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ORIGINAL ARTICLE

Antimicrobial resistance of *Moraxella catarrhalis* isolates in Taiwan

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Background/Purpose: The prevalence of ampicillin-resistant *Moraxella catarrhalis* has been higher in Taiwan than in other countries, with reports of 97.7% in the 1990s. The aims of this study were to assess resistance trends for *M. catarrhalis*, which causes respiratory tract infections, against several classes of oral antibiotics and to compare the minimum inhibitory concentration (MIC) of antimicrobial agents against *M. catarrhalis* isolates between 1993–1994 and 2001–2004.

Methods: Clinical isolates of *M. catarrhalis* ($n = 314$) were collected from 11 large medical centers in Taiwan between 2001 and 2004. β-Lactamase production tests were performed. The MICs for 13 different oral antibiotics were calculated using the agar dilution method. Pulsed-field gel electrophoresis (PFGE) was performed for 18 randomly selected high-level ampicillin-resistant (BRO-1 β-lactamase-positive, MIC $\geq$ 32 μg/mL) isolates to investigate their genetic relatedness.

Results: The overall rate of β-lactamase-producing isolates was 97.8% (307/314). All isolates were susceptible to amoxicillin + clavulanate, chloramphenicol, cefixime, ciprofloxacin, erythromycin, levofloxacin, moxifloxacin, and roxithromycin. The rate of resistance to cefaclor and cefuroxime was 8.3% and 1.3%, respectively, while no resistance was found in 1993–1994. Resistance to trimethoprim–sulfamethoxazole (SXT) and tetracycline was 18.5% and 19.8%, respectively. Comparison of 1993–1994 and 2001–2004 isolates revealed that the zone diameter for amoxicillin + clavulanate disks decreased from 43 mm in 1993–1994 to 32 mm in 2001–2004 ($p < 0.001$). However, MIC_{50} (0.25 μg/mL in both 1993–1994 and 2001–2004) and MIC_{90} (0.5 μg/mL in both 1993–1994 and 2001–2004) for amoxicillin + clavulanate did not differ

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between the study periods. The PFGE typing results demonstrate that at least two closely related BRO-1 clones are spreading in Taiwan.
Conclusion: The rates of resistance to cefaclor, cefuroxime, tetracycline and SXT are now increasing in Taiwan. Molecular typing showed that at least two closely related BRO-1 clones are circulating. Although amoxicillin + clavulanate remains the antimicrobial therapy of choice for *M. catarrhalis* infections, continued surveillance of antimicrobial susceptibility and application of control measures against further transmission are required to inhibit the emergence of the resistant strains.

Introduction

Moraxella catarrhalis (formerly *Branhamella catarrhalis*), a Gram-negative aerobic diplococcus, has been recognized as an increasingly important pathogen in respiratory infections including otitis media, sinusitis, acute bronchitis, and pneumonia.[1,2] It resides exclusively in humans and colonizes the nasopharynx and occasionally the conjunctiva and genital tract.[3] Colonization of the respiratory tract is believed to be a precursor of infection, although the mechanism is not well understood.[4] A high percentage (up to 75%) of colonization was found in infants and decreased with age.[3] The prevalence of colonization in healthy adults is low (1–3%).[5]

M. catarrhalis isolates frequently harbor a β-lactamase enzyme designated BRO (from *Branhamella* and *Moraxella*). Two distinct BRO-type β-lactamase enzymes, BRO-1 and BRO-2, have been found since 1976.[6] BRO-positive isolates have increased rapidly in recent years, and account for more than 80–90% of isolates in Europe and North America[7,8] and nearly 98% in Taiwan.[9] Although a third BRO enzyme (BRO-3) was postulated by Christensen et al,[10] BRO-3 is now considered to be a membrane-bound precursor rather than a distinct enzyme.[11] BRO-1 and BRO-2 can be detected by polymerase chain reaction (PCR) methods using specific primers.[12] Our previous investigation revealed that BRO-1 is the most common enzyme among β-lactamase-positive isolates (238/270 isolates, 88%), with only 12% (32/270) of β-lactamase-producing isolates containing BRO-2.[13] Both enzymes are encoded by chromosomal genes and are phenotypically identical and membrane-associated. The proteins differ by only a single amino acid.[14] Fung et al showed significant differences between the geometric mean minimum inhibitory concentration (MIC) of ampicillin for BRO-1- and BRO-2-producing strains, and smaller differences for amoxicillin + clavulanate, loracarbef, cefixime, and cefetamet.[15] The difference is attributed to the production of more enzymes as a consequence of the higher transcriptional activity of the *BRO-1* gene.[4,6]

The continuing increase in the antibiotic resistance of respiratory pathogens remains a global problem. Surveillance to monitor shifting trends in resistance is vital and ultimately influences the selection of antimicrobial agents available for use against a particular organism.[16] Jaecklin et al emphasized the importance of ongoing antimicrobial susceptibility surveillance studies at a regional level.[17]

In this study, we collected 314 *M. catarrhalis* isolates from 11 medical centers in Taiwan in 2001–2004 to assess resistance trends for this organism against 13 different oral antibiotics used to treat respiratory tract infections. The rapid increase in β-lactamase-producing strains of *M. catarrhalis* is a global problem. The ultimate aim of the study was to obtain a clearer understanding of BRO distributions to gain an insight into clonal spreading in Taiwan. Therefore, we compared MIC values to those of isolates obtained in 1993–1994 and determined the genetic relatedness of ampicillin-resistant isolates using pulsed-field gel electrophoresis (PFGE).

Material and Methods

Bacterial strains

A total of 314 clinical isolates of *M. catarrhalis* were consecutively collected from 11 geographically scattered laboratories in Taiwan in 2001–2004. One isolate was accepted for each episode of infection. The isolates were sent to Taipei Veterans General Hospital for further identification and antimicrobial susceptibility testing. Organisms were immediately subcultured onto chocolate agar plates to check viability and purity. Identification was confirmed by Gram stain, colony morphology, hydrolysis of tributyrin, and positive oxidase and DNase tests.[15] β-Lactamase production was confirmed by a nitrocefin test.[15]

Antimicrobial susceptibility testing

To assess the trends of ampicillin resistance of *M. catarrhalis*, isolates were inoculated into 1 mL of brain–heart infusion (BHI) broth with 5% Fildes' extract supplement and incubated for 5 h at 37°C. Each suspension was diluted 1:100 in peptone water and swabbed onto Muller-Hinton (MHA) agar supplemented with 0.25% lysed horse blood. Disks of 6 mm in diameter containing ampicillin (2 μg) or amoxicillin + clavulanate (2 μg + 1 μg) were applied. All plates were incubated in 5% CO_2 and 95% air at 37°C for 18 h. Zone diameters were measured and recorded to the nearest whole millimeter.

MIC values for the 13 antimicrobial agents were determined using an agar dilution technique with horse-blood-supplemented MHA agar. A Denley multipoint inoculator (Denley Instruments, Billingshurst, UK) was used to deliver 0.003 mL of 1:100 dilutions of each organism prepared as for disk testing (10^4 colony-forming units/spot) to the agar surface. All plates were incubated in 5% CO_2 and 95% air at