Short communication

Antibiotic susceptibility profiles of oral pathogens

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ABSTRACT

Periodontitis is a bacterial disease that can be treated with systemic antibiotics. The aim of this study was to establish the antibiotic susceptibility profiles of five periodontal pathogens to six commonly used antibiotics in periodontics. A total of 247 periodontal bacterial isolates were tested for susceptibility to the six antibiotics using the Etest method. MIC 50 and MIC 90 values (minimum inhibitory concentrations for 50% and 90% of the organisms, respectively) were calculated. Both European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints were used in the study to interpret results. β-Lactamase production was tested when amoxicillin resistance was found. MIC 90 values of the anaerobic bacteria were all well below breakpoint values, except for three isolates of Prevotella intermedia and one isolate of Fusobacterium nucleatum that were resistant to amoxicillin (CLSI breakpoints); these isolates were β-lactamase-positive. Two isolates of the capnophilic Aggregatibacter actinomycetemcomitans appeared to be amoxicillin-resistant but failed to show β-lactamase activity. Comparison with a previous study from The Netherlands showed minor differences in susceptibility profiles, but the MIC 90 values of A. actinomycetemcomitans for amoxicillin, clindamycin, azithromycin and tetracycline were higher. Geographical differences in the susceptibility profiles of Porphyromonas gingivalis and A. actinomycetemcomitans between European countries were noted. Comparison of European susceptibility profiles with that of a South American country (Colombia) revealed a much higher resistance in the latter. Owing to these differences in susceptibility profiles, it is of concern to regularly perform surveillance studies on antibiotic resistance.

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1. Introduction

Periodontitis is an inflammatory destructive disorder of the tissues that support the teeth. Loss of alveolar bone is a major characteristic of the disease. Periodontitis is caused by a subgingival biofilm consisting of anaerobic and facultative anaerobic bacteria [1]. The clinically most important cultivable periodontal bacterial species occurring at sites of periodontal disease activity are Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia (including the closely related Prevotella nigrescens), Fusobacterium nucleatum and Parvimonas micra [2]. Treatment of periodontitis involves reduction of the total periodontal bacterial load by supragingival and subgingival mechanical debridement. However, bacterial deposits in the depth of the pockets are often difficult to remove and may be responsible for a poor treatment outcome. Therefore, antibiotic treatment can be indicated for certain patient groups [3]. Several systemic antimicrobial interventions as an adjunct to scaling and root planing have proven effective, such as metronidazole and the combination of metronidazole and amoxicillin [4]. To provide the patient with an appropriate antibiotic therapy, it is critical to know the susceptibility profiles of clinically relevant oral pathogens. Knowledge of antibiotic susceptibility profiles of oral pathogens is, however, limited. Moreover, study outcomes on susceptibility profiles of oral pathogens vary considerably between countries, and significant variation in resistance to antibiotics has been shown [5]. It has been suggested that the antibiotic resistance of oral pathogens is increasing, especially in bacterial species isolated from patients with recurrent periodontitis [6].

Oral pathogens can be disseminated from the oral cavity to other body sites, causing distant (dental focal) infections [7] such as brain [8] and lung abscesses [9]. Treatment of these infections also requires knowledge of the antibiotic susceptibility profiles of oral anaerobes.

This study reports on the antibiotic susceptibility of selected periodontal bacterial species in The Netherlands to six commonly used antibiotics in periodontics, namely amoxicillin,
amoxicillin/clavulanic acid (AMC), clindamycin, azithromycin, metronidazole and tetracycline.

2. Materials and methods

2.1. Bacterial strains

The oral species *P. intermedia*, *F. nucleatum*, *P. micra*, *P. gingivalis* and *A. actinomycetemcomitans* were isolated from subgingival plaque samples from consecutive patients suffering from chronic or aggressive periodontitis. From each patient, one isolate of each species was used for analyses when detected. For each species, 50 isolates from 50 patients were tested, except for *A. actinomycetemcomitans* (*n* = 47). Samples were grown on 5% horse blood agar plates (no. 2; Oxoid Ltd., Basingstoke, UK) supplemented with 5 mg/L haemin and 1 mg/L menadione (BA plates) and incubated in 80% N₂, 10% H₂ and 10% CO₂ for 7 days at 37 °C. Identification of isolates was based on their colony morphology using a ring-light-equipped stereomicroscope, Gram staining and biochemical characteristics using Rapid ID 32A (bioMérieux, Marcy l’Étoile, France) [5,10]. *Aggregatibacter actinomycetemcomitans* was isolated from tryptic soy–serum–bacitracin–vancomycin agar incubated in air + 5% CO₂ at 35 °C for 5 days and was identified by typical colony morphology and catalase production upon exposure to 3% hydrogen peroxide [11].

2.2. Susceptibility testing

Antibiotic susceptibility of the test bacteria to amoxicillin, AMC, clindamycin, azithromycin, metronidazole and tetracycline was determined by Etest (AB BIODISK, Solna, Sweden) [12]. For anaerobic bacteria, a suspension from 48-h bacterial cultures of ca. 2 McFarland was prepared in pre-reduced Brucella broth and was applied to a pre-reduced BA plate. Minimum inhibitory concentrations (MICs) were determined after 48 h of incubation at 37 °C in an anaerobic atmosphere. If necessary, the incubation time was prolonged to 72 h, except for clindamycin. The quality control strain *Bacteroides fragilis* ATCC 25285 was included for AMC, clindamycin, metronidazole and tetracycline and *Streptococcus pneumoniae* ATCC 49619 for amoxicillin and azithromycin. For antibiotic susceptibility testing of *A. actinomycetemcomitans*, a suspension of 2 McFarland was prepared and applied to a BA plate. MIC₅₀ and MIC₉₀ values (MICs for 50% and 90% of the isolates, respectively) were determined after 72 h of incubation at 37 °C in air +5% CO₂.

Percentages of resistant isolates were calculated using breakpoints advised by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). It should be noted that breakpoints were not available for several species. In these cases, breakpoint values from closely related species were used (Table 1).

2.3. β-Lactamase testing

Isolates of *P. intermedia* and *F. nucleatum* showing a MIC > 2 mg/L towards amoxicillin were subjected to β-lactamase testing using DrySlide™ Nitrocefin test/cefinase disks™ (BBL Microbiology systems, Cockeysville, MD) according to the manufacturer’s recommendations.

3. Results

3.1. Quality control

MICs for the quality control strains *B. fragilis* ATCC 25285 and *S. pneumoniae* ATCC 49619 determined for amoxicillin, AMC, clindamycin, azithromycin, metronidazole and tetracycline were within the expected range.

3.2. Susceptibility

The MIC range and MIC₅₀ and MIC₉₀ values of the five tested bacterial species towards six antibiotics are summarised in Table 1. The MIC₉₀ for amoxicillin for all species was <1 mg/L. Three *P. intermedia* isolates had high MIC values of 2, 3 and 48 mg/L. One isolate of *F. nucleatum* had a high MIC of 4 mg/L. These isolates were all susceptible to AMC.

All anaerobic isolates had low MIC₉₀ values towards clindamycin. Two isolates of *P. intermedia* showed a MIC of ≥256 mg/L. One isolate of *P. micra* also showed clindamycin resistance (MIC of 12 mg/L). The MIC₅₀ and MIC₉₀ of *A. actinomycetemcomitans* for clindamycin were 4 mg/L and 12 mg/L, respectively, showing very low sensitivity towards this antibiotic.

Among the anaerobic isolates, *F. nucleatum* and *P. micra* showed the highest MIC₉₀ values for azithromycin. Two isolates of *P. intermedia* and one isolate of *P. micra* showed a MIC of ≥256 mg/L.

All anaerobic species showed high susceptibility towards metronidazole, with MIC₅₀ values ranging from <0.016 mg/L to 0.25 mg/L. In contrast, the MIC₅₀ and MIC₉₀ values of *A. actinomycetemcomitans* for metronidazole were 32 mg/L and 96 mg/L, respectively. The highest MIC₉₀ for tetracycline was found in *P. intermedia* (4 mg/L); four isolates showed a MIC of 6 mg/L.

*Porphyromonas gingivalis* showed the lowest MIC₅₀ and MIC₉₀ values for all six tested antibiotics. *Parvimonas micra* and *P. gingivalis* had the lowest MIC₅₀ and MIC₉₀ values for amoxicillin and AMC.

3.3. Resistant strains

The percentage of resistant isolates of each test species is shown in Table 1. Information on breakpoint concentrations is not available for several species, e.g. breakpoint concentrations (CLSI and EUCAST) for azithromycin for anaerobes, metronidazole and clindamycin for *A. actinomycetemcomitans*, and EUCAST breakpoints for tetracycline for anaerobes. Resistance towards amoxicillin was found in 4–6% of the *P. intermedia* and 2–6% of the *F. nucleatum* isolates. None of these isolates were resistant to AMC. Two isolates of *A. actinomycetemcomitans* (4.3%) showed amoxicillin resistance according to EUCAST criteria but not according to CLSI criteria. One of these isolates was also resistant towards AMC based on EUCAST criteria. Intermediate sensitivity towards tetracycline was found in 8% of *P. intermedia* (CLSI) and 14.9% of *A. actinomycetemcomitans* isolates (EUCAST).

3.4. β-Lactamase production

Three *P. intermedia* isolates were β-lactamase-positive. According to the CLSI breakpoints, these three isolates were resistant to amoxicillin, whereas two isolates were resistant according to the EUCAST breakpoints. One *F. nucleatum* isolate produced β-lactamase and was resistant to amoxicillin according both to the CLSI and EUCAST breakpoints. Susceptible isolates were all non-producers.
4. Discussion

Monitoring antimicrobial susceptibility of bacterial pathogens is necessary for infectious diseases that are treated with antibiotics. Establishing MICs on a regular basis reveals changes in susceptibility profiles and the emergence of antimicrobial resistance and this information is essential for the rational use of antimicrobial chemotherapeutics. Severe and refractory periodontitis is treated worldwide with systemic antimicrobial therapy as an adjunct to mechanical debridement and periodontal surgery. The present study was undertaken to establish the antibiotic susceptibility of a number of periodontal species associated with destructive periodontal disease [4].

Different techniques have been used to establish the antimicrobial susceptibility of periodontal pathogens, complicating the comparison of study results [5,6]. Also, different breakpoints for the various antibiotics are used in literature, i.e. CLSI and EUCAST, which also influences interpretation of the percentage of resistant/susceptible isolates. Therefore, both CLSI and EUCAST breakpoints are provided in Table 1 for comparison. Furthermore, we have primarily focused on studies that have used the same testing method, i.e. Etest, in order to compare antibiotic susceptibility based on MIC50 and MIC90 values.

Since antibiotic susceptibility data of periodontal pathogens were also published in a previous study in The Netherlands [5], we were able to study possible changes in antimicrobial resistance profiles among oral pathogens. The prevalence of amoxicillin resistance, based on EUCAST criteria, in the present study was 2% (5/247). Three of these resistant isolates appeared to be β-lactamase-positive [two P. intermedia isolates (4%) and one F. nucleatum isolate (2%)]. The prevalence is low in comparison with a study by van Winkelhoff et al. [13] who found 26% of P. intermedia isolates and 13% of F. nucleatum isolates to produce β-lactamase. However, β-lactamase production in the two amoxicillin-resistant A. actinomycetemcomitans isolates could not be detected.

In comparison with the 2005 study in The Netherlands [5], only minor differences were noted in MIC50 and MIC90 values and there were only a few indications for changes in susceptibility profiles (Table 1). P. gingivalis, a major periodontal pathogen, continues to be susceptible to all antibiotics tested at low MIC50 levels.
Table 2
Minimum inhibitory concentration (MIC) range and MIC50 and MIC90 values of four antibiotics for Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans tested in different countries.

<table>
<thead>
<tr>
<th>Pathogen/antibiotic</th>
<th>The Netherlands (this study), Etest</th>
<th>The Netherlands [5], Etest</th>
<th>Switzerland [19], Etest</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. gingivalis</td>
<td><strong>MIC range</strong></td>
<td><strong>MIC50</strong></td>
<td><strong>MIC90</strong></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&lt;0.016–0.38</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>AMC</td>
<td>&lt;0.016–0.25</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>&lt;0.016–0.032</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>A. actinomycetemcomitans</td>
<td><strong>MIC range</strong></td>
<td><strong>MIC50</strong></td>
<td><strong>MIC90</strong></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.5–3</td>
<td>0.75</td>
<td>1</td>
</tr>
<tr>
<td>AMC</td>
<td>0.38–2</td>
<td>0.75</td>
<td>1</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>1.5–64</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>4 to &gt;256</td>
<td>32</td>
<td>96</td>
</tr>
<tr>
<td>Spain [5], Etest</td>
<td>P. gingivalis</td>
<td>Amoxicillin</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Germany [15], agar dilution</td>
<td>AMC</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Colombia [14], Etest</td>
<td>A. actinomycetemcomitans</td>
<td>Amoxicillin</td>
<td>0.064–32</td>
</tr>
<tr>
<td></td>
<td>AMC</td>
<td>0.094–6</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>0.016–256</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>0.5–256</td>
<td>48</td>
</tr>
</tbody>
</table>

MIC50, MIC90: MICs for 50% and 90% of the organisms, respectively; AMC, amoxicillin/clavulanic acid.

* Data taken from Eick et al. [15], suggesting that a typographical error may have been made in that particular paper.

A. actinomycetemcomitans, a pathogen associated with aggressive periodontitis, was also susceptible to all antibiotics tested except for metronidazole. However, MIC90 values of amoxicillin, clindamycin, azithromycin and tetracycline were higher in the present study in comparison with a previous study in The Netherlands [5]. Also, two A. actinomycetemcomitans isolates showed reduced susceptibility (CLSI) or resistance (EUA CAST) towards amoxicillin. P. intermedia showed slightly lower MICs for amoxicillin and metronidazole in the present study. P. micra showed very similar MICs in both studies.

Geographical differences in antimicrobial resistance among periodontal pathogens have been described [5] and are probably due to a difference in antibiotic consumption. Table 2 summarises MICs of four antibiotics determined with the Etest system in different countries in Europe as well as a South American country (Colombia). A. actinomycetemcomitans and P. gingivalis were selected for this comparison since both pathogens are associated with disease progression [2]. All studies report a high susceptibility of P. gingivalis in European countries to amoxicillin and AMC, with MIC50 values of <0.016 mg/L to ≤0.25 mg/L and of ≤0.016 mg/L, respectively. Striking results were found in a recent study on antibiotic susceptibility of periodontal pathogens in Colombia [14]. The authors used clinical bacterial isolates of periodontitis patients to determine MICs, and levels of resistance were calculated based on breakpoints even higher than used in the present study, except for clindamycin. These authors found a MIC90 of amoxicillin for P. gingivalis of >256 mg/L and 25% resistant isolates. Because all tested isolates appeared susceptible to AMC, β-lactamase production by this species is the explanation for amoxicillin resistance, although testing for β-lactamase in these strains was not reported. Earlier, Eick et al. [15] reported amoxicillin resistance based on β-lactamase production in 7.6% of P. gingivalis isolates in Germany. Amoxicillin resistance in P. gingivalis has not been found in The Netherlands (present study) [5], Finland [16] or Spain [5,17]. Another striking observation by Ardila et al. [14] was the level of resistance of P. gingivalis towards metronidazole, a chemotherapeutic drug that is often used in periodontics [4]. They reported a MIC90 of ≥16 mg/L and resistance towards this antimicrobial agent in 21.6% of the tested isolates. Metronidazole resistance in P. gingivalis has only been observed in Spain in 9% of strains (1/11) [17].

Major differences in amoxicillin susceptibility of A. actinomycetemcomitans were also noted among the different geographical studies. MIC90 values in The Netherlands were low (0.38–1.0 mg/L), but were significantly higher in Spain (32 mg/L), Germany (16 mg/L) and Colombia (32 mg/L), where the percentage of resistant A. actinomycetemcomitans isolates amounted to 77%. Since AMC showed good activity, β-lactamase production is probably the cause of amoxicillin resistance, but production of β-lactamase was not tested in these studies.

Also, clindamycin resistance showed a high prevalence in Colombia among isolates of P. gingivalis (23%), P. intermedia (22%), Prevotella melaninogenica (22%) and F. nucleatum (36%). This level of resistance towards clindamycin has not been observed in The Netherlands and only occasionally in Spain [5]. All anaerobic bacterial species tested in the study by Ardila et al. [14] were, however, susceptible to protected amoxicillin acid and moxifloxacin. The higher level of resistance among periodontal pathogens in Colombia has been explained by the high and uncontrolled antibiotic use in Latin America [18].

In conclusion, it can be stated that antimicrobial susceptibility testing of periodontal pathogens towards commonly used antibiotics against anaerobes seems important. Significant differences in susceptibility profiles of relevant antibiotics have been documented in different geographical areas. These differences are explained by uncontrolled drug use and poor compliance. Regular surveillance of antimicrobial susceptibility in different geographical locations seems important in order to treat patients with the most optimal antibiotic regimen.

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