January 2014

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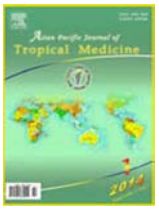
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading doi: 10.1016/S1995-7645(14)60237-6

β-Lactamase production and antimicrobial susceptibility pattern of *Moraxella catarrhalis* isolates: report from Pakistan

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ARTICLE INFO

Article history:
Received 6 Dec 2013
Received in revised form 15 Mar 2014
Accepted 3 May 2014
Available online 28 Jul 2014

Keywords
Moraxella catarrhalis
Antimicrobial resistance in Pakistan
β-lactamase enzyme
Moraxella catarrhalis resistance pattern in Pakistan

ABSTRACT

Objective: To assess the frequency of β-lactamase production and antimicrobial resistance in *Moraxella catarrhalis* isolated from clinical specimens in Pakistan.

Methods: This cross sectional study (January to December 2010) was conducted in clinical microbiology laboratory of Aga Khan University Hospital. A total of 97 clinical respiratory specimens growing *Moraxella catarrhalis* were included. Frequency of β-lactamase production and antimicrobial resistance rates against ampicillin, erythromycin, ciprofloxacin and tetracycline were noted by performing minimum inhibitory concentration (MIC). MICs were calculated as MIC_{50} and MIC_{90}.

Results: β-Lactamase production was detected in 84% of isolates, which correlated well with high MIC of ampicillin. Majority of isolates were susceptible to erythromycin (97%) and tetracycline (96%) with MIC_{90}=0.12 mg/L and MIC_{90}=1 mg/L respectively. All isolates were found susceptible to ciprofloxacin (MIC_{90}=0.06 mg/L).

Conclusions: Result suggests that empirical use of ampicillin should be discouraged while treating respiratory tract infections. This also emphasizes the importance of continuous surveillance in order to detect emerging resistance in *Moraxella* isolates.

1. Introduction

Over the last few decades the increasing pathogenicity and capacity to produce β-lactamase enzyme has evolved *Moraxella catarrhalis (M. catarrhalis)* from a benign commensal to a genuine pathogen[1,2]. Currently, it is considered as one of the common cause of upper and lower respiratory tract infections. Though rarely reported, it can also cause severe infections including pneumonia, endocarditis, septicaemia and meningitis *etc*[1-4]. *M. catarrhalis* also contributes in mixed respiratory tract infections with other pathogens such as *Streptococcus pneumoniae* (*S. pneumoniae*) and/or *Haemophilus influenzae (H. influenzae)*. In such cases, β-lactamase produced by this organism may render ampicillin ineffective against susceptible *S. pneumoniae* and *H. influenzae*[5].

Sweden was the first country to report the presence of β-lactamase in *M. catarrhalis* in 1977[6]. Since then β-lactamase production has been reported from various countries with increasing frequency (even above 90%)[7-9]. Though it remains rare, reports of increasing resistance to other oral antibiotics useful to treat community acquired respiratory tract infections (CARTI) are also documented in *M. catarrhalis*[10,11].

Information regarding frequency of β-lactamase production in *M. catarrhalis* is limited from Pakistan. A study from Pakistan reported 93.5% ampicillin susceptibility in *M. catarrhalis* but authors did not report β-lactamase activity in those isolates[12]. Another study reported 68.7% β-lactamase production in *M. catarrhalis*. However this study had the limitations of small sample size and use of less sensitive filter paper acidometric test for β-lactamase detection[13]. The local antimicrobial susceptibility data of other oral antibiotics used for CARTI against *M. catarrhalis* is also missing. Surveillance to monitor shifting trends in resistance is vital as it ultimately influences the selection of antimicrobial agents

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Foundation Project: Supported by the departmental seed money grant of Aga Khan University Hospital, Karachi, Pakistan (Grant No. SM2011-04).

available for use against a particular organism. Therefore, this study aimed to assess the frequency of β-lactamase production in *M. catarrhalis* and also drug resistance against ampicillin, erythromycin, ciprofloxacin and tetracycline, so as to guide empirical therapy in CARTI.

2. Material and methods

This cross sectional study was conducted from January 2010 to December 2010 in the clinical microbiology laboratory of Aga Khan University Hospital (AKUH). The AKUH laboratory has an extensive network of more than 200 collection units throughout Pakistan. Through this it caters a vast outpatient population across the country.

2.1. Respiratory specimens

Clinical respiratory specimens (sputum, tracheal aspirate, bronchoalveolar lavage, middle ear fluid, nasopharyngeal swab/aspirate and sinus aspirate) from community that were received in AKUH laboratory for culture and sensitivities during study period were included.

2.2. Isolation of M. catarrhalis

The identification of the *M. catarrhalis* was confirmed by colony morphology, Gram stain appearance and conventional biochemical tests such as positive oxidase, catalase, and DNAase test. Clinical isolates were saved in glycerol-phosphate buffer at −80 °C. Duplicate samples from same patient were excluded. Demographic data including gender and age and seasonal distribution were also noted.

2.3. Antimicrobial susceptibility testing

Ampicillin, erythromycin, ciprofloxacin, and tetracycline powder were obtained from Sigma-Aldrich, UK. Minimum inhibitory concentrations (MICs) of the antimicrobials were performed according to agar dilution method recommended by British Society for Chemotherapy (BSAC) [14]. Agar plates were prepared with the antimicrobials to be tested incorporated in the media supplemented with 5% defibrinated blood in double dilution series. Bacterial suspension 10^4 CFU/spot was prepared and applied by the multipoint inoculator. For testing ampicillin, inoculum of 10^6 CFU was used. Plates were incubated at 37 °C aerobically for 18 h. MIC breakpoints provided by the BSAC[15] were used for interpretation and isolates were categorized as being sensitive (S), intermediately susceptible (I), or resistant (R).

Interpretation of the MIC breakpoints was as follows: Ampicillin: S≤1 mg/L, R>1 mg/L; Ciprofloxacin: S≤0.5 mg/L, R>0.5 mg/L; Tetracycline: S≤1 mg/L, I=2 mg/L, R≥2 mg/L; Erythromycin: S≤0.25 mg/L, I=0.5 mg/L, R≥0.5 mg/L.

β-Lactamase production was detected by nitrocefin (Oxoid) test. The reference strains used for quality control included *S. pneumoniae* ATCC strain 49619 and *Staphylococcus aureus* ATCC strain 29213. MICs were calculated as MIC_{50} and MIC_{90} (MIC causing inhibition of 50% and 90% of isolates, respectively).

2.4. Data management and statistical analysis

Data was entered and analysed by the statistical software SPSS version 19.0. Frequencies with percentages for age and seasonal distribution, β-lactamase production and resistance for each drug were computed.

2.5. Ethical approval

This study was approved by the Ethics Review Committee of the Aga Khan University, Pakistan.

3. Results

A total of 4 440 respiratory specimens were received during study period. Out of these, 30% (1 333) were reported positive for respiratory pathogens. About 60% (2 680) specimens were received from male patients.

A total of 97/1 333 clinical respiratory specimens yielded growth of *M. catarrhalis*. Amongst studied isolates of *M. catarrhalis*, 71% (69) were from male and 65% (63) were recovered from patients aged greater than 65 years. The age distribution is shown in Table 1.

Table1

Distribution of *M. catarrhalis* isolates (*n*=97) among different age groups.

Age range in years	Number (%)
0-15	11 (11.5)
16-30	11 (11.5)
31-45	12 (12.0)
46-65	24 (25.0)
>65	39 (40.0)

Majority of isolates (50.5%, 49) were grown in the winter months (November-February) with the peak incidence in the month of January (Figure 1). β-Lactamase production was detected in 84.5% (81) of isolates, which correlated well with the high ampicillin MIC among them. Ninety-seven percent of isolates were found susceptible to erythromycin with MIC_{90} of 0.12 mg/L and ninety-six percent susceptible to tetracycline with MIC_{90} of 1 mg/L. All isolates were susceptible to ciprofloxacin. The MIC distribution for the

four antibiotics tested is given in Table 2.

Table 2

Distribution of MICs (μg/mL)) for antimicrobials tested against *M. catarrhalis* isolates (*n*=97).

MIC	Ampicillin	Ciprofloxacin	Tetracycline	Erythromycin
128	–	–	–	–
64	2	–	–	–
32	3	–	–	–
16	4	–	–	–
8	17	–	1	–
4	21	–	2	–
2	34	–	1	–
1	9	–	10	2
0.5	4	3	57	1
0.25	–	2	13	1
0.125	3	5	8	41
0.06	–	11	5	40
0.03	–	32	–	12
0.015	–	44	–	–
MIC_{90}	8	0.06	1	0.125
MIC_{50}	2	0.03	0.5	0.06
MIC range	0.125–64	0.015–0.5	0.06–8	0.03–1
Sensitive (%)	15.5	100	96	97

BSAC break points of MICs for ampicillin (mg/L): S≤1, R>1, ciprofloxacin (mg/L): S≤0.5, R>0.5, tetracycline (mg/L): S≤1, I=2, R≥2 and erythromycin (mg/L): S≤0.25, I=0.5, R≥0.5.

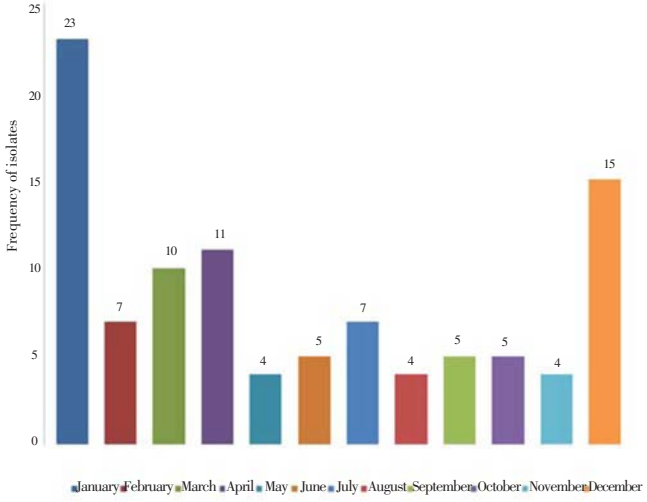

Figure 1. Monthly rate of isolation of *M. catarrhalis*.

4. Discussion

This study suggests that empirical use of ampicillin/amoxicillin in CARTI would miss β-lactamase producing *M. catarrhalis* strains and may lead to treatment failure. It may also hamper the treatment of certain concomitant organisms, such as penicillin susceptible *S. pneumoniae* and *H. influenzae* in cases of co-infection[1,5].

This study demonstrates that frequency of β-lactamase production among *M. catarrhalis* is entirely consistent with what reported globally[7-9]. Previous study from Pakistan reported 68.7% β-lactamase production. However, in that study they used less sensitive acidometric test for enzyme detection[13]. Increasing frequency of enzyme production was reported from the neighbouring country India. Larson *et al.* reported enzyme production in 68% isolates in 1999 and Anita *et al.* demonstrated 86% of isolates with enzyme activity in 2011[16-17].

In this study, high ampicillin MIC correlates well with high β-lactamase production. Previously published work from Pakistan reported 93.5% susceptibility to this drug. However, that study had major limitation that authors did not test the β-lactamase production in their isolates by the standard method[12].

Another important and promising finding of our study is that, resistance to other commonly used oral antibiotics in CARTI such as tetracycline and erythromycin remained low. Our finding regarding the tetracycline resistance is comparable to those obtained from most Asia pacific regions (3.2%) except Taiwan where it is around 19%[9,18]. Similarly macrolide resistance is comparable to China 5.8%, however it is much lower than that reported previously from Pakistan (25%)[7,12].

Although ciprofloxacin is an accessible over the counter drug in Pakistan and easily available with or without a prescription, fortunately none of the isolate was found to be ciprofloxacin resistant. This finding is consistent with other reports published globally[7-9]. Though Tabussum *et al.* from Karachi reported 7% and a study from India reported 20% ciprofloxacin resistance[12,19].

Globally, recovery of majority of the isolates from respiratory samples from male patients was also observed in this study[20]. We also found that *M. catarrhalis* infections are more frequent among older age group. Similar finding was reported from India and Nepal[20,21]. The predilection of CARTI in the older age group has been studied and several reasons have been proposed including age related changes in systemic immunity, respiratory tract mucosal changes due to smoking and several other co-morbidities[1,2,22].

Majority of isolates were recovered in winter season, which is similar to finding of others[20-22]. A viral co-infection has been anticipated as the mechanism for the seasonal variation, it causes weakening of local immunity thereby promoting infection by these pathogens[22].

This study is limited by its inability to perform molecular detection of BRO 1 and 2 for identification of β-lactamase enzymes. But it may be anticipated that majority were BRO 1 β-lactamase enzyme as they had high MICs for ampicillin[23].

In summary, result of this study supports the recommendation of BSAC in 2011 that clinical laboratories are no more required to check the susceptibility of ampicillin against *M. catarrhalis* and should report them resistant[24]. Therefore appropriate alternative antibiotic should be

selected empirically in regard of treating CARTI. Finally we emphasize the judicious use of antibiotics in this region and continued surveillance to detect emerging resistance.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

This research project is supported by the departmental seed money grant of Aga Khan University hospital, Karachi, Pakistan (Grant No. SM2011-04).

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