contributed to the resistance.

A few ParC mutations, i.e. Thr-57 to Ser, Thr-66 to Ile, Ser-80 to Arg or Tyr, and Glu-80 to Lys, Val or Gly, also confer quinolone resistance. All our strains contained three significantly different residues at these positions: Gln-57, Val-66 and Ala-80. Despite the current lack of analogy, these residues may potentially contribute to the quinolone resistance.

**Acknowledgements**

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**References**


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**Table 4. Effect of efflux pump inhibitor Phe-Arg-β-naphthylamide (20 mg/L)**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>ATCC 11568</th>
<th>2192</th>
<th>1007</th>
<th>0824</th>
<th>2271</th>
<th>1126</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>2</td>
<td>2.7</td>
<td>1.5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cefepime</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Gatifloxacin</td>
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<td>1.3</td>
<td>no</td>
<td>1.3</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
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<td>2</td>
<td>2</td>
<td>no</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>no</td>
<td>no</td>
<td>no</td>
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</tbody>
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