In vitro antibacterial activity of the peptide PsVP-10 against antimicrobial-resistant Enterococcus faecalis isolated from clinical samples

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Objective: To analyse the antimicrobial activity of the peptide PsVP-10 against 67 resistant Enterococcus faecalis strains isolated from clinical samples.

Methods: The qualitative disc diffusion method and MIC determinations were used.

Results: The presence of several multidrug-resistant phenotypes of E. faecalis was demonstrated, in which there were high MICs to chloramphenicol, tetracycline, vancomycin, cefaloridine, ampicillin and gentamicin. In comparison, the peptide PsVP-10 showed lower MICs against all the multidrug-resistant and susceptible E. faecalis.

Conclusions: There is an urgent need for the development of novel antimicrobial agents against the highly resistant E. faecalis. The present study shows that the peptide PsVP-10 might make a contribution to the solution of this serious problem.

Keywords: antibiotics, multiresistance and human infections

Introduction

Enterococcus faecalis is the most common species of the genus Enterococcus in human infections. E. faecalis causes 80%–90% of human enterococcal infections, and E. faecium accounts for most of the remainder. In humans, enterococci are frequently associated with intra-abdominal and pelvic infections, but other microorganisms are often present. Usually, only in endocarditis and urinary infections are they the sole microorganism involved.1

In the 1970s and 1980s, enterococci became clearly established as major nosocomial pathogens. They are now the fourth leading cause of hospital-acquired infection and the third leading cause of bacteremia in the USA.2

The intrinsic resistance of enterococci to many commonly used antimicrobial agents may have given them an advantage for the further acquisition of genes encoding high levels of resistance to aminoglycosides, penicillins, tetracycline, chloramphenicol and vancomycin.3 The mechanisms of this resistance are mediated by mobile resistance genes on plasmids and transposons, which have the capacity to transfer resistance.4 Given this scenario, it is important to assess other possibilities for the control and treatment of E. faecalis infections.

During the last few years, we have investigated bacteriocins produced by bacteria isolated from the sediment of well water.5 In these studies, one Pseudomonas sp. strain was selected that is capable of inhibiting several Gram-positive and -negative bacterial strains. This microorganism produces a peptide called PsVP-10, of 2.4 kDa, which is very heat stable, resistant to proteolytic enzymes and tolerant to pH.

The principal aim of this study was to determine the activity of peptide PsVP-10 against several antimicrobial-resistant E. faecalis strains isolated from different clinical origins.

Material and methods

The E. faecalis strains were isolated from clinical samples taken from patients of different sexes and ages in hospitals in Talca, Linares and Chillán in central Chile. The samples were isolated from urinary tract infections (55 isolates), the bloodstream (19 isolates) and soft tissues (11 isolates). All of the 85 strains were identified according to Facklam & Collins.6 Pseudomonas sp. R-10 was used for the production of PsVP-10 bacteriocin and the peptide was purified according to Hubert et al.7 as a pure powder.

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E. faecalis susceptible to the peptide PsVP-10

Table 1. MICs of chloramphenicol, tetracycline, gentamicin, ampicillin, vancomycin, cefaloridine and the peptide PsVP-10 against different resistance phenotypes of E. faecalis

<table>
<thead>
<tr>
<th>Resistance phenotype</th>
<th>Number of strains</th>
<th>Median MIC (range) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ch</td>
<td>T</td>
</tr>
<tr>
<td>ChTG</td>
<td>12</td>
<td>64(16–128)</td>
</tr>
<tr>
<td>ATG</td>
<td>7</td>
<td>32(16–64)</td>
</tr>
<tr>
<td>ChAT</td>
<td>5</td>
<td>64(32–128)</td>
</tr>
<tr>
<td>AVCh</td>
<td>5</td>
<td>16(8–128)</td>
</tr>
<tr>
<td>AV</td>
<td>4</td>
<td>16(8–128)</td>
</tr>
<tr>
<td>TCh</td>
<td>5</td>
<td>64(32–128)</td>
</tr>
<tr>
<td>A</td>
<td>8</td>
<td>8(8–64)</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>64(32–128)</td>
</tr>
</tbody>
</table>

Ch, chloramphenicol; T, tetracycline; G, gentamicin; A, ampicillin; V, vancomycin; C, cefaloridine and peptide PsVP-10.

The following antimicrobial compounds were used in the study: ampicillin, chloramphenicol, gentamicin, tetracycline, chloramphenicol and vancomycin. The susceptibility tests for the E. faecalis strains were carried out by the qualitative diffusion method described by Bauer et al. The antimicrobial compounds were prepared according to NCCLS guidelines or the manufacturer’s recommendations. All the MICs were performed in duplicate following NCCLS guidelines, using the broth microdilution method.

Results and discussion

Of the 85 E. faecalis strains, 67 (78.8%) were resistant to one or more antimicrobials (see Table 1). Only 18 strains (21.1%) were susceptible to all the antimicrobials studied. The antimicrobial-resistant E. faecalis strains were isolated from three different types of clinical samples (urinary tract infection, the bloodstream and soft tissues) and no difference in resistance was observed between them. Table 1 shows the various resistance phenotypes. Fourteen of the strains studied were resistant to three antibacterial drugs and two were resistant to two. It is possible that the resistance was encoded on a plasmid, as has been found previously in E. faecalis. Table 1 shows that chloramphenicol is the drug with the lowest antibacterial activity, whereas vancomycin is the most active compound. From the results it is important to note that in this area of Chile the proportion of vancomycin-resistant E. faecalis is currently low.

In recent years, E. faecalis has been identified as an agent of nosocomial infection with increasing frequency, paralleling the increase of antimicrobial resistance to most currently approved agents. Therefore, it is very important to assess novel possibilities for the future treatment of infections caused by E. faecalis. It was demonstrated previously that the peptide PsVP-10 displays interesting antimicrobial activity against Gram-positive and -negative bacteria. The results obtained in the present study show, in their own right, independent of susceptibility to other antimicrobials, noteworthy results. Table 1 reveals that a PsVP-10 MIC of 0.06 mg/L is the average in most of the resistant phenotypes (AVCh, AV, A, G and V). Also, in this context it is important to emphasize that when the vancomycin-resistant phenotype was present the MIC was always 0.06 mg/L. Thus, these results indicate possible future treatment for vancomycin-resistant E. faecalis. The occurrence of resistant E. faecalis strains has caused alarm in the global healthcare community because it reduces drastically the options for disease treatment and because of the subsequent rapid expansion of resistant populations. The results show that all susceptible E. faecalis strains are susceptible to peptide PsVP-10, and demonstrate an average MIC of 0.03 mg/L.

In conclusion, the peptide PsVP-10 has been shown here to have good in vitro activity against all the sensitive and resistant E. faecalis isolates examined, and so presents an interesting possibility for the future. However, the peptide needs investigating further, and more in vitro and in vivo experiments are necessary to provide detailed information on the potential of this peptide as an antimicrobial drug.

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References

