The production of a novel carbapenem-hydrolysing β-lactamase in A. aeromonas veronii biovar sobria, and its association with imipenem resistance


Abstract


Correspondence

Sir,

Species of the genus A. aeromonas are widely dispersed in aquatic environments, but are now increasingly implicated in clinical infections that include gastroenteritis, wound infections and bacteraemia. A. aeromonas veronii biovar sobria is a major pathogenic species of this genus and, as with most A. aeromonas species, has the ability to produce a chromosomally encoded inducible carbapenem-hydrolysing β-lactamase. This metallo-β-lactamase belongs to molecular class B; however, paradoxically, when standard in-vitro susceptibility testing is performed, these bacteria remain susceptible to the carbapenems. In this report we identify two such imipenem-resistant strains that produce a novel carbapenem-hydrolysing β-lactamase, first described in 1997.

A. veronii biovar sobria strains 13 and 99 were isolated from a reservoir in Vellore, July 1996, during a study on antibiotic resistance in environmental bacteria isolated from water sources in Southern India. Strains 13 and 99 were found to be resistant to imipenem (MIC 8 mg/L) by the agar dilution method, employing a conventional inoculum of 10^4 cfu/mL. To date, only a handful of naturally occurring carbapenem-resistant aeromonas isolates have been recorded when testing with a conventional sized inoculum. β-Lactamase extracts from strains 13 and 99 demonstrated efficient hydrolysis of imipenem when assayed spectrophotometrically. β-Lactamase could not be further induced and therefore, strains 13 and 99 constitutively produce a carbapenemase.

Isoelectric focusing (IEF) demonstrated that both strain 13 and 99 possess two β-lactamases, detectable with nitrocephin staining, with isoelectric point (pl) values of 5.84 and 8.3. A. aeromonas agar overlay modification to IEF was applied and showed the presence of a single imipenem hydrolysing enzyme of pl 5.84 in both strains 13 and 99. The carbapenemase in these two strains is unlike all other previously described aeromonas carbapenemases. Furthermore, the pl value is considerably different from the aeromonas carbapenemases that have so far been reported (typically 8.0 or greater).

IEF/inhibitor overlays facilitated further characterisation of the 5.84 and 8.3 β-lactamases. The gel was overlaid with 100 mM EDTA which is known to inhibit metallo-β-lactamases, and the serine-β-lactamase inhibitor BRL42715 (100 μM), prior to staining with nitrocephin. A TEM-1 (serine-based) β-lactamase and a cell extract from Stenotrophomonas maltophilia strain 5115 that produces two β-lactamases, (i) L1 metallo-β-lactamase (pl 6.4) and (ii) L2 serine-β-lactamase (pl 9.7), were included as controls. The pl 8.3 β-lactamase of both A. veronii biovar sobria strains were found to be serine-based because they were inhibited by BRL42715 but not EDTA; however, the pl 5.84 carbapenemase is not inhibited by either EDTA or BRL42715, and therefore, cannot be classified as either a metallo- or serine-β-lactamase (Figure). These results indicate the presence of a completely novel carbapenemase in A. veronii biovar sobria that may constitute a new β-lactamase molecular class. The hyper-production of the carbapenemase can be clearly correlated with a decrease in sensitivity, which in a clinical setting could lead to therapeutic failure. The novel carbapenem-hydrolysing β-lactamase is to be designated AVS-1.

References


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**In-vitro activity of the combination of ampicillin and arbekacin against high-level gentamicin-resistant enterococci**

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Sir,

The optimal antibiotic therapy of patients with serious enterococcal infections, particularly endocarditis, is currently a combination of a cell wall-active agent, such as ampicillin or vancomycin, and an aminoglycoside, most
commonly gentamicin. Such combinations usually produce synergic bactericidal effects, so long as a strain is not resistant to one of the constituents. E. faecalis exhibiting high-level resistance to gentamicin (MICs > 2000 mg/L), which precludes synergy, have become increasingly common. This resistance is mediated by the aac(6’)-aph(2’)* gene which encodes the bifunctional aminoglycoside-modifying enzyme A A C(6’)-A PH(2’).* Enterococci carrying aac(6’)-aph(2’)** are also resistant to other widely used aminoglycosides, including tobramycin, amikacin, netilmicin, kanamycin and, variably, streptomycin.

Arbekacin is a novel aminoglycoside derived from dibekacin (dideoxykanamycin B). As most strains of Staphylococcus aureus in Japan are susceptible to this drug, it has been used to treat patients with infections caused by gentamicin- and methicillin-resistant isolates. G entamicin resistance in staphylococci is also mediated by aac(6’)-aph(2’)† and, although arbekacin is modified by A A C(6’)-A PH(2’)*, the rate of modification is ≈ 17% of that of gentamicin. In the present study, we investigated the bactericidal effect of the combination of ampicillin and arbekacin against high-level gentamicin-resistant (HLGR) enterococci.

Thirty-eight enterococcal clinical isolates (36 strains of Enterococcus faecalis and two of Enterococcus faecium) exhibiting high-level gentamicin resistance were studied. The presence of aac(6’)-aph(2’)* in each strain was confirmed by the polymerase chain reaction according to a method described previously.6 A mpcillin was obtained from Sigma Chemical Co., St Louis, Mo, USA; gentamicin from Fluka, Milwaukee, WI, USA and arbekacin from Meiji Seika K aisha, Tokyo, Japan. MICs were determined by a microbroth dilution method recommended by the National Committee for Clinical Laboratory Standards; the medium was Mueller–Hinton broth (Difco, Detroit, MI, USA) and E. faecalis ATCC 29212 was used as a control. Synergy was evaluated by a time–kill method described previously.6 The medium was Mueller–Hinton broth and the inoculum c. 1 × 10⁵ cfu/L. The concentration of ampicillin used was equivalent to 1 × MIC for each strain and arbekacin was used at two concentrations, 8 mg/L and 16 mg/L, which were chosen on the basis that the mean serum concentration of arbekacin following a single 400 mg iv dose is 28 mg/L. Each strain was tested at least twice. Synergy was defined as a ≥ 2 log₁₀ decrease in the number of cfu/L in the presence of the combination compared with the number of cfu/L in the presence of arbekacin alone after incubation for 24 h at 37°C (the growth curve in the presence of arbekacin alone being indistinguishable from that of the antibiotic-free control).

The MICs of ampicillin for the E. faecalis strains ranged from 0.5 mg/L to 2 mg/L and for the E. faecium strains were 64 mg/L. The MICs of gentamicin for all 38 isolates were >2000 mg/L, while those of arbekacin ranged from 16 to 1024 mg/L. Sixteen (42%) of the 38 isolates exhibited synergy, eight (21%) at an arbekacin concentration of 8 mg/L and a further eight at an arbekacin concentration of 16 mg/L. For seven isolates, decreases in the numbers of cfu/L of ≥ 1 log₁₀ but < 2 log₁₀, were observed. Neither of the E. faecium strains exhibited synergy and no effect exceeding that produced by ampicillin alone was observed with six isolates for which the arbekacin MICs were >512 mg/L. Thus, 50% (16 of 32) of the isolates for which the arbekacin MICs were ≤ 256 mg/L exhibited synergy. Why this phenomenon was not observed with the other strains for which the MICs of arbekacin were ≤ 256 mg/L is not known.

The results of this study suggest that the combination of ampicillin and arbekacin would be effective therapy of some patients with serious infections caused by HLGR enterococci that remain susceptible to ampicillin. A synergy was not observed with enterococcal isolates for which the MICs of arbekacin were > 512 mg/L, synergy studies involving ampicillin and arbekacin could be limited to those strains for which the MICs of arbekacin are ≤ 256 mg/L.

References
A ntibiotic susceptibilities and plasmid profiles of Shigella flexneri isolates from children with diarrhoea in Islamabad, Pakistan


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Sir,

Shigellosis is a major problem in developing countries and is associated with high incidences of morbidity and mortality. In Pakistan, Shigella spp., particularly Shigella flexneri, are frequently isolated from patients with diarrhoeal illnesses. Effective antibiotic therapy of infections caused by these pathogens is often compromised by resistance to commonly used agents. However, there are no published data on the incidence of antimicrobial resistance amongst shigellae in Pakistan. The aim of this study, which we believe to be the first of its kind to be undertaken in this country, was to determine the susceptibilities to 11 antibiotics and the plasmid profiles of S. flexneri strains isolated from patients with diarrhoea in the Islamabad region.

Fifteen non-replicate S. flexneri strains were isolated from the stools of patients with diarrhoea at the National Institute of Health, Islamabad, Pakistan between June and August 1995 and were identified according to standard laboratory procedures. The patients, all of whom were under 3 years of age, had not received antibiotics for at least 3 months before the samples were provided. The antibiotics studied, which were obtained from Sigma Chemical Co., included amikacin, ampicillin, chloramphenicol, gentamicin, kanamycin, novobiocin, penicillin, streptomycin, spectinomycin, tetracycline and trimethoprim. Susceptibilities were determined by an agar dilution method;² the medium used was Mueller–Hinton (Oxoid) and the inoculum was 10⁵–10⁶ cfu. The MIC was taken as the lowest concentration of each antibiotic that completely inhibited growth after incubation for 18 h at 37°C. Plasmid DNA was isolated according to the method of Portnoy & White,³ separated by electrophoresis on 0.7% agarose gels in Tris–acetate buffer and visualized under UV light after staining with ethidium bromide.

The resistance phenotype of each isolate, assigned according to a system described by Koneman et al.,² is shown in the Table. The incidences of resistance to the antimicrobials tested were high. With the exception of one strain which was resistant only to trimethoprim, all of the isolates were resistant to at least five drugs. Three strains, for which the patterns of resistance were indistinguishable (A p Cm G n Nb P n Sm Sp Tc Tp; see Table for abbreviations), were resistant to nine antibiotics; the MICs of chloramphenicol, novobiocin, penicillin, spectinomycin and

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Resistance phenotype</th>
<th>Plasmids (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH-02</td>
<td>A p Cm G n P n Sm Sp Tc Tp</td>
<td>2.5, 1.9, 1</td>
</tr>
<tr>
<td>SH-15</td>
<td>Cn Sm Sp Tc Tp</td>
<td>4.4, 2.7, 2.5, 1.6</td>
</tr>
<tr>
<td>SH-28</td>
<td>A p Cm G n Nb P n Sm Sp Tc Tp</td>
<td>56, 38, 32, 23.5, 19.5, 7</td>
</tr>
<tr>
<td>SH-31</td>
<td>A p Cm G n P n Sm Sp Tc Tp</td>
<td>2.5, 1.9, 1.2, 1</td>
</tr>
<tr>
<td>SH-36</td>
<td>A p Cm G n P n Sm Sp Tc Tp</td>
<td>35, 27, 18.2, 11, 7.8, 4.4, 2.5, 1.6</td>
</tr>
<tr>
<td>SH-38</td>
<td>A p Cm G n P n Sm Sp Tc Tp</td>
<td>56, 35, 27, 18.2, 11, 7.8, 4.4, 4.2, 1.6</td>
</tr>
<tr>
<td>SH-50</td>
<td>Cm G n Sm Sp Tp</td>
<td>4, 2.5, 1.9, 1.6, 1</td>
</tr>
<tr>
<td>SH-65</td>
<td>Tp</td>
<td>ND</td>
</tr>
<tr>
<td>SH-68</td>
<td>G n Nb P n Sm Tp</td>
<td>ND</td>
</tr>
<tr>
<td>SH-76</td>
<td>A p Cm G n P n Sm Sp Tc Tp</td>
<td>2.5, 1.9, 1.2, 1</td>
</tr>
<tr>
<td>SH-77</td>
<td>Cm G n P n Sm Sp Tc Tp</td>
<td>2.5, 1.9, 1.2, 1</td>
</tr>
<tr>
<td>SH-125</td>
<td>A p Cm G n Nb P n Sm Sp Tc Tp</td>
<td>18.2, 5.9, 5.5, 3, 2.6, 1.6</td>
</tr>
<tr>
<td>SH-128</td>
<td>A p Cm P n Sm Tc Tp</td>
<td>30, 4.2, 2.5</td>
</tr>
<tr>
<td>SH-129</td>
<td>Cm G n Sm Sp Tc Tp</td>
<td>7.8, 4, 2.5, 1.9, 1.6, 1</td>
</tr>
<tr>
<td>SH-130</td>
<td>A p Cm G n Nb P n Sm Sp Tc Tp</td>
<td>25, 18.2, 5.5, 4.4, 2.6, 1.6</td>
</tr>
</tbody>
</table>

Abbreviations: A k, amikacin; A p, ampicillin; C m, chloramphenicol; G n, gentamicin; K m, kanamycin; N b, novobiocin; P n, penicillin; S m, streptomycin; S p, spectinomycin; T c, tetracycline; T p, trimethoprim; N D, none detected.
trimethoprim for these strains were the same (data not shown). A further five isolates, all exhibiting the same resistance phenotype (Ap Cm G n Pn Sm Sp Tc Tp), were resistant to eight antibiotics; the MICs of chloramphenicol, gentamicin and spectinomycin for all of these strains were the same (data not shown). One strain exhibited resistance to seven agents, two to six and three to five. All 15 isolates were resistant to trimethoprim, 14 to streptomycin, 13 to gentamicin and 12 each to chloramphenicol, spectinomycin and tetracycline. On the other hand, all of the strains were susceptible to amikacin and kanamycin.

The plasmids identified in each of the isolates are shown in the Table. Thirteen isolates harboured three or more plasmids ranging in size from c. 1 kb to 56 kb. The maximum number of plasmids in any one isolate was nine and the minimum was none (two strains). The plasmid profiles of all of the strains which harboured plasmids were distinctive, although plasmids of the same size were present in multiple strains; for example, a 2.5 kb band was identified in eight strains, a 1.6 kb band in seven and a 1.9 kb band in six. Moreover, all plasmids of the same size exhibited the same intensities following ethidium bromide staining (data not shown). However, there was no correlation between the antibiotic resistance profiles and the plasmid DNA analyses.

Notwithstanding the small number of isolates examined, the results of the present study are in accord with those of other investigators who have reported multidrug resistance amongst shigellae isolated from patients with diarrhoea.\textsuperscript{1,4,5} All or most of the strains examined were resistant to trimethoprim, streptomycin, gentamicin, ampicillin, chloramphenicol and tetracycline, all of which are used widely to treat patients with both diarrhoeal and non-diarrhoeal infections. Novel fluoroquinolones, which were not included in this study, are currently the only oral agents to which Shigella spp. isolates remain uniformly susceptible; in a recent report from Bangladesh, none of 14,915 shigellae exhibited resistance to ciprofloxacin.\textsuperscript{6} The present situation of high levels of resistance to multiple antibiotics has arisen in Pakistan principally because of the excessive and inappropriate use of these drugs. Practitioners in the community frequently prescribe multiple antibiotics as therapy of infections, including diarrhoea, in the absence of the results of laboratory investigations. Antibiotics are also administered to patients with viral infections with the aim of preventing secondary bacterial infections. Finally, these drugs are freely available from chemists without prescription and the public frequently takes advantage of this facility.

Bacterial typing systems allow investigators to demonstrate epidemiological relatedness amongst isolates, thereby confirming outbreaks of infection, and to track the spread of the strains causing the outbreaks. Several such systems, in particular various molecular techniques, of which plasmid profile analysis is one, have been developed during the past decade. Plasmid profiles have been used to identify and track the causes of outbreaks of diarrhoeal illnesses such as shigellosis.\textsuperscript{7} In the present study, plasmids of certain sizes (e.g., 2.5 kb, 1.9 kb and 1.6 kb) were detected at higher frequencies and this characteristic might allow them to be used in the investigation of outbreaks caused by Shigella spp. in this region.

References