Nitroimidazole resistance genes (nimB) in anaerobic Gram-positive cocci (previously Peptostreptococcus spp.)

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Objectives: To investigate metronidazole resistance and the prevalence of nitroimidazole (nim) genes in clinically isolated anaerobic Gram-positive cocci.

Methods: Metronidazole susceptibility was determined in 99 strains of anaerobic Gram-positive cocci and PCR amplification for the nim gene carried out on 61 strains (metronidazole MIC ≥0.5 mg/L).

Results: The nimB gene was detected in 34% (21/61) of the strains. These included two highly resistant Finegoldia magna strains (MICs >128 mg/L). The nimB gene was, however, also demonstrated in 90% (19/21) of susceptible strains.

Conclusions: Although the nimB gene may be implicated in the high-level metronidazole resistance in 2 F. magna strains, the alarmingly high prevalence of the nimB gene in anaerobic Gram-positive cocci cannot be directly associated with resistance and the possibility of a silent nimB gene should be considered.

Keywords: anaerobic Gram-positive cocci, metronidazole resistance, nim genes

Introduction

Anaerobic Gram-positive cocci form part of the endogenous human flora and can cause a wide range of infections ranging from female genital tract infections to brain abscesses. Metronidazole, a 5-nitroimidazole, has been one of the preferred antimicrobials for serious anaerobic infections, often used empirically, but anaerobes can no longer be considered universally susceptible as resistance has been noted in strains of Bacteroides fragilis, Clostridium spp. and Peptostreptococcus spp. Resistance mechanisms have evaded conclusive identification, but reports increasingly implicate the nitroimidazole (nim) genes that encode a 5-nitroimidazole reductase to be a possible cause of resistance in Bacteroides. The presence of nim genes in clinical isolates of B. fragilis, Prevotella bivia, Clostridium bifermentans, Actinomyces odontolyticus and Propionibacterium spp. found in our laboratory, and reports of metronidazole resistance in Peptostreptococcus spp. in South Africa prompted the investigation of these anaerobic Gram-positive cocci (previously all classified as Peptostreptococcus spp.) for the prevalence of metronidazole resistance genes.

Materials and methods

Bacterial isolates

Ninety-nine anaerobic Gram-positive cocci were isolated during 1996–1997 (as Peptostreptococcus spp.) from clinically significant infections in patients in the Universitas and Pelonomi Hospitals in Bloemfontein.

MIC determination

MICs of metronidazole (supplied by Sigma, St Louis, MO, USA) were determined by the National Committee for Clinical Laboratory Standards (NCCLS) agar dilution methods. The susceptibility breakpoint for metronidazole (≤16 mg/L) was used as suggested by the NCCLS. Wilkins Chalgren agar (Mast Diagnostics, Merseyside, UK) was supplemented with 5% lysed horse blood to enhance growth of these fastidious bacteria. Sixty-one isolates comprising 35 Peptostreptococcus anaerobius, nine Finegoldia magna, eight Anaerococcus prevotii, four Peptoniphilus asaccharolyticus, two Peptoniphilus indolicus and three Micromonas strains with metronidazole MICs ≥0.5 mg/L were selected for PCR amplification.

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nimB gene PCR and sequencing

Cell lysates were obtained from overnight cultures, as described by Lubbe et al. Lysis was enhanced by the addition of 10 mg/L mutanolysin to the cell suspension. PCR assays were carried out using the primers Nim-3 and Nim-5 as described by Trinh & Reyssset. The primers were designed for the universal amplification of all known nim genes to produce a PCR product of 458 bp. Amplification conditions were as previously described by Lubbe et al., using an annealing temperature of 52°C. PCR products from Bacteroides vulgatus (BV-17) and B. fragilis (BF-8) control strains were used as references.

Fragments of approximately 458 bp in any of the isolates were regarded as presumptive positives and excised from a 1% agarose gel, using a GFX PCR DNA and Gel Band Purification kit (Amersham Pharmacia Biotech, NJ, USA). Automated sequencing of PCR products was carried out using a BigDye Terminator sequencing kit (Applied Biosystems, CA, USA). Reaction mixtures for sequencing were analysed on a Sequence Navigator gel and computer analysis was carried out with ABI PRISM, Model 377 (Perkin-Elmer). Sequences were electronically submitted for identification into the GenBank nucleotide database via the website of the National Center for Biotechnology Information (NCBI).

Results and discussion

Nucleotide sequencing confirmed the nimB gene to be present in 21/61 (34%) of the strains with metronidazole MICs >≥0.5 mg/L. These strains included 11 P. anaerobius, five F. magna, three A. prevotii, one M. micros, and one P. asaccharolyticus (Table 1). The nimB gene was found in two highly resistant F. magna strains (MIC >128 mg/L), but not in a P. anaerobius strain with reduced metronidazole susceptibility (MIC 8 mg/L). All 19 of the other strain with reduced metronidazole susceptibility (MIC 8 mg/L).

Should the nimB gene be confirmed to be implicated in the development of such high-level resistance, the problem could be more far reaching than was previously thought. Proportionately, the nimB genes were also more prevalent in the F. magna strains (56%) than in the other strains investigated (Table 1).

The role of the nimB gene in metronidazole resistance development in these anaerobic Gram-positive cocci is not immediately evident as the nimB gene was surprisingly found in 33% (19/58) of the metronidazole-susceptible strains, yet not in the one P. anaerobius strain with reduced susceptibility to metronidazole (MIC 8 mg/L). According to Haggoud et al., the positioning of nim genes relative to insertion sequence elements, with either strong or weak promoters, is crucial when assessing the input nim genes may have on phenotypic levels of metronidazole resistance, as every nim gene has been shown to be associated with an IS element. However, both nimA and nimB are directly preceded by the insertion sequence IS1168 and the electronic gene identification results also indicated the nimB gene being associated and preceded by the insertion sequence IS1168, in the resistant as well as the susceptible Gram-positive cocci.

In a recent study in our laboratory, nim genes were demonstrated in a wide range of clinically important anaerobic bacteria, such as B. fragilis group (nimA and nimB), P. bivia (nimA), C. bifermentans (nimA), A. odontolyticus (nimA) and Propionibacterium spp. (nimA) although worldwide they have only been described in Bacteroides spp. This is the first report of the nim gene being found in any of the anaerobic Gram-positive cocci, that have all previously been classified under the genus Peptostreptococcus, and they can now be added to the South African list. Of interest is the fact that they appear to be a very important source of the nimB gene in particular. The detection of nim genes in numerous anaerobic bacterial species calls for periodic metronidazole susceptibility testing in conjunction with monitoring for the prevalence of different nim gene types.

Table 1. Details of the Gram-positive anaerobic cocci screened for the presence of nim genes

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of strains</th>
<th>No. of strains possessing a nimB gene</th>
<th>MICs (mg/L)</th>
<th>nimB identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. anaerobius</td>
<td>35</td>
<td>11</td>
<td>0.5–1</td>
<td>82–98</td>
</tr>
<tr>
<td>F. magna (resistant)</td>
<td>2</td>
<td>2</td>
<td>&gt;128</td>
<td>96–98</td>
</tr>
<tr>
<td>F. magna (susceptible)</td>
<td>7</td>
<td>3</td>
<td>0.5</td>
<td>86–95</td>
</tr>
<tr>
<td>A. prevotii</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>90–98</td>
</tr>
<tr>
<td>Pn. asaccharolyticus</td>
<td>4</td>
<td>1</td>
<td>0.5</td>
<td>84</td>
</tr>
<tr>
<td>M. micros</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>92</td>
</tr>
<tr>
<td>Pn. indolicus</td>
<td>2</td>
<td>0</td>
<td>0.5</td>
<td>–</td>
</tr>
</tbody>
</table>

References


