RESEARCH NOTE

Evaluation of susceptibility patterns and BRO β-lactamase types among clinical isolates of *Moraxella catarrhalis*

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ABSTRACT

The aims of this study were to detect BRO β-lactamase types and to evaluate any correlation with the susceptibility patterns of 90 clinical isolates of *Moraxella catarrhalis*. The overall prevalences of the *bro*-1 and *bro*-2 genes were 78% and 12%, respectively. Penicillin G MICs for BRO-1$^+$ isolates were significantly higher than those for BRO-2$^+$ isolates. All the isolates were susceptible to amoxycillin–clavulanate, levofloxacin and cefixime. Resistance to clarithromycin, tetracycline and trimethoprim–sulphamethoxazole was 1.1%, 2.2% and 1.1%, respectively. One-step, length-based PCR was an efficient method to screen for BRO β-lactamase genes.

Keywords Antibiotic susceptibility, β-lactamase, BRO, *Moraxella catarrhalis*, PCR, resistance

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Moraxella catarrhalis has been recognised as an increasingly important human pathogen during the last 25 years. *M. catarrhalis* can cause upper and lower respiratory tract infections in adults and acute otitis media in children. Although *M. catarrhalis* strains are generally susceptible to most antibiotics used for the treatment of respiratory infections, they are resistant to penicillin because of the widespread production of BRO β-lactamases [1]. Production of β-lactamase can be detected using nitrocefin disks and isoelectric-focusing patterns, or by DNA restriction endonuclease analysis [2–4]. Levy and Walker [5] have described an easy, one-step, length-based PCR assay that can be used to distinguish BRO-1 and BRO-2 β-lactamase-producing strains. The present study aimed to use this assay to identify the BRO-type β-lactamases produced by clinical isolates of *M. catarrhalis* from a Turkish University Hospital and to compare their antibiotic susceptibility patterns.

Ninety *M. catarrhalis* isolates from different patients with upper and lower respiratory tract infections were investigated. The isolates were identified using the Crystal ID System for *Neisseria–Haemophilus* (Becton Dickinson, Franklin Lakes, MD, USA). Identification was confirmed by growth at room temperature, hydrolysis of tributyrin and positive DNase tests [6]. BRO-negative, BRO-1$^+$ and BRO-2$^+$ control strains for PCR assays were kindly provided by East Tennessee State University (Johnson City, TN, USA) [5]. All of the isolates were stored at −70°C until required.

Antimicrobial susceptibility testing was performed using Etests (AB Biodisk, Solna, Sweden). MICs of penicillin G, amoxycillin–clavulanate, trimethoprim–sulphamethoxazole, clarithromycin, levofloxacin, cefixime and tetracycline were determined according to the manufacturer's instructions. MICs were interpreted according to CLSI breakpoints [7] for *Haemophilus* spp., except those for penicillin G, which were interpreted according to the breakpoints for *Staphylococcus* spp. *Staphylococcus aureus* ATCC 29213 was used as a quality control strain for susceptibility tests.

Screening for β-lactamase production was performed using nitrocefin disks (Becton-Dickinson) according to the manufacturer's recommended procedure. A colour change from yellow to pink within 5–60 min was considered to be a positive reaction. *S. aureus* ATCC 29213 and *Haemophilus influenzae* ATCC 10211 were used as positive and negative quality control strains, respectively, for nitrocefin disk assays.

DNA extraction was performed using an EZ-10 Spin Column Genomic DNA Minipreps Kit (Bio-Basic, East Markham, Ontario, Canada). Primer sequences used to detect BRO β-lactamase genes were 5′-CACCCYGTRGGACAAGC and 5′-AA-TGACGGCGTTGCATC, with PCR amplification

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comprising 94°C for 10 min, followed by 25 cycles of 94°C for 30 s, 55°C for 60 s and 72°C for 40 s, with a final extension at 72°C for 7 min [5]. PCR products of 235 bp (*bro-1*) or 214 bp (*bro-2*) were distinguished on agarose 2% w/v gels. All negative PCRs were repeated. MICs of each antimicrobial agent were compared for BRO-1^+ and BRO-2^+ isolates using Student's *t*-test for independent samples.

Overall, 85 (94%) of the isolates were β-lactamase-positive according to the nitrocefin disk assay, and 81 (90%) were positive for a BRO β-lactamase gene by PCR. One of the five isolates that were negative by PCR and the nitrocefin assay was resistant to penicillin (MIC 0.5 mg/L). The PCR and nitrocefin results were consistent for 86 (96%) of the isolates.

Since the first report of β-lactamase-producing *M. catarrhalis* isolates in 1977 [8], the frequency of β-lactamase production has increased worldwide [1]; currently >90% of *M. catarrhalis* isolates produce a β-lactamase [2,3,9,10]. Koseoglu *et al*. [4] reported that 96.9% of clinical isolates and 90.6% of carrier strains produced β-lactamase in Turkey. In the present study, four penicillin-resistant isolates failed to yield PCR products, but were nitrocefin-positive, suggesting the presence of a third BRO enzyme, as postulated by Christensen *et al*. [11], although BRO-3 is now considered to be a membrane-bound precursor molecule rather than a new type of enzyme [12]. Levy and Walker [5] concluded that PCR failure is probably caused by mismatches between primer and target sequences, rather than the absence of a functional gene, but this was not investigated further in the present study.

The overall prevalences of the *bro*-1 and *bro*-2 genes in the present study were 78% and 12%, respectively. Similarly, the majority of β-lactamase-producing isolates in many countries produce BRO-1 [1–4,9,10]. The MIC ranges and MIC_{50} and MIC_{90} values of each antimicrobial agent for BRO-1^+, BRO-2^+ and BRO^- isolates are shown in Table 1. Penicillin MICs for BRO-1^+ isolates were significantly higher than those for BRO-2^+ isolates ($t = 3.06$, $p < 0.001$; mean ± SD: BRO-1^+ = 5.89 ± 5.06, BRO-2^+ = 1.18 ± 0.82). The MIC_{90} value of penicillin G for BRO-1^+ isolates was 16 mg/L, compared with 2 mg/L for BRO-2^+ isolates. One of five BRO^- isolates was resistant to penicillin, suggesting the existence of a different resistance mechanism. It has been reported that 8% of β-lactamase-negative *M. catarrhalis* isolates in Europe are resistant to penicillin, but the mechanism of resistance remains unknown [13]. Two (2.9%) BRO-1^+ isolates, which were also positive according to the nitrocefin disk assay, were susceptible to penicillin (MIC 0.03 mg/L); however, it is known that BRO β-lactamases are relatively weak enzymes that may result in only low MICs [1].

In the present study, all of the isolates were susceptible to amoxycillin–clavulanate, levofloxacin and cefixime. One (1.1%) BRO-1^+ isolate was found to be intermediately susceptible to clarithromycin, one (1.1%) BRO-1^+ isolate was resistant to trimethoprim–sulphamethoxazole, and two (2.2%) BRO-1^+ isolates were resistant to tetracycline. However, there were no significant differences among the MICs of antimicrobial agents for BRO-1^+ and BRO-2^+ isolates, except for penicillin G. Although *M. catarrhalis* isolates are known to be highly susceptible to macrolides, tetracycline, fluoroquinolones, second- and third-generation cephalosporins and β-lactam/β-lactamase inhibitor combinations, resistance to erythromycin [14], tetracycline [15] and ciprofloxacin [16] has been reported occasionally.

Table 1. MIC range, MIC_{50} and MIC_{90} values (mg/L) of seven antimicrobial agents for *Moraxella catarrhalis* clinical isolates, grouped according to their BRO β-lactamase type

	BRO-1^+ (n = 70)			BRO-2^+ (n = 11)			BRO^- (n = 9)		
Antimicrobial agent	**MIC range**	**MIC_{50}**	**MIC_{90}**	**MIC range**	**MIC_{50}**	**MIC_{90}**	**MIC range**	**MIC_{50}**	**MIC_{90}**
Penicillin G	0.125–16	4	16	0.25–2	1	2	0.03–1	0.125	0.5
AMC/CL	<0.01–1	0.06	0.125	<0.01–0.125	0.06	0.06	<0.01–0.06	0.01	0.06
TMP/SMX	0.008–4	0.06	0.25	<0.002–0.125	0.125	0.25	<0.002–0.125	0.06	0.125
Clarithromycin	<0.01–16	0.03	0.06	<0.01–0.06	0.06	0.06	<0.01–0.06	0.06	0.06
Levofloxacin	0.008–0.25	0.03	0.06	<0.02–0.06	0.03	0.06	0.002–0.06	0.03	0.03
Cefixime	<0.01–1	0.06	0.25	<0.01–0.125	0.06	0.125	<0.01–0.25	0.03	0.125
Tetracycline	0.03–16	0.125	0.25	<0.01–0.25	0.125	0.125	0.06–0.25	0.125	0.25

AMC/CL, amoxycillin–clavulanate; TMP/SMX, trimethoprim–sulphamethoxazole.

Resistance to the trimethoprim–sulphamethoxazole combination is also now being reported with greater frequency [10,13].

In conclusion, the increasing rates of resistance to several antibiotics among *M. catarrhalis* isolates make continuous monitoring of resistance patterns important. Although the nitrocefin disk assay is adequate for routine testing to detect the production of β-lactamase, it is important to distinguish the BRO β-lactamases in order to predict susceptibility patterns and to monitor long-term trends in susceptibility among *M. catarrhalis* strains. The one-step, length-based PCR assay seems to be an easy and efficient method for distinguishing the BRO β-lactamases.

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