Table 1. Antibiotic susceptibility of E. coli 1736, E. coli transformant and E. coli recipient strain DH10B

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>E. coli 1736</th>
<th>E. coli transformant</th>
<th>E. coli DH10B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.25</td>
<td>0.19</td>
<td>0.095</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.38</td>
<td>0.19</td>
<td>0.095</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Piperacillin/</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&lt;4</td>
</tr>
<tr>
<td>tazobactam</td>
<td>64</td>
<td>64</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Amikacin</td>
<td>8</td>
<td>1.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;4</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;8</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>16</td>
<td>0.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Meropenem</td>
<td>16</td>
<td>0.5</td>
<td>0.023</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;32</td>
<td>1</td>
<td>0.008</td>
</tr>
<tr>
<td>Doripenem</td>
<td>4</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.38</td>
<td>0.047</td>
<td>0.047</td>
</tr>
</tbody>
</table>

*aSusceptibility testing of all antibiotics was performed using Vitek-2; carbapenem MICs were additionally tested by agar dilution; MICs of imipenem, meropenem and ertapenem below 0.5 mg/L and MICs of ceftriaxone, ceftazidime, tigecycline and colistin were determined by Etest.

Since the emergence of carbapenem-resistant K. pneumoniae in Israel during 2006, an active surveillance programme was implemented in high-risk patients newly admitted to our institution, and at the national level in long-term care facilities. blAOXA-48 was not detected in Enterobacteriaceae prior to and after this single report. Thus, although the patient was hospitalized in our hospital ~2 months prior to the isolation of the OXA-48-producing E. coli strain, it is most likely that the strain or the plasmid was carried prior to hospitalization.

Class D OXA-48 carbapenemase among Enterobacteriaceae was first reported in 2004 in K. pneumoniae from Turkey and is continuously spreading in the Mediterranean area, as well as in other countries in Europe. Recent reports in the Middle East region stress the urgent need for regional collaboration to confront the spread of resistance.

Acknowledgements

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We would like to thank the laboratory of Professor P. Nordmann, Hospital de Bicetre, Paris, France, for confirming the results on Tn19992.

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Transparency declarations

None to declare.

References


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Molecular characterization of high-level fluoroquinolone resistance in a clinical isolate of Haemophilus parainfluenzae

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Keywords: prostatitis, quinolones, QRDRs, quinolone resistance-determining regions

Sir,

Haemophilus parainfluenzae is commonly implicated as a causative organism in respiratory tract infections. H. parainfluenzae has also been frequently associated with the genitourinary tract,
but it is not a frequent pathogen in prostatic secretions,\textsuperscript{2} Ampicillin resistance mediated by TEM-1 \(\beta\)-lactamase is common in this species;\textsuperscript{4} however, to our knowledge, only a few studies previously examined the fluoroquinolone susceptibility of \textit{H. parainfluenzae}. Studies in Europe and the USA indicated that most \textit{H. parainfluenzae} isolates were susceptible to levofloxacin and other fluoroquinolones, so resistance to fluoroquinolones in \textit{H. parainfluenzae} has rarely been reported.\textsuperscript{5} Another study from Japan described some fluoroquinolone-resistant \textit{H. parainfluenzae} isolated from clinical samples, but the molecular basis of this resistance was not clarified.\textsuperscript{6}

We report herein a multidrug-resistant \textit{H. parainfluenzae} (strain 617) isolated from a clinical sample. A patient in his early 30s with a diagnosis of chronic prostatitis was re-evaluated because of persistence of suprapubic pain. Culture of the prostatic secretion yielded a heavy growth of \textit{H. parainfluenzae}. API NH (bioMérieux, Marcy l’Etoile, France) was used for identification. Disc diffusion and \textit{Haemophilus} test medium (HTM) broth microdilution methods were used to determine the antibiotic susceptibility of the isolate according to CLSI guidelines.\textsuperscript{7} \textit{Haemophilus influenzae} ATCC 49247 was used as a reference strain for susceptibility testing. \textit{H. parainfluenzae} strain 617 was resistant to tetracycline, clarithromycin, telithromycin and co-trimoxazole, and susceptible to \(\beta\)-lactams (ampicillin, cefuroxime and cefotaxime) (Table 1). This isolate showed high-level resistance to most of the fluoroquinolones, but not to clinafloxacin (MIC of 1 mg/L) (Table 1).

As the molecular mechanisms of fluoroquinolone resistance have not been characterized in \textit{H. parainfluenzae}, and given that mutations in the DNA gyrase and topoisomerase IV genes are the most common mechanisms of fluoroquinolone resistance in bacteria, the corresponding quinolone resistance-determining regions (QRDRs) of the gyr\(A\) and \(\text{parC}\) genes of strain 617 were analysed by PCR. The primers used were: Gyra-Hp-A (5’-TCT GAC GGT TTA CAT GCC-3’) and Gyra-Hp-B (5’-ACC ACG ACC TGG TTA GAT CAT-3’) for gyr\(A\) (expected amplicon of 563 bp); the complete genome of the fluoroquinolone-susceptible strain \textit{H. parainfluenzae} T3T1 has recently become available (http://www.sanger.ac.uk) and was used for comparison. The sequence of the quinolone-resistant isolate showed two substitutions in Gyr\(A\) (Ser84Phe and Asp88Tyr) and three in Par\(C\) (Ser84Phe, Ser138Thr and Met198Leu) compared with \textit{H. parainfluenzae} T3T1 sequences. Ser84 and Asp88 positions in Gyr\(A\) and the Ser84 position in Par\(C\) are some of the most frequent sites associated with fluoroquinolone resistance in Gram-negative bacteria. Ser84Phe and Asp88Tyr substitutions in Gyr\(A\) have also been identified previously in quinolone-resistant isolates of \textit{H. influenzae};\textsuperscript{8,9} however, to our knowledge, the Ser84Phe substitution in Par\(C\) has not been previously identified in \textit{H. influenzae}. Additionally, the QRDRs of the gyr\(B\) and par\(E\) genes were also analysed. The primers used were: Gyr\(B\)-Hp-A (5’-CCA GCA CTT TCA GAA CTT TAC-3’) and Gyr\(B\)-Hp-B (5’-CCA TCT AAC GCA AGC ATT AAT TC-3’) for gyr\(B\) (expected amplicon of 446 bp); and Par\(E\)-Hp-A (5’-TAG TGA GTG GTC CTG TAC-3’) and Par\(E\)-Hp-B (5’-AAA GAG GGC ACA TTG TAG GCT-3’) for par\(E\) (expected amplicon of 347 bp). No change was found in the QRDR of the gyr\(B\) gene, while two substitutions were found in Par\(E\) (Asp420Asn and Ala451Ser).

In order to check for additional mechanisms involved in this fluoroquinolone resistance phenotype, the presence of plasmid-mediated quinolone resistance \textit{qnrA}, \textit{qnrB}, \textit{qnrS}, \textit{qnrC}, \textit{qnrD}, \textit{qnrVC}, \textit{qepA} and \textit{aac(6’)-Ib-cr} genes was investigated, but no positive result was obtained. Considering that the overexpression of multidrug efflux systems plays an important role in fluoroquinolone resistance in Gram-negative bacteria,\textsuperscript{10} antimicrobial susceptibility testing was performed on HTM agar with or without the presence of several efflux pump inhibitors (25 \(\mu\)M carbonyl cyanide \(m\)-chlorophenylhydrazone, 20 mg/L reserpine and 20 mg/L phenylalanine arginyl \(\beta\)-naphthylamide). Addition of these inhibitors to the medium did not significantly decrease the MICs of fluoroquinolones. According to this result, no efflux pumps were implicated in quinolone resistance. The differences observed in the susceptibility between clinafloxacin and the other quinolones could be due to the higher activity of this quinolone against \textit{H. parainfluenzae}.

This is the first report of molecular characterization of an \textit{H. parainfluenzae} isolate with high-level fluoroquinolone resistance due to mutations in at least the topoisomerase gyr\(A\) and \textit{parC} genes, and possibly the par\(E\) gene. This bacterial resistance might be related to the selective pressure derived from the use of fluoroquinolones in this patient.

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This work was supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (project PI060580) and the Consejería de Innovación Ciencia y Empresa, Junta de Andalucía (P07-CTS-02908), Spain. It was partly supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III—FEDER, Spanish Network for Research in Infectious Diseases (REIPI RD06/0008).

\textbf{Transparency declarations}
None to declare.

\begin{table}[ht]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Quinolones} & \textbf{H. parainfluenzae 617} & \textbf{H. influenzae ATCC 49247} \\
\hline
naldixic acid & 256 & 1 \\
norfloxacin & 32 & 0.06 \\
ofloxicin & 64 & 0.03 \\
cipirofloxicin & 32 & 0.015 \\
levofloxicin & 32 & 0.015 \\
moxifloxicin & 64 & 0.015 \\
gemifloxicin & 16 & 0.002 \\
cilinafloxacin & 1 & 0.004 \\
\hline
\textbf{Other antimicrobial agents} & & \\
ampicillin & 0.25 & 2 \\
cefuroxime & 0.25 & 1 \\
cefotaxine & <0.015 & 0.125 \\
clarithromycin & 256 & 8 \\
tetracycline & 128 & 16 \\
co-trimoxazole & 1024 & 0.06 \\
\hline
\end{tabular}
\caption{MICs of quinolones and other antimicrobial agents for \textit{H. parainfluenzae} 617 and \textit{H. influenzae} ATCC 49247}
\end{table}
References


Keywords: K. pneumoniae, carbapenemases, ST258

Sir,

The emergence and dissemination of Enterobacteriaceae isolates producing carbapenemases in various geographical regions represent a significant threat to the management of nosocomial infections. Carbapenem-hydrolysing β-lactamases include metallo-β-lactamases, expanded-spectrum oxacillinases and Ambler class A enzymes. Among class A enzymes, the most common are KPC β-lactamases, which hydrolyse all β-lactams except cephamycins. The blaKPC–like genes have been reported most often from enterobacterial species recovered from many states in the USA. In addition, KPC-producing Klebsiella pneumoniae isolates are endemic in Greece and Israel, and have been reported from many countries worldwide, including South America, China and Western Europe. The rapid dissemination of KPC enzymes among different enterobacterial species is related to the localization of blaKPC genes on transferable broad-host-range plasmids and their association with a particular transposon, but is also linked with a disseminated international clone of KPC-producing K. pneumoniae sequence type (ST) 258. We describe here the first identification of a KPC-producing K. pneumoniae in Switzerland.

In mid-2010, a patient with methicillin-resistant Staphylococcus aureus pneumonia requiring mechanical ventilation was transferred from a hospital in Sicily to the Neuchâtel public hospital in Switzerland. During his 11 day stay in hospital in Sicily, he had been treated with ciprofloxacin, clarithromycin and teicoplanin. Upon arrival in Switzerland the patient was febrile and was treated empirically with cefepime and linezolid for 5 days. A week later, while the patient was afebrile, urine and sputum cultures grew a pan-resistant K. pneumoniae. This was considered as colonization, thus no antibiotic treatment was initiated and the patient eventually fully recovered.

The antibiogram determined by the disc diffusion method and MICs determined by Etest and the Vitek2 system (AST-EXN8 card) and interpreted according to the CLSI guidelines revealed that this K. pneumoniae strain was resistant to all penicillins and expanded-spectrum cephalosporins, imipenem (MIC, 12 mg/L), ertapenem (>32 mg/L), meropenem (32 mg/L) and doripenem (16 mg/L). This strain was of intermediate susceptibility to tigecycline (MIC of 2 mg/L), but was susceptible to gentamicin (MIC of 3 mg/L), and the MIC of colistin was >16 mg/L. A blaKPC-2 gene was identified by PCR and sequencing as previously described. The isolate possessed additional β-lactamase genes, including those encoding the narrow-spectrum β-lactamases SHV-11 (naturally occurring), TEM-1 and OXA-9.

PCR mapping and sequencing of the blaKPC flanking regions using combinations of primers showed that the blaKPC-2 gene was located inside a Tn4401 transposon identical to that found in the K. pneumoniae reference strain YC from France. Multilocus sequence typing (MLST), performed according to the protocol described on the K. pneumoniae MLST web site (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html), showed that this K. pneumoniae strain was of the ST258 type, known to be disseminated worldwide. The plasmid location of the blaKPC-2 gene was confirmed by electroporation of a plasmid from a clinical isolate to E. coli DH5α.

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Plasmid-mediated carbapenem-hydrolysing β-lactamase KPC-2 in a Klebsiella pneumoniae isolate from Switzerland

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Keywords: K. pneumoniae, carbapenemases, ST258