Antimicrobial susceptibility

Antibiotic susceptibility of clinically relevant anaerobes in Estonia from 1999 to 2001

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Abstract

At present little or no data is available regarding the resistance profiles of anaerobic bacteria in relation to the general usage of antibiotics. The objective of this study was to assess whether any potential relationship exists between the dynamics of antibiotic resistance of anaerobic bacteria and the consumption of antibiotics during the last 3 years within the Estonian population.

In total, 416 anaerobic isolates were investigated from various clinical samples. The anaerobes were isolated on Wilkins-Chalgren Agar, incubated in an anaerobic glove box and identified by standard methods. \(\beta\)-lactamase negative strains were tested against metronidazole, clindamycin, benzylpenicillin and the positive strains were further tested against metronidazole, clindamycin, and ampicillin/sulbactam by E-tests. The results of the susceptibility tests were interpreted according to the current criteria of NCCLS.

Data from the Estonian State Agency of Medicines was used to assess the antibiotic consumption rate in the population (Defined Daily Doses per 1000 inhabitants annually).

The following species of anaerobes were isolated: \(B.\) fragilis group, \(Bacteroides\) sp., \(Fusobacterium\) sp., \(Porphyromonas\) sp., \(Prevotella\) sp., \(Peptostreptococcus\) sp., in addition to various unidentified Gram-positive rods.

Metronidazole resistance was not found among Gram-negative bacteria despite a relatively high consumption of this antimicrobial agent in Estonia. Only ampicillin/sulbactam demonstrated excellent in vitro activity against all anaerobes. Unexpectedly despite a relatively low rate of consumption of clindamycin a high rate of resistance to this agent occurred; a similar situation was noted for penicillin.

In the present study we did not observe a relationship between the changes in antibiotic consumption (DDD/1000) rate and the resistance pattern of anaerobic bacteria to metronidazole, clindamycin, penicillin and ampicillin/sulbactam during a 3-year follow-up period. High resistance to penicillin among some species and also to clindamycin is similar to the global trend and argues for limited use of these antibiotics in empirical treatment. We would suggest that monitoring of local susceptibility pattern is necessary for the selection of initial empirical therapy.

Keywords: Anaerobic bacteria; Antibiotic susceptibility and antibiotic consumption

1. Introduction

Anaerobic bacteria play an important role in the pathogenicity of mixed aerobic–anaerobic infections, mainly as intra-abdominal and gynecologic infections. The majority of anaerobic infections arise from the indigenous flora of the host [1]. There is a general agreement that increasing antimicrobial resistance is related to the selective pressure exerted by the use of antibiotics [2]. Therefore, the susceptibility pattern of anaerobes may be reflected in the general consumption of antibiotics. No data comparing the resistance of anaerobic bacteria to different antimicrobial agents with the dynamics of antibiotic consumption in the population is currently available.

A significant level of resistance to clindamycin, cephalosporins, penicillins, and \(\beta\)-lactam antibiotics has been reported among anaerobic bacteria [3].
Although still rare, resistance of *Bacteroides* sp. to metronidazole has been reported sporadically since 1978 [4].

Since susceptibility testing of anaerobes is both time consuming and expensive, therapy is often guided by local patterns of published susceptibility reports. However, the local patterns may differ significantly and dynamic changes of resistance pattern for particular anaerobes may occur.

In the present work, the first objective was to assess the prevalence of anaerobic pathogens and their susceptibility pattern in Estonia from 1999 to 2001. The second goal was to assess any potential relationship between the susceptibility pattern and the rate of antibiotic use over the course of 3 years.

2. Materials and methods

A total of 47, 178 and 191 consecutive strains of anaerobic bacteria (a total of 416) were isolated from abscesses, deep and superficial wounds, and paranasal sinus secretions clinical materials in 1999, 2000 and 2001, respectively. Strains were collected in an anaerobic Stuart Transport Medium and taken to the laboratory within 2 h. The anaerobes were isolated on Wilkins-Chalgren Agar (Oxoid, Basingstoke, Hampshire, UK) supplemented with 5% horse blood and incubated in anaerobic chamber (Sheldon Manufacturing Inc., Cornelius, Oregon, USA) under anaerobic conditions (with anaerobic chamber (Sheldon Manufacturing Inc., Cor-

supplemented with 5% horse blood and incubated in an
anaerobic chamber (Sheldon Manufacturing Inc., Cor-
nelius, Oregon, USA) under anaerobic conditions (with
gas mixture: 5% CO₂, 5% H₂, 90% N₂) for 2–4 days.
Aerotolerance testing was performed using chocolate
agar plates (Oxoid) in a 5–10% CO₂ atmosphere.
Identification was performed according to colony and
morphological, potency disk pattern, and biochemical profiles [5].

Susceptibility tests were performed and interpreted
according to the current criteria of NCCLS.

The production of β-lactamase was determined
with nitrocefin disks (AB Biodisk, Solna, Sweden). For
susceptibility testing, a suspension in 0.9% sodium
chloride solution (2mL) was prepared equivalent to
no. 1 McFarland turbidity standard. The suspension
was subsequently transferred onto the surface of a
Wilkins-Chalgren Agar (Oxoid) plate and streaked with
a cotton swab. Antibiotic strips (E-tests AB Biodisk,
Solna, Sweden) were applied onto the surface of each dried
agar plate. The plates were incubated in an anaerobic
chamber (Sheldon Manufacturing Inc.) at 37°C for 48 h
prior to examination. β-Lactamase-negative strains
were further tested for resistance to metronidazole, clindamycin,
benzylpenicillin and positive strains tested against metronidazole, clindamycin, and ampicillin/sulbactam. Data
from the Estonian State Agency of Medicines was used
to assess the antibiotic consumption rate in the population
(DDD/1000/annually).

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides fragilis</em> group</td>
<td>86</td>
<td>20.7</td>
</tr>
<tr>
<td><em>Bacteroides</em> sp.</td>
<td>81</td>
<td>19.5</td>
</tr>
<tr>
<td><em>Fusobacterium</em> sp.</td>
<td>66</td>
<td>15.9</td>
</tr>
<tr>
<td><em>Porphyromonas</em> sp.</td>
<td>16</td>
<td>3.8</td>
</tr>
<tr>
<td><em>Prevotella</em> sp.</td>
<td>59</td>
<td>14.2</td>
</tr>
<tr>
<td><em>Peptostreptococcus</em> sp.</td>
<td>97</td>
<td>23.3</td>
</tr>
<tr>
<td>Gram-positive rods*</td>
<td>11</td>
<td>2.6</td>
</tr>
<tr>
<td>Total</td>
<td>416</td>
<td>100</td>
</tr>
</tbody>
</table>

*Clostridium* sp. (six strains), *Propionibacterium* sp. (two strains), *Eubacterium* sp. (three strains).

3. Results

The distribution and frequency of test isolates are depicted in Table 1. Seventy-four percent were anaerobic
Gram-negative bacilli (predominantly *B. fragilis* group), and 23% were Gram-positive cocci (*Peptostreptococcus* sp.), whereas Gram-positive rods occurred rarely.

The results of susceptibility tests prepared in different years are listed in Table 2. The spectrum of pathogenic bacteria and their susceptibility patterns in 1999 and in 2001 was similar. Overall, the isolated anaerobes were susceptible to the majority of antimicrobial agents tested. Metronidazole resistance was not found among Gram-negative rods, despite high resistance of Gram-positive anaerobic cocci and rods. The lowest activity occurred with penicillin and the highest with ampicillin/sulbactam. No differences were found between the resistance rates of anaerobes for the 3-year time period.

β-lactamase production was 48.8% for the *B. fragilis*
group, 40.7% for *Bacteroides* sp., 22.7% for *Fusobacterium* sp., 37.5% for *Porphyromonas* sp., 62.7% for *Prevotella* sp., 18.6% for *Peptostreptococcus* sp. and 18.2% for Gram-positive rods.

The consumption rates of some antibiotics in Estonia during 1999, 2000, and 2001 are presented in Table 3. Metronidazole was the most frequently used anti-anaerobic antibiotic. The dynamics of consumption of these antibiotics was different. The use of metronidazole and penicillin was quite stable, whereas the use of clindamycin and ampicillin/sulbactam increased approximately two-fold.

The comparison of antibiotic consumption and the
total percentage of the resistance among all tested
anaerobic bacteria is presented in Fig. 1.

4. Discussion and conclusion

Antimicrobial resistance among both aerobes and
anaerobes is a growing health problem worldwide.
Within European countries the European Antimicrobial
Resistance Surveillance System (EARSS) has monitored the levels of resistance among aerobic bacteria since 1998. EARSS results show that antibiotic resistance of *Streptococcus pneumoniae* to penicillin is correlated with the use of β-lactam antibiotics and macrolides both at the state and at the European level [6]. Seppälä et al. [7] found a similar correlation between the usage and resistance of erythromycin to *Streptococcus pyogenes*. Such information is not yet available for anaerobic bacteria. For example, in Europe the resistance pattern of aerobes and *Helicobacter pylori* is very variable [6,8]. These differences may be due to antibiotic pressure and the country-specific drug usage as well as different resistance mechanisms for a single group of bacteria. Little or no such data is available regarding the resistance pattern of anaerobic bacteria in Eastern European countries whatsoever.

In our study, metronidazole resistance among Gram-negatives was not observed despite the relatively high consumption of this antimicrobial agent in Estonia. This was not the anticipated result; we expected that the intensive use of this antibiotic within Estonia would influence the microbial resistance pattern as suggested by other authors for other regions [6,7]. Up to now most data report antimicrobial activity of metronidazole against Gram-negative anaerobes [3,9]. It has been noted that resistance to this antibiotic among *B. fragilis* strains is a cause of treatment failures [10].

In the present study, there was an incongruity between the relatively high resistance and the low

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**Table 2**

The susceptibility pattern (MIC50 and MIC90, mg/L) and β-lactamase production of anaerobic bacteria in 1999, 2000, and 2001

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>No. of strains/β-lactamase-positive strains</th>
<th>MIC (mg/L)</th>
<th>Metronidazole</th>
<th>Clindamycin</th>
<th>Pencillin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ampicillin&lt;sup&gt;b&lt;/sup&gt;/sulbactam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td><em>B. fragilis</em> group</td>
<td>1999</td>
<td>9</td>
<td>4</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>256.0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>45</td>
<td>21</td>
<td>0.023</td>
<td>1.0</td>
<td>0.032</td>
<td>256.0</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>32</td>
<td>17</td>
<td>0.19</td>
<td>0.5</td>
<td>0.094</td>
<td>256.0</td>
</tr>
<tr>
<td><em>Bacteroides</em> sp.</td>
<td>1999</td>
<td>15</td>
<td>8</td>
<td>0.094</td>
<td>0.5</td>
<td>0.016</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>35</td>
<td>12</td>
<td>0.047</td>
<td>0.19</td>
<td>0.016</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>31</td>
<td>13</td>
<td>0.032</td>
<td>0.5</td>
<td>0.016</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Fusobacterium</em> sp.</td>
<td>1999</td>
<td>4</td>
<td>1</td>
<td>0.016</td>
<td>0.032</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>25</td>
<td>6</td>
<td>0.016</td>
<td>0.064</td>
<td>0.016</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>37</td>
<td>8</td>
<td>0.023</td>
<td>0.125</td>
<td>0.016</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Porphyromonas</em> sp.</td>
<td>1999</td>
<td>3</td>
<td>1</td>
<td>0.064</td>
<td>0.19</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>4</td>
<td>1</td>
<td>4.0</td>
<td>4.0</td>
<td>0.016</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>9</td>
<td>4</td>
<td>0.016</td>
<td>0.094</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td><em>Prevotella</em> sp.</td>
<td>1999</td>
<td>5</td>
<td>3</td>
<td>0.016</td>
<td>0.5</td>
<td>0.016</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>19</td>
<td>13</td>
<td>0.016</td>
<td>0.125</td>
<td>0.016</td>
<td>0.023</td>
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<tr>
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<td>2001</td>
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<td>0.016</td>
<td>0.038</td>
<td>0.016</td>
<td>0.023</td>
</tr>
<tr>
<td><em>Peptostreptococcus</em> sp.</td>
<td>1999</td>
<td>9</td>
<td>5</td>
<td>0.19</td>
<td>256.0</td>
<td>0.032</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>44</td>
<td>1</td>
<td>256.0</td>
<td>256.0</td>
<td>0.032</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>44</td>
<td>12</td>
<td>12.0</td>
<td>256.0</td>
<td>0.47</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Gram-positive rods</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1999</td>
<td>2</td>
<td>0</td>
<td>4.0</td>
<td>256.0</td>
<td>0.094</td>
<td>256.0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>6</td>
<td>1</td>
<td>6.0</td>
<td>256.0</td>
<td>0.125</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>3</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>β-lactam-negative strains.<br><sup>b</sup>β-lactam-positive strains.<br><sup>c</sup>*Clostridium* sp., *Propionibacterium* sp., *Eubacterium* sp.

---

**Table 3**

The differences in the Defined Daily Doses per 1000 inhabitants annually (DDD/1000) from 1999 to 2001 in Estonia

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>DDD/1000</th>
<th>Consumption dynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1999</td>
<td>2000</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.885</td>
<td>0.873</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.025</td>
<td>0.040</td>
</tr>
<tr>
<td>Pencillin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.491</td>
<td>0.742</td>
</tr>
<tr>
<td>Ampicillin&lt;sup&gt;b&lt;/sup&gt;/sulbactam</td>
<td>0.257</td>
<td>0.395</td>
</tr>
</tbody>
</table>

<sup>a</sup>Includes benzylpenicillin, phenoxymethylpenicillin, benzathin benzylpenicillin, and procain penicillin.
consumption of clindamycin. A rise in resistance to clindamycin was noted in the Bacteroides fragilis group and also among Clostridium sp. and anaerobic cocci [3,11]. Only ampicillin/sulbactam demonstrated excellent activity in vitro against all anaerobes. Nevertheless, the consumption of ampicillin/sulbactam was higher than that of clindamycin, and increased two-fold from 1999 to 2001, the resistance pattern decreased in three consecutive years. Likewise, there is an incongruity between the resistance and the consumption of penicillin. Despite low consumption of penicillin, the resistance pattern was high for 1999 and 2000. The B. fragilis group in addition to certain Clostridium and Fusobacterium species are resistant to β-lactam antimicrobial agents primarily due to β-lactamase production. β-Lactamase production was common in different anaerobic species throughout the 3-year period studied. However, several β-lactamase-negative Fusobacterium strains appeared resistant to penicillin indicating some other resistance mechanisms [12]. To summarize, we did not observe a relationship between the changes in antibiotic consumption (DDD/1000) rate and the resistance pattern of anaerobic bacteria to metronidazole, clindamycin, penicillin and ampicillin/sulbactam during a 3-year period. One reason may be that anaerobic infections are predominantly derived from the host’s own indigenous microflora, which is relatively stable [13–15]. Another explanation may be that the effect of antibiotic pressure on indigenous microflora acts slowly. We could hypothesize that the resistance rate of anaerobes may correlate with the drug use of the patients from their date of birth to the testing period, and also with the antibiotic consumption of their parents.

High resistance to penicillin and among some species also to clindamycin is similar to the global trend and suggests limited use of those antibiotics in empirical treatment. Therefore, monitoring of local susceptibility patterns may be necessary for the selection of initial empirical therapy.

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References


