1610
ECCMID 2006
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370,
fax 319.665.3371
ronald-jones@jmilabs.com

Contemporary Prevalence of BRO ß-Lactamases in *M. catarrhalis*: Report from the SENTRY Antimicrobial Surveillance Program (USA; 1997-2004)

LM DESHPANDE, HS SADER, RN JONES
JMI Laboratories, North Liberty, IA, USA

SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objective: To evaluate the prevalence of BRO-1 and BRO-2 among ß-lactamase (ßl)-producing *M. catarrhalis* (MCAT) in the USA. Although the ßl-mediated penicillin (PEN) resistance (R) in MCAT has been stable at 95%, the BRO-1 and -2 occurrence has not been determined in USA isolates since 1998. BRO-2 rates have been reported at < 15 (1980s), 4.8 (1984-94), 2.1 (1994-95) and 3.1% (1997-98).

Methods: Community-acquired MCAT isolates (SENTRY Program 1997-2004) were tested by CLSI broth microdilution methods including: 7,860 worldwide and 3,671 in North America (NA). BRO-1 and -2 were detected by PCR methods (Levy and Walker; 2004), compared to epidemiologic tests, and MIC values. 100 ß-lactamase-positive (BL+) MCAT samples per year from USA (39 sites) and Canada (7 sites) were tested for the odd-numbered years.

Results: The BRO-2 rate was 4, 4, 3, and 3% for 1997, 1999, 2001 and 2003, respectively; rates in Canada (8 isolates) > USA (6). Several agents remained active: amoxicillin/clavulanate (MIC_{90}, ≤ 0.25 mg/L), ceftriaxone (CTRI; 0.5), cefuroxime (2), erythromycin (≤ 0.25-0.5), levofloxacin (≤ 0.03 - 0.06), tetracycline (2) and trimethoprim/sulfamethoxazole (TMP/SMX; ≤ 0.5/9.5). Penicillin MIC distribution was tri-modal (≤ 0.03, 1-2, >4 mg/L) and ceftriaxone bi-modal (0.016, 0.5), yet BRO-1 and -2 MIC/zone distributions overlap (best discriminated by methicillin [mean zone, 10.6 vs. 19.4 mm] and penicillin [13.9 vs. 24.1] disks).

Antimicrobial agent	MIC_{90} (mg/L) for: Worldwide (7,860)	North America (3,671)	PCR sample (400)
Penicillin	>4	>4	>4
Amoxicillin/Clavulanate	≤0.25	≤0.25	≤0.25
Ceftriaxone	0.5	0.5	0.5
Cefuroxime	2	2	2
Erythromycin	≤0.25	0.5	0.5
Levofloxacin	0.06	≤0.03	0.06
Tetracycline	≤2	≤2	≤2
TMP/SMX	≤0.5	≤0.5	≤0.5

Possible BRO-2 epidemic clusters could not be excluded due to a very common ribotype in 3 centers (Canada, 2 sites; USA, 1).

Conclusions: This BRO-1 and -2 enzymes NA prevalence update in MCAT isolates (1997-2003) shows stability at 96 - 97% and 3 – 4 %, respectively. Phenotypic tests (zones or MICs) can not easily distinguish between these ß-lactamase types, necessitating the use of molecular applications.

INTRODUCTION

Moraxella catarrhalis is a commensal of the respiratory tract and historically had been considered a relatively harmless organism. Over time, however, this Gram-negative diplococcus became recognized as the 3rd most common upper respiratory tract pathogen in children and in adults with chronic obstructive pulmonary disease by inducing inflammatory response of bronchial epithelial cells. Nasopharyngeal carriage of *M. catarrhalis* is, therefore, a risk factor for serious respiratory tract infections and otitis media among children.

Although susceptible to a number of antimicrobial agents, greater than 90% of all *M. catarrhalis* isolates are resistant to penicillin by means of BRO-1 or BRO-2 ß-lactamase production. Sequence and genetic context of BRO enzymes suggests that BRO-2 was acquired by interspecies gene transfer possibly from a Gram-positive species and BRO-1 evolved from BRO-2 with spread by horizontal transfer via subsequent transformation events. BRO-1 shows a "promoter fit" mutation and is produced in quantities 2-3 times greater than BRO-2. Thus, isolates carrying BRO-1 are usually more resistant to ampicillin than those carrying BRO-2.

BRO-2 among *M. catarrhalis* have been reported at less than 15% in the 1980s, 4.8% in 1984-94, 2.1% in 1994-95 and 3.1% in 1997-98. Although ß-lactamase-mediated penicillin resistance in *M. catarrhalis* has been stable at >95%, the BRO-1 and -2 occurrence rates have not been determined in North America since 1998.

MATERIALS AND METHODS

Bacterial Isolates. In the 1997-2004 period, the SENTRY Antimicrobial Surveillance Program collected 7,860 *M. catarrhalis* isolates from patients with community-acquired pneumonia from medical centers located worldwide and 3,671 in North America. Only isolates considered clinically significant were included in the study. Species identification was confirmed by standard biochemical tests such as oxidase and butyrate disk tests (Remel). ß-lactamase production was detected by nitrocefin disk test (Remel, Lenexa, KS, USA). One hundred ß-lactamase-positive *M. catarrhalis* samples per year from USA (39 sites) and Canada (7 sites) were tested for the odd-numbered surveillance years.

Susceptibility Testing. The *M. catarrhalis* isolates were susceptibility tested against more than 25 antimicrobials by the broth microdilution procedure as described by the CLSI (2006) using validated dry-form panels manufactured by Trek Diagnostics (Cleveland, OH, USA). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 were routinely included in testing for quality assurance. Disk diffusion tests were performed using penicillin, ceftriaxone and methicillin disks (Remel) by the CSLI (2006) methods. Interpretations of susceptibility to all antimicrobials tested were those CLSI (2006) criteria used for *Haemophilus influenzae*.

Characterization of BRO ß-lactamase: 0.5 McFarland suspensions of *M. catarrhalis* isolates were heated at 95°C for 5 minutes. The heat-treated suspension was used to detect BRO-1 and -2 by PCR methods using primers and interpretations described by Levy and Walker (2004).

Molecular typing: All isolates producing BRO-2 and seven isolates from different medical centers producing BRO-1 were molecularly typed by using Riboprinter™ Microbial Characterization System according to the manufacturer's instructions (Dupont Qualicon, Wilmington, DE, USA). The isolates were also typed by pulsed-field gel electrophoresis (PFGE).

RESULTS

- The North American subset chosen for PCR analysis was representative of the worldwide collection in terms of susceptibilities to key antimicrobials (Table 1). Several agents remained very potent against *M. catarrhalis* strains: amoxicillin/clavulanate (MIC_{90}, ≤ 0.25 mg/L), ceftriaxone (0.5), cefuroxime (2), azithromycin (≤ 0.12), levofloxacin (≤ 0.03 - 0.06), tetracycline (2) and trimethoprim/sulfamethoxazole (≤ 0.5/9.5).

Table 1. Susceptibility of *M. catarrhalis* isolates to selected antimicrobial agents.

Antimicrobial agent	MIC_{90} (mg/L)/ % susceptible for: Worldwide (7,860)	North America (3,671)	PCR sample (400)
Penicillin	>4/ 5.2[a]	>4/ 5.3[a]	>4/ 100[a]
Amoxicillin/Clavulanate	≤0.25/ 100.0	≤0.25/ 100.0	≤0.25/ 100.0
Ceftriaxone	0.5/ 99.9	0.5/ 100.0	0.5/ 99.5
Cefuroxime	2/ 98.9	2/ 99.5	2/ 98.8
Azithromycin	≤0.12/ 100.0	≤0.12/ 100.0	≤0.12/ 100.0
Levofloxacin	0.06/ 100.0	≤0.03/ 100.0	0.06/ 100.0
Tetracycline	≤2/ 91.8	≤2/ 99.7	≤2/ 100.0
Trim/sulfa[b]	≤0.5/ 92.9	≤0.5/ 92.8	≤0.5/ 94.8

a. Susceptibility to penicillin was determined by the ß-lactamase test results.
b. Trimethoprim/sulfamethoxazole.

- The BRO-2 occurrence rates were only 4, 4, 3, and 3% for 1997, 1999, 2001 and 2003, respectively. The overall rate in Canada (8/101 isolates, 7.9%) was significantly higher than in the USA (6/298, 2.0%). BRO-2 isolates were more commonly detected from sites in Manitoba, Nova Scotia, Ontario, Ohio and Massachusetts.

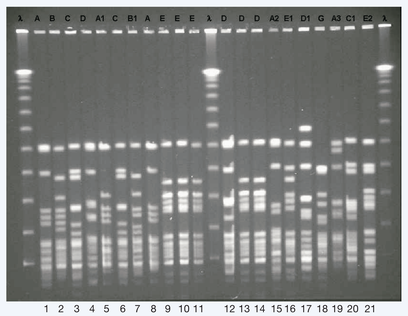
- BRO-2 producing isolates are considered more susceptible to ß-lactam agents than BRO-1 producers. Differences among penicillin and ampicillin MIC distributions were observed between the two enzyme types (Table 2). Penicillin MIC distribution was tri-modal (≤ 0.03, 1-2, >4 mg/L) and ceftriaxone bi-modal (0.016, 0.5), yet MIC values and zone diameter distributions of BRO-1 and -2 strains significantly overlapped (data not shown). They were best discriminated by the methicillin (mean BRO-1/BRO-2 zone, 10.6/19.4 mm) and penicillin (13.9/24.1 mm) disk tests (data not shown).

- Ribotyping produced an acceptable banding pattern (7- 8 bands) for *M. catarrhalis* isolates, but did not discriminate the isolates well. All the isolates tested (14 BRO-2 producers and seven BRO-1 producers) belonged to the same ribogroup (105.572.8). In contrast, PFGE revealed seven different patterns and eight subtypes among the 21 isolates (Figure 1). BRO-2 producing strains could be divided into five patterns and two subtypes with two BRO-2 clusters easily identified, both from Canada.

Table 2. Distribution of penicillin and ampicillin MIC values among BRO-1 and -2 producing *M. catarrhalis* isolates.

Antimicrobial Agent	BRO-type	Cumulative % inhibited at MIC (mg/L) ≤0.5	1	2	4	>4
Penicillin	BRO-1	4.9	18.7	30.6	41.0	100.0
	BRO-2	50.0	78.6	85.8	100.0	-
Ampicillin	BRO-1	33.3	46.7	70.4	89.3	100.0
	BRO-2	80.0	90.0	100.0	-	-

Figure 1: PFGE patterns of BRO-2 and BRO-1 producing isolates from United States and Canada. λ – 48.5 Kb lambda molecular weight ladder. Lanes 1-14 represent BRO-2 producing strains, lanes 15-21 represent BRO-1 producing strains. Letter designations at the top are PFGE types assigned.



CONCLUSIONS

- BRO-1 prevalence increased in the 1980's at dramatic rates due to horizontal gene transfer, and BRO-2 occurrence reportedly declined for the United States. This BRO-1 and -2 enzyme prevalence update for North America (1997-2003) shows stability at 96 – 97% and 3 – 4 %, respectively.
- Prevalence of BRO-2 in Canada was significantly higher compared to the USA isolate pool.
- Phenotypic tests (zone diameter or MICs) can not distinguish accurately between these BRO ß-lactamase types, necessitating the use of molecular applications.
- Also, automated ribotyping analysis failed to demonstrate any usefulness as an epidemiological test for *M. catarrhalis*. PFGE proved superior for isolate discrimination.
- Clonal spread among community-acquired isolates producing BRO-1 and -2 was evident from this small sample of strains tested.

SELECTED REFERENCES

Bootsma HJ, van Dijk H, Verhoef J, Fleer A, Mooi FR (1996). Molecular characterization of the BRO beta-lactamase of *Moraxella* (*Branhamella*) *catarrhalis*. *Antimicrob Agents Chemother* 40: 966-972.

Bootsma HJ, Aerts PC, Posthuma G, Harmsen T, Verhoef J, van Dijk H, Mooi FR (1999). *Moraxella* (*Branhamella*) *catarrhalis* BRO beta-lactamase: a lipoprotein of gram-positive origin? *J Bacteriol* 181: 5090-5093.

Bootsma HJ, van Dijk H, Vauterin P, Verhoef J, Mooi FR (2000). Genesis of BRO beta-lactamase-producing *Moraxella catarrhalis*: evidence for transformation-mediated horizontal transfer. *Mol Microbiol* 36: 93-104.

Koseoglu O, Ergin A, Gurkan Aydin N, Hascelik G (2004). Molecular characterization of BRO beta-lactamases of *Moraxella catarrhalis* strains isolated from carrier children. *Mikrobiyol Bul* 38: 335-340.

Levy F, Walker ES (2004). BRO beta-lactamase alleles, antibiotic resistance and a test of the BRO-1 selective replacement hypothesis in *Moraxella catarrhalis*. *J Antimicrob Chemother* 53: 371-374.

Richter SS, Winokur PL, Brueggemann AB, Huynh HK, Rhomberg PR, Wingert EM, Doern GV (2000). Molecular characterization of the beta-lactamases from clinical isolates of *Moraxella* (*Branhamella*) *catarrhalis* obtained from 24 U.S. medical centers during 1994-1995 and 1997-1998. *Antimicrob Agents Chemother* 44: 444-446.