ORIGINAL ARTICLE

Effect of inoculum size on the antibiotic susceptibilities of β-lactamase positive isolates of Moraxella catarrhalis

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ABSTRACT

It is well known that bacteria producing β-lactamases in general show marked inoculum effect in susceptibility testing. The aim of this study is to determine the effect of inoculum size on the susceptibility of β-lactamase positive strains of Moraxella catarrhalis to oral β-lactam and non-β-lactam antibiotics. MICs of antibiotics were determined by a twofold microdilution technique with two different inoculum sizes were tested: 5x 10⁵ CFU/ml-standard inoculum and 5 x10⁷ CFU/ml-high inoculum. The highest increase (4-fold) was observed with penicillins alone (amoxycillin and ampicillin) or combined with inhibitor and cefitubene, followed by older cephalosporins, erithromycine and chloramphenicol (2-fold). Tetracycline did not show a significant increase in MIC when a higher inoculum size was applied. In spite of the increase in MIC with high inoculum all strains were still susceptible to amoxycillin combined with clavulanate. MICs of cephalosporins were also below the resistance breakpoint for most of the strains at the higher inoculum. Based on that, we can conclude that therapeutic implications of the inoculum effect were not significant. These data suggest that high inocula should be used to determine MICs of ampicillin and amoxycillin for Moraxella catarrhalis but that this precaution is unnecessary with the cephalosporins tested or with amoxycillin/clavulanate.

Key words: Moraxella catarrhalis, β-lactamases, inoculum effect, penicillin, cephalosporin

INTRODUCTION

Moraxella catarrhalis, formerly known as Branhamella catarrhalis, was generally considered a harmless oropharyngeal commensal bacterium. However, in the past decades it has been recognized as the etiologic agent of otitis media in children (1) acute bronchitis and pneumonieae (2-7) acute laryngitis (8), acute sinusitis (9), septicemia (10), meningitis (11) and endocarditis (12-13). Most clinical isolates produce a β-lactamase (14-18) which is a penicillinase according to the substrate profile (19-20) and is encoded on the plasmids (19,23). The enzymes are produced in small amounts and remain strongly cell associated (19,23). Diseases caused by β-lactamase producing strains are refractile to therapy with ampicillin and amoxycillin, two antibiotics that are frequently used for the therapy of infections such as those with which Moraxella catarrhalis is associated (6, 26-27). In vitro antibiotic susceptibilities of β-lactamase positive isolates of Moraxella catarrhalis have been extensively studied (28-34). However, it is well known that bacteria producing β-lactamases in general show marked inoculum effect (35-37) in susceptibility testing. If there is a high density of bacteria at the infections site there will be a lot of β-lactamase produced to protect the bacteria from the bactericidal activity of antibiotic. There are only a few reports on the effect of inoculum size on the antimicrobial susceptibility of Moraxella catarrhalis (38-39). The aim of this study is to determine the effect of inoculum size on the susceptibility of β-lactamase positive strains of Moraxella catarrhalis to oral β-lactam and non-β-lactam antibiotics.
MATERIALS AND METHODS

Bacteria

Fifty penicillin resistant *M. catarrhalis* strains were collected in Children’s University Hospital Zagreb during 1990-1992 from various clinical specimens (nasopharyngeal swabs, middle ear fluids, sputum, bronchoaspirates). Bacteria were identified by conventional biochemical tests.

Detection of β-lactamases

β-lactamases were detected with commercially available chromogenic cephalosporin disk β-lactamase test (40) containing nitrocefin as the substrate (Cefinase disks; BBL Microbiology Systems, Cockeysville, Md). The test was repeated with crude enzyme preparations. 50 µl of a nitrocefin solution (500 mg/L) was mixed with unpurified enzyme sample from each strain. The test was considered to be positive if the yellow substrate colour turned to red (17).

Minimum inhibitory concentrations (MICs)

MICs of ampicillin, amoxycillin, amoxycillin/clavulanate, cephalaxin, cefuroxime, cefadroxil, cefprozil, cefitubten tetracycline, erythromycine and azithromycin were determined by a twofold microdilution technique using microtiter plates and Mueller-Hinton broth (41-42). Two different inoculum sizes were tested: 5x10^5 CFU (colony forming units)- ml standard inoculum and 5 x10^7 CFU/ml- high inoculum. The tested range of antibiotic concentrations was 0.0075-128 mg/L. Clavulanic acid was added to amoxycillin in the fixed concentration of 4 mg/L. The antibiotic powders were provided by following manufacturers: ampicillin, amoxycillin, clavulanate, cephalaxin, cefuroxime, tetracycline, erythromycine, azithromycin and chloramphenicol Pliva, Zagreb, Croatia and cefadroxil and cefprozil Bristol Myers Squibb, Zagreb, Croatia. Cefitubten was kindly provided by Prof. Ellen Stobbering (University Hospital Maastricht, Maastricht, The Netherlands). Two referal strains *M. catarrhalis* Ravasio and *M. catarrhalis* 1908 were used as quality control strains from MIC determination. The strains were kindly provided by Dr Christine Cooper (SmithKline Beecham Laboratories, Surrey, UK).

RESULTS

The susceptibilites with two different inocula are shown in Table 1. It can be seen the MICs of β-lactam antibiotics have higher values with the high inoculum. Table 2 shows the range and median increase of MIC when higher bacterial density was applied. The highest decrease (4-fold) was observed with penicillins alone (amoxycillin and ampicillin) or combined with inhibitor and cefitubten, followed by older cefalosporins, erythromycine and chloramphenicol (2-fold). Tetracycline did not show a significant increase in MIC when higher the inoculum size was applied. Azithromycine had off-scale MIC values. The percentage of strains resistant
to oral antibiotics with two different inocula is shown in Table 3. At the higher inoculum the percentage of strains resistant to unprotected penicillins rose to 100%. In spite of the increase in MIC with $5 \times 10^9$ CFU/ml, inoculum all strains were still susceptible to amoxycillin combined with clavulanate. MICs of cephalosporins were also below the resistance breakpoint for most of the strains at the higher inoculum as shown in Table 3.

**DISCUSSION**

Ampicillin and amoxycillin alone and combined with clavulanate have shown the most pronounced inoculum effect. That can be explained by the fact that BRO β-lactamases which are typical for *M. catarrhalis* are penicillinases according to the substrate profile. The inoculum effect was less convincing with cephalosporins but unexpectedly it was of a higher magnitude with newer cephalosporins such as ceftibuten which is supposed to be more resistant to hydrolysis by β-lactamases, than with the older compounds. None of β-lactam antibiotics did exhibit marked inoculum effect. It was observed that strains with lower ampicillin and amoxycillin MICs were more susceptible to the inoculum effect probably because they exhibit low level of β-lactamase activity. As a result, higher inocula are required to abrogate the inhibitory activity of β-lactam antimicrobial agents susceptible to hydrolysis by β-lactamases, such as ampicillin and amoxycillin. Other authors reported a significant inoculum effect with β-lactamase positive strains of *M. catarrhalis* for penicillin G, ampicillin, cephalotin, cefamandole, cefuroxime, cefaclor and cefixime (38,39). As far as amoxycillin/clavulanate is concerned, our results were in concordance with those obtained by Livermore et al (39), but different from those reported by Doern et al who did not detect

**REFERENCES**


10. Doern GV, Miller MJ, Winn RE. *Branhamella (Neisseria) ca-

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**Table 3 Percentage of resistant strains at the following inocula.**

<table>
<thead>
<tr>
<th>Antibiotic and NCCLS breakpoint ng/l</th>
<th>Inoculum size (CFU/ml)</th>
<th>5x10²</th>
<th>5x10⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (4)</td>
<td>39/50 (78%)</td>
<td>50/50 (100%)</td>
<td></td>
</tr>
<tr>
<td>Amoxycillin (8)</td>
<td>40/50 (80%)</td>
<td>50/50 (100%)</td>
<td></td>
</tr>
<tr>
<td>Amoxycillin/clavulanate (8/4)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td></td>
</tr>
<tr>
<td>Cephalaxin (32)</td>
<td>0/50 (0%)</td>
<td>6/50 (12%)</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime (32)</td>
<td>1/50 (2%)</td>
<td>3/50 (6%)</td>
<td></td>
</tr>
<tr>
<td>Cefprozil (32)</td>
<td>1/50 (2%)</td>
<td>2/50 (4%)</td>
<td></td>
</tr>
<tr>
<td>Cefadroxil (732)</td>
<td>2/50 (4%)</td>
<td>1/50 (2%)</td>
<td></td>
</tr>
<tr>
<td>Cefituban (32)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline (8)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td></td>
</tr>
<tr>
<td>Erythromycin (8)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td></td>
</tr>
<tr>
<td>Azithromycin (8)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol (8)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td></td>
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</tbody>
</table>
25. Farmer T, Reading C. Inhibition of β-lactamases of Branhamella catarrhalis by clavulanic acid and other inhibitors. Drugs 1986; 31(Suppl.3):70-78.
36. Goldstein EJ; Citron DM, Cherubin CE. Comparison of the inoculum effects of members of the family Enterobacteriaceae on cefoxitin and other cephalosporins, β-lactamase inhibitor combinations, and the penicillin derived components of these combinations. Antimicrob Agent Chemother 1991;35:560-566.