Emergence of VIM-producing Aeromonas caviae in Israeli hospitals

Amos Adler1*, Marc V. Assous2, Svetlana Paikin3, Anastasiya Shulman1, Tamar Miller-Roll1, Sarah Hillel2, Rima Aronov3, Yehuda Carmeli1 and Mitchell J. Schwaber3

1National Center for Infection Control, Ministry of Health, Tel-Aviv, Israel; 2Microbiology and Immunology Laboratory, Shaare Zedek Medical Center, Jerusalem, Israel; 3Division of Medical Laboratories, Laniado Hospital, Netanya, Israel

*Corresponding author. Tel: +972-3-6925644; Fax: +972-3-6974332; E-mail: amosa@tasmc.health.gov.il

Received 18 October 2013; returned 15 November 2013; revised 3 December 2013; accepted 3 December 2013

Objectives: Resistance to carbapenems in Aeromonas species is rare and mediated mostly by the chromosomal cphA gene. Our aims were to describe the molecular characteristics of the first cases of VIM-producing Aeromonas caviae isolated from human samples.

Methods: Carbapenem-resistant Aeromonas (CRA) spp. were isolated from rectal surveillance cultures. Bacterial identification was done by dnaJ sequencing. Detection of metallo-carbapenemase and other β-lactamase genes was done by PCR. Molecular typing was done by PFGE. The genetic environment of the blaVIM gene was determined by sequencing.

Results: Five CRA were isolated from surveillance cultures in 2010–13; four were from Shaare Zedek Medical Center and one was from Laniado Hospital. All five isolates were identified as A. caviae and comprised four different pulstotypes. MICs ranged from 0.5 to 8 mg/L for imipenem and from 0.25 to 8 mg/L for meropenem. All isolates were resistant to gentamicin, susceptible to amikacin and ciprofloxacin (except one), and were positive for carbapenemase production in the modified Hodge and Carba NP tests. The carbapenemase genes blaVIM-1 and blaVIM-35 were located inside a class I integron with two different sizes to its variable region.

Conclusions: This is the first report of blaVIM in A. caviae from human samples and the first report of VIM-producing Gram-negative bacteria in Israel. This finding is alarming as this species may spread via water or sewage systems. Although infection due to Aeromonas spp. is rare, the presence of the gene on a mobile element is of concern due to the potential for dissemination to clinically important Gram-negative pathogens.

Keywords: Aeromonas spp., VIM carbapenemases, integrins

Introduction

Aeromonads are ubiquitous in the microbial biosphere and can be isolated from every environmental niche, including aquatic habitats, food, vertebrate and invertebrate species and natural soils. Consequently, humans may be constantly exposed to aeromonads and may acquire them from a variety of sources. Although the genus Aeromonas includes >20 different species, 3 species (Aeromonas hydrophila, Aeromonas caviae and Aeromonas veronii bv. sobria) account for the vast majority of human infections and clinical isolates. Aeromonads may cause a variety of clinical infections in both immunocompetent and immunocompromised individuals, typically including gastroenteritis, soft tissue infection and bacteremia. Aeromonas spp. may produce different types of broad-spectrum β-lactamases, including AmpC and extended-spectrum β-lactamase (ESBL)-type enzymes. In addition, Aeromonas spp. may harbour a unique inducible metallo-β-lactamase (MBL) gene, designated cphA, that can be found in the majority of A. hydrophila isolates. Although commonly referred to as a carbapenemase, actual in vitro resistance to carbapenems is seen in only a minority of cphA-induced isolates. As blaCphA is located on the chromosome and is found in this genus alone, its clinical significance is probably limited, as is concern for its likely dissemination. In contrast, the MBL VIM confers resistance to all β-lactam antimicrobials (with the exception of aztreonam) and has been identified across the world in a wide variety of species, thereby representing one of the greatest problems in the realm of antimicrobial resistance. To date, only a single case of a VIM-producing aeromonad, A. hydrophila, has been reported. In this study, we report the first cluster of VIM-producing A. caviae, identified in five patients in two medical centres in Israel, and describe their microbiological and molecular characteristics.

Methods

Set-up and microbiological methods

Laniado Hospital (LH) is a 300 bed hospital in the city of Netanya, covering a population of ~200000 people. The Shaare Zedek Medical Center (SZMC) is a 550 bed hospital, the second largest in the city of Jerusalem, covering...
a population of ~400,000 people. Carbapenem-resistant bacteria are routinely sent from these hospitals to the National Center for Infection Control laboratory for molecular characterization. Surveillance rectal cultures were inoculated in both centres onto CHROMagar KPC medium (HyLabs, Rehovot, Israel) and pigmented colonies were further tested for species identification and antimicrobial susceptibility testing (LH, Vitek 2 system; SZMC, manual biochemical tests and disc diffusion). Phenotypic characterization included the modified Hodge test (MHT), ertapenem–EDTA synergy and the Carba NP imipenem hydrolysis assay.8 Identification and antimicrobial susceptibility testing (LH, Vitek 2 system; SZMC, manual biochemical tests and disc diffusion). Phenotypic characterization included the modified Hodge test (MHT), ertapenem–EDTA synergy and the Carba NP imipenem hydrolysis assay.8

**Table 1.** Microbiological and molecular features of VIM-producing *A. caviae* isolates in Israel

<table>
<thead>
<tr>
<th>Isolate</th>
<th>8</th>
<th>669</th>
<th>1045</th>
<th>1254</th>
<th>81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical centre</td>
<td>SZMC</td>
<td>LH</td>
<td>SZMC</td>
<td>SZMC</td>
<td>SZMC</td>
</tr>
<tr>
<td>Isolation date (mm/yy)</td>
<td>04/10</td>
<td>01/12</td>
<td>08/12</td>
<td>12/12</td>
<td>01/13</td>
</tr>
<tr>
<td>Ertapenem MIC(^a) (mg/L)</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Imipenem MIC(^a) (mg/L)</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Meropenem MIC(^a) (mg/L)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>Gentamicin(^b)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Amikacin(^b)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin(^b)</td>
<td>I</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole(^b)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>bla(_{VIM}) allele</td>
<td>35</td>
<td>1</td>
<td>1</td>
<td>35</td>
<td>1</td>
</tr>
<tr>
<td>Integron size (bp)(^c)</td>
<td>4650</td>
<td>4650</td>
<td>3300</td>
<td>3300</td>
<td>3300</td>
</tr>
</tbody>
</table>

S, susceptible; I, intermediate; R, resistant.

\(^a\)MICs were determined by agar dilution.

\(^b\)Antimicrobial susceptibility testing was performed using the Vitek 2 system.

\(^c\)Size of the variable region of the class I integron.

Results and discussion

Carbapenem-resistant *Aeromonas* (CRA) spp. isolates were detected in rectal surveillance cultures as hazy blue colonies on CHROMagar KPC medium. The isolation date and institution for each isolate are listed in Table 1. Contact precautions were implemented for all CRA carriers. Clinical infections due to CRA were not identified in any of these patients or in any other patient in these institutions. Isolates were first identified as *Aeromonas* spp. at the local laboratories and were later identified as *A. caviae* by dnapl sequencing.13 Carbapenem MICs ranged from 0.5–8 mg/L for ertapenem and imipenem and 0.25–8 mg/L for meropenem (Table 1). All isolates were resistant to the β-lactams ceftriaxone, ceftazidime and piperacillin/tazobactam, as well as to gentamicin and trimethoprim/sulfamethoxazole; all were susceptible to amikacin and ciprofloxacin (except one). All were positive by the MHT and the Carba NP test and all but one (isolate 669) showed synergy between ertapenem and EDTA.

All isolates yielded positive results on PCR testing for bla\(_{VIM}\) carrying either bla\(_{VIM-1}\) or a single nucleotide variant, bla\(_{VIM-35}\). PCR testing for other metallo-carbapenemase genes, including bla\(_{NDM}\), bla\(_{IMP}\) and bla\(_{SHV}\), were negative. All isolates produced bla\(_{TEM-1}\) and tested negative for the presence of ESBL genes. Comparison of the SpeI macrorestriction patterns revealed a heterogeneous population, with only two isolates showing similarity (81 and 1254, data not shown). The bla\(_{VIM}\) gene was located inside a class 1 integron, with two different sizes to its variable region (Table 1 and Figure 1). The 4650 bp integron included an IS\(_{Pa21}\) type transposase gene and was almost identical to a bla\(_{VIM}\)-harbouring integron that was identified in *Enterobacter cloacae* isolated in the United Arab Emirates.17 This integron was also identical to the 3300 bp integron except for a missing 1350 bp fragment in the latter. The bla\(_{VIM}\) gene was not transferable by electroporation using a DH10B E. coli recipient strain. In one isolate (1045), a few bla\(_{VIM}\) PCR-positive transconjugant colonies were identified. However, Southern blotting of the I-CEul-digested genome showed a chromosomal location of the gene (data not shown) and the transconjugant strains were completely susceptible to carbapenem. Also, no bla\(_{VIM}\)-harbouring plasmid was extracted from the recipient strain, despite multiple attempts.
This study represents the first reported cluster of VIM-producing \textit{Aeromonas caviae}, with five cases detected in two medical centres, and the first report of VIM-producing bacteria in Israel. Two of the isolates (8 and 669) were fully susceptible to carbapenems and only one (isolate 81) was resistant to imipenem and meropenem. Although these findings differ from the previous report of VIM in \textit{Aeromonas}, which involved \textit{A. hydrophila}, relatively low MIC values have also been reported in VIM-producing \textit{Klebsiella pneumoniae} isolates. Similar to previous reports of MBL-producing \textit{VIM-producing Aeromonas} spp., these isolates tested positive by the MHT and the ertapenem–EDTA synergy test (except for isolate 669).

As previously described in both \textit{Aeromonas} and other Gram-negative organisms, the \textit{bla}_{VIM} gene was located inside two variants of a class I integron. Both types also harboured additional antimicrobial resistance (AMR) genes and a transposable gene indicative of its plasticity as well as its transmissibility potential. Only two isolates had similar PFGE patterns, but they differed in their \textit{bla}_{VIM} allele. This heterogeneity in molecular features indicates that both clonal spread and horizontal gene transfer play a role in the dissemination of this resistance mechanism. The connection between these patient carriers remains elusive, probably since these findings were incidental and hence targeted screening for other carriers was not performed.

All of the isolates in this study were recovered from rectal culture only, as an incidental finding of the carbapenem-resistant Enterobacteriaceae surveillance programmes implemented in these centres in the context of a national infection control intervention. Although foodborne outbreaks of \textit{Aeromonas} gastroenteritis have been reported, there are almost no data regarding the transmission of this organism in the hospital environment. In 1987, Sherlock et al. reported a high proportion (8%) of intestinal colonization by \textit{A. hydrophila} in patients with haematological malignancies. Although molecular typing was not done in this study, it highlights the possibility of nosocomial transmission of \textit{Aeromonas} spp. by colonized patients, similar to patterns seen with other enteric organisms, such as enterococci and Enterobacteriaceae. Also, \textit{Aeromonas} spp. can potentially spread via the water or sewage systems within the hospital or out to the community. Although clinical infections due to \textit{Aeromonas} spp. are relatively uncommon, the dissemination of VIM-producing \textit{Aeromonas} spp. is worrisome due to the potential for horizontal transmission of this gene to more clinically important pathogens, e.g. \textit{K. pneumoniae}. As with other AMR Gram-negative organisms, strict adherence to infection control practices and meticulous environmental cleaning are likely to have some benefit in controlling spread. Whether these measures will suffice or additional, organism-specific measures will be required has yet to be determined.

### Funding

This work was supported in part by European Commission FP7: SATURN—Impact of Specific Antibiotic Therapies on the Prevalence of Human Host Resistant Bacteria research grant 241796.

### Transparency declarations

None to declare.

### References