CASE REPORTS

Isolation of Buttiauxella gaviniae from a Spinal Cord Patient with Urinary Bladder Pathology

Thierry De Baere,1 Georges Wauters,2 Peter Kämpfer,3 Caroline Labit,1 Geert Claeys,1 Gerda Verschraegen,1 and Mario Vaneechoutte1*

Department of Microbiology, Ghent University Hospital, Ghent,1 and Department of Microbiology, Université Catholique de Louvain, Brussels,2 Belgium, and Institut für angewandte Mikrobiologie, Justus Liebig Universität, Giessen, Germany3

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A gram-negative Buttiauxella gaviniae-like organism (LBV449) was isolated from a urine sample of a patient suffering from urine bladder pathology and neurological problems. The isolate was positive for adonitol fermentation and l-arginine dihydrolase and negative for melibiose and l-ornithine decarboxylase. The API 20E code was 3004113. Retrospectively, another isolate (ENT107), from a leg wound, was recovered from our collections and was shown to have similar biochemical characteristics. DNA-DNA hybridization showed 77% similarity between both strains, and strain LBV449 revealed 74% DNA-DNA similarity to the type strain of B. gaviniae. Neither 16S rRNA gene sequencing nor fatty acid analysis were useful for identification. The characteristic tRNA-PCR patterns obtained for these two clinical isolates consisted of fragments with lengths of 102.2, 105.4, 116.6, and 136.9 bp and most resembled the tRNA-PCR pattern obtained for B. gaviniae, but they lacked the B. gaviniae fragments of 88.2 and 239.5 bp. To our knowledge, no clinical cases with Buttiauxella strains have been described thus far.

CASE REPORT

In April 1998, a 31-year-old man was admitted to our hospital with a bifrontal contraction lesion and a D12-L4 fracture. Due to this trauma and neurological syndrome, he developed epileptic attacks, hyperflexion of the lower limbs, and a neurological urinary syndrome, wherefore a suprapubic catheter was placed. During a recent hospitalization the person suffered again from urinary retention and a urine sample was taken for further investigation. Microscopic examination of the urine sediment yielded 121 leukocytes per μl (normal count, less than 25 leukocytes per μl, as determined with UF100 [TOA Medical Electronics, Kobe, Japan]) and 107 bacteria per μl. Direct microscopy and culture indicated gram-negative rods at a concentration of 106 CFU/ml. All of these results are indicative of a urinary tract infection in a spinal cord patient.

The suprapubic catheter was removed and the patient was treated with ofloxacin (200 mg, twice a day) for 2 weeks. Therapy was successful, and no bacteria and no elevation in number of white blood cells were seen in later samples.

Discussion. Culture of the urine sample on tryptic soy agar with 5% sheep blood (Becton Dickinson, Erembodegem, Belgium) and on MacConkey agar (Becton Dickinson) yielded a lactose-negative gram-negative rod, designated LBV449. Identification with API 20E (Biomérieux, Marcy l’Étoile, France) resulted in code 3004113 or 1004113, due to a variable β-galactosidase reaction (β-nitrophenyl-β-D-galactopyranosidase [ONPG] hydrolysis) that was negative in the latter case, which gave a good identification as Escherichia vulneris (96.5%) or a weak identification as E. vulneris (95%), Buttiauxella agrestis (85%), Klebsiella pneumoniae subsp. ozaenae (80%), or Pantoea sp. strain 4 (89%), respectively. Although lactose fermentation was scored negative, macro-testing showed that it was positive after 6 days of incubation. Lactose fermentation was found in this study to be slowly positive among B. noackiae strains but remained negative for the four B. gaviniae reference strains tested (S1/1 986, S1/1 987, S1/1 988, and S1/1 998).

The results of the biochemical characterization are presented in Table 1. The case report strain was negative for l-ornithine decarboxylase, melibiose, raffinose, and pyrrolidonyl aminopeptidase, which makes differentiation from B. agrestis possible. The case report strain could be differentiated from B. noackiae by a positive adonitol test and by the absence of phenylalanine deaminase. It was negative for ONPG hydrolysis and citrate in API 20E, but in macro-tests it was weakly or slowly positive for ONPG and citrate. No definite biochemical differentiation with B. gaviniae could be found when comparing these findings with previously published data (2–4, 8).

DNA-DNA hybridization was carried out as described previously (11). The isolation of DNA was done using the method of Marmur (6), with the additional steps of RNase A (Sigma, Steinheim, Germany) and RNase T1 (Sigma) treatment for 2 h at 37°C.

Our clinical isolate (LBV449) had DNA-DNA similarities of 42.3% to the type strain of B. agrestis (CDC 1176-81), 62% to...
<table>
<thead>
<tr>
<th>Biochemical characteristic</th>
<th>B. agrestis</th>
<th>B. gaviniae</th>
<th>B. noackiae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WG 189 ENT1185, wound, right toe</td>
<td>WG 190 ENT1194, human</td>
<td>ENB 141 S3/3 203, snail, Germany</td>
</tr>
<tr>
<td></td>
<td>ENB 143 S3/3 162, snail, Germany</td>
<td>ENB 142 CDC 17681, soil, France</td>
<td>ENB148 ENT116, urine</td>
</tr>
<tr>
<td>Differentiating</td>
<td></td>
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<tr>
<td>Adonitol (macroscopic)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phenylalanine deaminase (APP)</td>
<td>–</td>
<td>w⁺&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Pyrrolidonyl aminopeptidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Rafines</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Variable</td>
<td></td>
<td></td>
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<tr>
<td>Citrate utilization (Simmons)</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate (API, 30 and 37°C)</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(% Positive strains of 86)&lt;sup&gt;a&lt;/sup&gt; molusks</td>
<td>65</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ENB153 ENT107, wound, leg</td>
<td>100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LBV 449, bladder urine</td>
<td>0</td>
<td>Delayed</td>
<td>Delayed</td>
</tr>
<tr>
<td>ENB 146 NSW 11, snail, Australia</td>
<td>Delayed</td>
<td>Delayed</td>
<td>Delayed</td>
</tr>
<tr>
<td>ENB 144 BR626, snail, Brazil</td>
<td>1 Delayed</td>
<td>+</td>
<td>Delayed</td>
</tr>
<tr>
<td>ENB 145 BR 489, snail, Brazil</td>
<td>Delayed</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from Müller et al. (8).<sup>b</sup> w⁺, weak positive.<sup>c</sup> ND, not determined.
the type strain of \textit{B. noackiae} (NSW 11), and 77.3\% to a strain (ENT107) previously isolated from a leg wound which had been at that time identified as belonging to enteric group 59. The DNA-DNA similarity of LBV449 to the type strain of \textit{B. gaviniae} (ATCC 51604) was 74\%, and similarities to the type strains of all further \textit{Buttiauxella} species (8) were in the range of 43 to 58\%.

Cellular fatty acids were determined as described previously (5) and were of the straight-chain type, both saturated and unsaturated, with 16:0 and 16:19\text{cis}, as major components. There was no significant difference between the cellular fatty acid profile of the various \textit{Buttiauxella} species tested (4), although the data indicated that the highest similarity was between the clinical isolate and \textit{B. noackiae}.

Genotypic identification was attempted by amplification of the 16S rRNA gene and sequencing (10). The 16S rRNA gene was amplified using the primers 5’ AGT TTG ATC CTG GCT CAG 3’ and 5’ TAC CTT GTT ACG ACT TCG TCC TCA 3’. Comparison of the sequence with all bacterial sequences available from the GenBank database using the Blast 2.0 program (National Center for Biotechnology Information, Bethesda, Md.) revealed similarities of between 99.2 and 99.8\% with the published sequences of the \textit{Buttiauxella} species tested (4). The overall similarity of the published sequences of the \textit{Buttiauxella} species is above 99\% (9), but our data indicate that even several of the described differences are not really present. The difference between our results and those of Spröer et al. (9) remains unexplained, since those authors also applied direct sequencing and did not start from cloned 16S rRNA genes, which otherwise could have explained how they overlooked the ambiguities present.

\textbf{tRNA-PCR} (1, 7) enabled clear differentiation from \textit{B. agrestis} and \textit{B. noackiae} and from all clinically important gram-negative species (unpublished data, available upon request). The patterns obtained for the two clinical isolates (tRNA intergenic spacer lengths of 102.4, 105.4, 116.5, and 136.8 bp) resembled best those of \textit{B. gaviniae}, with some distinct differences (Table 2).

Using the Kirby-Bauer disk diffusion method, the strain was found to be susceptible to ampicillin, cotrimoxazole, cefuroxime, gentamicin, furadantin, temocillin, and quinolones.

\textbf{Clinical} strain isolated from a urine sample from a spinal

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Species & Strain designation & GenBank Accession Number & Sequence & \textit{E. coli} position \\
\hline
\textit{B. agrestis} & DSM 4586 & AJ233400 & TACCTGACAYCAGACAGATCTCAGAGATGCCTTGACCTGAGAC & 980
\hline
\textit{B. agrestis} & ATCC 333207 & AJ293685 & ++++++++S+**C+*ST**+++AS+********+++YS+**** & 990
\hline
\textit{B. agrestis} & ENB 141 & AJ293684 & ++++++++C+*Y+GY+++GC+------------GG+++ & 1000
\hline
\textit{B. agrestis} & ENB 143 & AJ293686 & ++++++++CR+*Y+GY+++GC+------------YY+++ & 1010
\hline
\textit{B. gaviniae} & DSM 9397 & AJ233403 & ++++++++CA+*C+CG+++GC+----------TG+++ & 1020
\hline
\textit{B. gaviniae} & DSM 9401 & AJ293683 & ++++++++SA+*C+ST+++RG+++---------------- & 1030
\hline
\textit{B. noackiae} & DSM 51607 & AJ293689 & ++++++++CG+*Y+GY+++GC+------------GC+++ & 980
\hline
\textit{B. noackiae} & ATCC 51607 & AJ293687 & ++++++++CR+*Y+GY+++GC+------------GC+++ & 990
\hline
\textit{B. noackiae} & ENB 144 & AJ293688 & ++++++++SR+*Y+GC+++GC+------------GC+++ & 1000
\hline
\textit{B. noackiae} & ENB 145 & & & \\
\hline
\end{tabular}
\caption{16S rRNA gene sequence alignments for \textit{B. agrestis}, \textit{B. gaviniae}, \textit{B. noackiae}, and the \textit{B. gaviniae} clinical isolate. Sequences with accession numbers shown in bold are those determined in this study. International Union of Microbiological Societies (IUMS) codes in the sequences: R = A or G; S = G or C; Y = C or T.}
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\hline
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\hline
\textit{B. noackiae} & ENB 144 & AJ293688 & ++++++++SR+*Y+GC+++GC+------------GC+++ & 1000
\hline
\end{tabular}
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cord patient with urinary bladder pathology had clinical significance and responded well to treatment. To our knowledge, only one clinical isolate (CDC 1650-80) belonging to *B. noackiae* has been described thus far (8). *B. gaviniae* has not been reported from clinical specimens until now, and all but one of the *B. gaviniae* isolates studied by Müller et al. (8) were isolated from snails.

In summary, 16S rRNA gene sequencing was indecisive because of the high degree of similarity and the presence of polymorphism in the described regions allowing differentiation. Also, fatty acid analysis was of little help. The two clinical isolates were positive for adonitol fermentation and L-arginine dihydrolase and negative for L-ornithine decarboxylase and melibiose and, thus, were biochemically indistinguishable from *B. gaviniae*. The tRNA-PCR patterns of these clinical isolates most resembled those of *B. gaviniae* and could be used to identify them as such.

**Nucleotide sequence accession number.** The DNA sequence of the clinical isolate obtained in this study was deposited in GenBank under accession number AJ293683.

**REFERENCES**


