EPIDEMIOLOGY OF *MORAXELLA CATARRHALIS* INFECTIONS AMONG THAI PATIENTS TREATED AT SIRIRAJ HOSPITAL, THAILAND DURING 2012-2015

Chanwit Tribuddharat and Somporn Srifuengfung

Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract. *Moraxella catarrhalis* is an important pathogen but data from Thailand is scarce. We studied epidemiology, β-lactamase production, and presence of bla_{BRO} gene among *M. catarrhalis* isolated from patients of all ages. A total of 173 non-duplicate isolates from all clinical specimens were identified by standard bacteriological methods. β-lactamase production and bla_{BRO} gene were detected by nitrocefin and polymerase chain reaction (PCR), respectively. Incidence of *M. catarrhalis* was highest among patients aged <10 years (31.2%; 54/173), followed by patients aged >80 years (16.2%; 28/173). The month *M. catarrhalis* was isolated most commonly was January, followed by February and December. From 173 *M. catarrhalis* isolates, 93.1% were positive for β-lactamase production. All 30 β-lactamase-producing *M. catarrhalis* isolates randomly selected to determine the bla_{BRO} gene using PCR were positive. Molecular study by DNA sequencing showed the presence of the β-lactamase gene (bla_{BRO-1}).

Keywords: *Moraxella catarrhalis*, epidemiology, Thai patients

INTRODUCTION

In the past, *Moraxella catarrhalis* was considered a saprophyte of the upper respiratory tract and thought not to cause pathology (Constantinescu *et al*, 2016). Now *M. catarrhalis* is considered an important pathogen causing bacteremia (Sano *et al*, 2010), arthritis (Melendez and Johnson, 1991), respiratory infections (Murphy and Parameswaran, 2009), middle ear infections (Lee *et al*, 2014), sinusitis (Verduin *et al*, 2002), eye infections (McGregor *et al*, 1998), and central nervous system infections (Verduin *et al*, 2002). *M. catarrhalis* is a common cause of otitis media and sinusitis in children and upper and lower respiratory tracts infections in adults (Constantinescu *et al*, 2016). In the United States, *M. catarrhalis* is the third most common cause of otitis media and sinusitis, following *Streptococcus pneumoniae* and *Haemophilus influenzae* (Constantinescu *et al*, 2016). *M. catarrhalis* infections is more common in adults with underlying conditions, especially in the elderly (Constantinescu *et al*, 2016). *M. catarrhalis* infections can occur at any age but colonization is more common among children than adults (Constantinescu *et al*, 2016). *M. catarrhalis* tends to cause more

Correspondence: Somporn Srifuengfung, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Road, Bangkok Noi, Bangkok 10700, Thailand.
Tel: +66 (0) 2419 9684; Fax: +66 (0) 2411 3106
E-mail: somporn.sri@mahidol.ac.th

upper respiratory tract infection in children and lower respiratory tract infection in adults (Khan *et al*, 2010). Diseases caused by *M. catarrhalis eg*, otitis media tends to predominate in children, with chronic obstructive pulmonary disease being found much more commonly in adults (Khan *et al*, 2010).

The first β-lactamase-producing *M. catarrhalis* isolate was reported from Sweden in 1976 (Khan *et al*, 2010). This enzyme mediates resistance to penicillin (Khan *et al*, 2010). Over the past 30 years, there has been a dramatic increase in the percentage of β-lactamase-producing *M. catarrhalis* clinical isolates (Khan *et al*, 2010). Studies from Australia, Europe, USA, England, and Scotland have reported that β-lactamase production is found in more than 90% of *M. catarrhalis* isolates (Verduin *et al*, 2002; Koseoglu *et al*, 2004). Although β-lactamase production is common among *M. catarrhalis* isolates, many β-lactam antibiotics continue to have good efficacy against *M. catarrhalis*, including β-lactam/β-lactamase-inhibitor combinations (*eg*, amoxicillin-clavulanate), cephalosporins (*eg*, cefixime), macrolides, quinolones (*eg*, ciprofloxacin, ofloxacin), and trimethoprim-sulfamethoxazole (Tille, 2014). Given the efficacy of several drugs to treat *M. catarrhalis*, susceptibility testing is not routinely required to guide therapy (Tille, 2014; CLSI, 2015). Although not common, resistance to erythromycin and trimethoprim-sulfamethoxazole may occur (Tille, 2014). Two major forms of β-lactamase (BRO-1 and BRO-2) are described on the basis of their isoelectric focus patterns and their levels of β-lactamase production (Khan *et al*, 2010). The BRO-1 enzyme is found in the majority of β-lactamase-producing isolates and is associated with a higher level of resistance than BRO-2 isolates (McGregor *et al*, 1998; Khan *et al*, 2010). The two enzymes differ by only a single amino acid substitution and are encoded by chromosomal genes (bla_{BRO}) that can be transferred from cell to cell by conjugation (Verduin *et al*, 2002). β-lactamase in *M. catarrhalis* may indirectly benefit other pathogens, such as *S. pneumoniae,* by helping susceptible isolates evade penicillin and ampicillin therapy, possibly contributing to treatment failure (Hol *et al*, 1994).

The aim of this study was to investigate the epidemiology, β-lactamase production, and presence of the bla_{BRO} gene among *M. catarrhalis* isolates obtained from patients presenting at Siriraj Hospital, Thailand during 2012-2015.

MATERIALS AND METHODS

A total of 173 *M. catarrhalis* clinical isolates were obtained, each from a different patient, at the Bacteriology Laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand from 1 January 2012 to 31 December 2015. Sputum was considered an acceptable culture specimen if it contained >25 polymorphonuclear cells and <25 epithelial cells per low-power field. For broncho-alveolar lavage (BAL) fluid, a 1 μl standard calibrated loop was used. A minimum cut-off value to be considered a positive culture for *M. catarrhalis* was 10^4 colony formimg unit (CFU)/ml. Isolation and identification for *M. catarrhalis* was performed following standard microbiological techniques (Tille, 2014). *M. catarrhalis* isolates were maintained at -70°C in 5% trypticase soy broth plus 20% (V/V) glycerol until use. Reviewing of all positive culture for *M. catarrhalis* was performed by age-groups of patients (<1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80 and >80 year).

β-lactamase production, testing (Tille, 2014) was performed using Nitrocefin disks (Becton, Dickinson, Franklin Lakes, NJ). *S. pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 25923 were used as negative and positive controls in β-lactamase production, respectively.

Thirty β-lactamase-producing study isolates were tested for the presence of the bla_{BRO} gene using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN). Purified genomic DNA was used for the polymerase chain reaction (PCR). The primers used for the amplification (Koseoglu *et al*, 2004) were: BRO-F 5′-ATAATGATGCAACGCCGTCAT-3′ and BRO-R 5′-GGCTTGTTGGGTCATAAATT-3′for the bla_{BRO} gene, which resulted in a 996 bp product size. Amplification consisted of denaturation at 93°C for 5 minutes; followed by 30 cycles of 1 second at 93°C, 1 minute at 52°C, and 72°C for 1 minute; with a final extension at 72°C for 7 minutes. The purified PCR product was verified by DNA sequencing with the BRO-F and BRO-R primers in order to confirm the sequence of the bla_{BRO} gene (BioDesign, Pathum Thani, Thailand). The DNA sequences of bla_{BRO} positive genes were analyzed using public domain software available from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignment was analyzed using the BioEdit software program (Ibis Biosciences, Carlsbad, CA).

RESULTS

A total of 173 patients were included in this study. The mean (± standard deviation) age of the studied patients was 45.2 (± 33.0) years (range: 1 month-106 years (Table 1). Eighty-nine patients (51.5%) were male. The M:F gender ratio

Table 1
M. catarrhalis isolates by year among studied subjects.

Year	No. of isolates	Age range of patients	Mean ± standard deviation	Male to female ratio	Sources of specimens		
					Specimens	No. of isolates	%
					Sputum; bronchial wash; bronchoalveolar lavage; tracheal suction	117; 2; 11; 6 (total 136)	78.6
2012	44	1 mo - 89 yrs	45.8 ± 33.4	1.00:1.0	Adenoid tissue	19	11
2013	60	1 mo - 87 yrs	43.7 ± 32.2	1.73:1.0	Eye	8	4.6
2014	47	1 mo - 106 yrs	50.2 ± 34.0	0.74:1.0	Ear	4	2.3
2015	22	1 yr - 88 yrs	37.3 ± 32.7	0.69:1.0	Nose; aspiration sinus; urine	2; 3; 1 (total 6)	3.5
Total	173	1 mo - 106 yrs	45.2 ± 33.0	1.06:1.0	Total	173	100

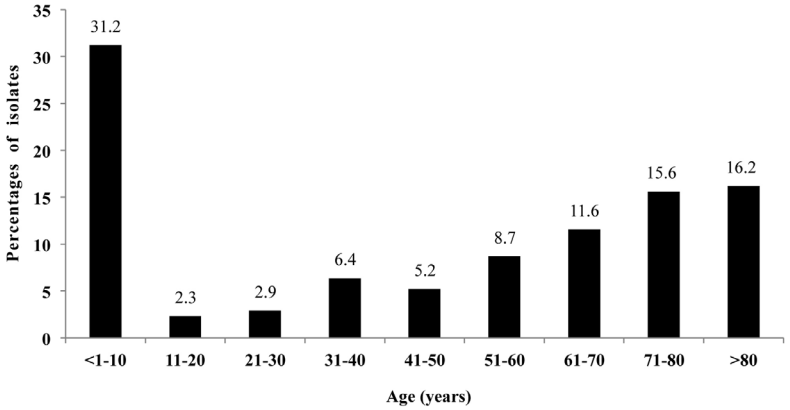

Fig 1–Age distribution of studied patients.

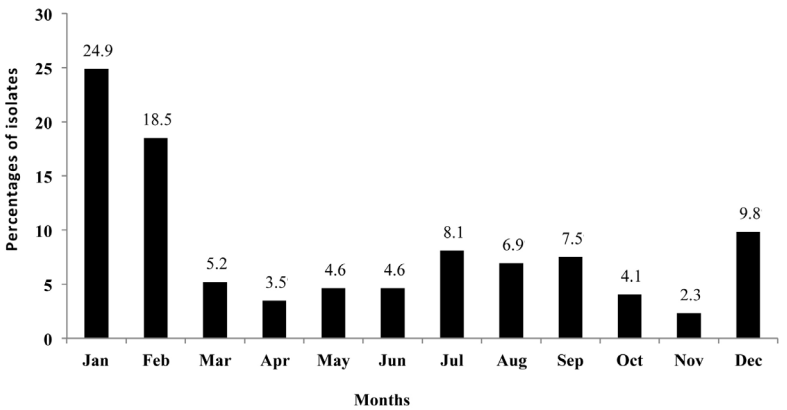

Fig 2–Percentages of *M. catarrhalis* isolates by month.

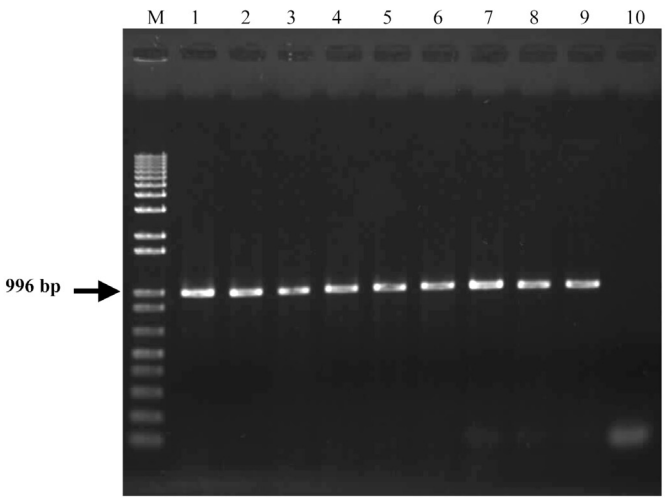

Fig 3–PCR products of bla_{BRO} genes among *M. catarrhalis* isolates showing a 996 bp product size. Lane M, the standard 1 Kb plus DNA size marker. Lanes 1-9, bla_{BRO} gene-positive isolates. Lane 10, the negative control.

was 1.06:1. The sources of the clinical specimens included sputum (117; 67.6%), bronchial washing (2; 1.2%), bronchoalveolar lavage (BAL) (11; 6.4%), tracheal suction (6; 3.5%) and adenoid biopsy tissue (19; 11%). For BAL quantitative culture, a range of *M. catarrhalis* $3x10^4$ to $\geq 10^5$ CFU/ml positive results was found, with $\geq 10^5$ CFU/ml being the most common bacterial count (72.7%) (Table 2). The reason for giving data for BAL only is that BAL needs quantitative culture to identify true *M. catarrhalis* infection, not bacterial contamination during BAL collection from patients.

Incidence of *M. catarrhalis* was highest among patients aged <10 years (31.2%; 54/173), followed by patients aged >80 years (16.2%; 28/173) (Fig 1). The month *M. catarrhalis* was isolated most commonly was January, followed by February and December (Fig 2).

One hundred sixty-one of 173 (93.1%) of *M. catarrhalis* isolates were positive for β-lactamase production. All 30 β-lactamase-producing *M. catarrhalis* isolates randomly selected to determine the bla_{BRO} gene using PCR were positive (Fig 3).

Table 2
Quantitative culture result from broncho-alveolar lavage.

Colony-forming units/ml	No. of isolates (%)
$\geq 10^5$	8 (72.7)
7×10^4	1 (9.1)
4×10^4	1 (9.1)
3×10^4	1 (9.1)
Total	11 (100)

We randomly selected two isolates for DNA sequencing of the bla_{BRO} gene. The 996 nucleotide base pairs of the bla_{BRO} gene were DNA sequenced, translated into amino acid sequence, and compared to the GenBank database. We found a 100% nucleotide and amino acid sequence match with *M. catarrhalis* ATCC 43527 (GenBank accession no. U49269) (Fig 4).

DISCUSSION

At the studied hospital there were 22-60 *M. catarrhalis* isolates per year, mostly from the sputum. A previous study reported the respiratory tract was the most common site of infection for *M. catarrhalis* (Murphy and Parameswaran, 2009). A previous study from Japan reported a M:F gender ratio of 1.19:1 (Yamada and Saito, 2014) similar to our study (1.06:1). However, a study from Greece reported a M:Fgender ratio of 1.79:1 (Maraki and Papadaki, 2014).

A previous study reported *M. catarrhalis* infection is common in pediatric patients (Hu *et al*, 2016). Another study (Yamada and Saito, 2014) found 71.4% of patients with *M. catarrhalis* infections were aged ≤10 years, while in our study the percentage was lower at 31.1%. However, in a study from Greece 44.8% of subjects with *M. catarrhalis* infections were aged ≥61 years (Maraki and Papadaki, 2014).

Our finding that *M. catarrhalis* was the most common in January is similar to a previous study finding a peak in infections during autumn and winter (Verhaegh *et al*, 2011). Higher detection rates of *M. catarrhalis* during the colder months may be associated with much nasal secretions due by more viral illnesses during cold months (Hendley *et al*, 2005) with greater risk of secondary bacterial infections.

The high rate of β-lactamase-producing *M.catarrhalis* in our study (93.1%) is comparable to the reported worldwide estimated prevalence of *bro* gene carriage among clinical isolates of *M. catarrhalis* of nearly 95% (Khan *et al*, 2010), but far higher than the rate reported from Greece of 47.8% (Maraki and Papadaki, 2014).

More than 90% of *M. catarrhalis* are resistant to penicillin due to the presence of BRO-1 or BRO-2 β-lactamase production (Deshpande *et al*, 2006). Our identification of the bla_{BRO-1} gene in this study was consistent with previous reports that BRO-1 β-lactamase production was 96-100% in USA and Thailand (Deshpand *et al*, 2006; Srifuengfung *et al*, 2016).

In conclusion, this study was the first report of epidemiology as well as the presence of the β-lactamase gene (bla_{BRO-1}) in *M. catarrhalis* clinical isolates in Thailand. Surveillance of this bacterium should be continued to evaluate *M. catarrhalis* infection.

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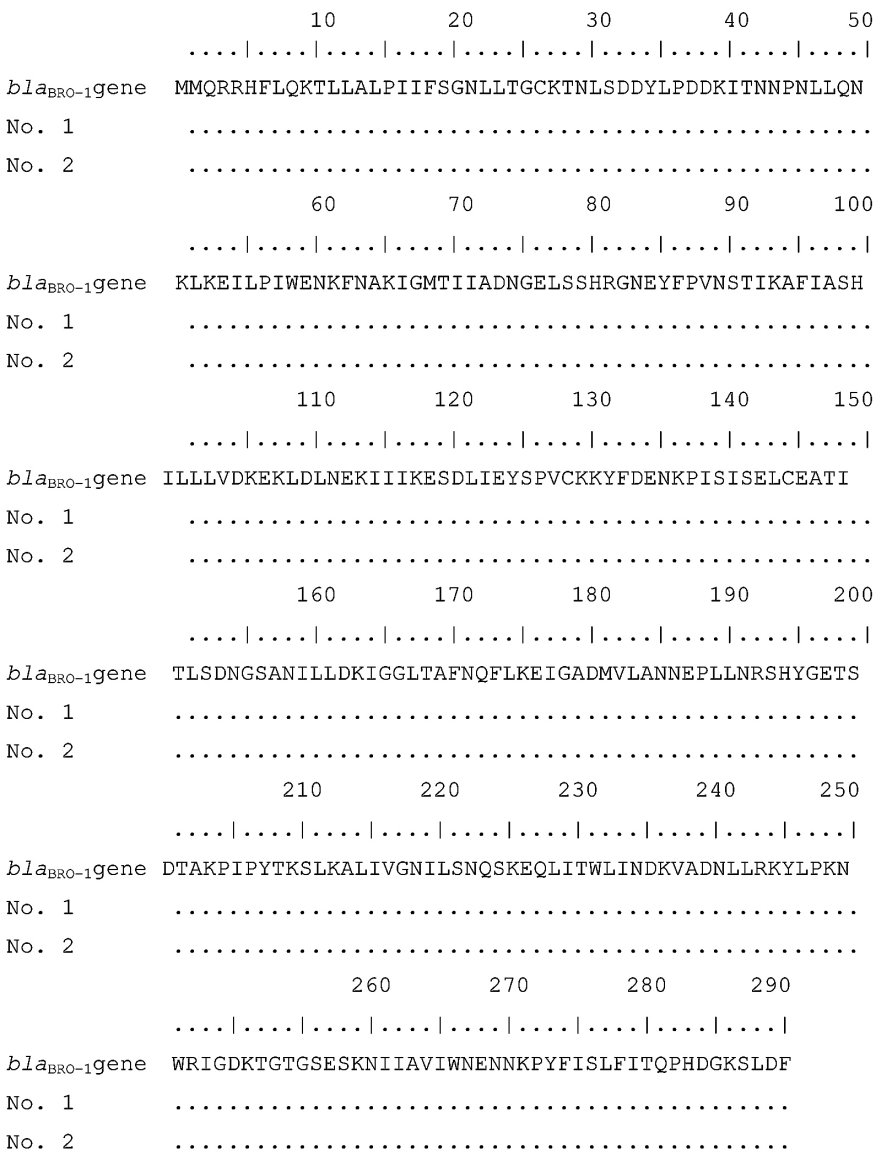

Fig 4–Multiple alignments of the bla_{BRO} gene sequences from sequences of the BRO gene in BRO-1 (*M. catarrhalis* ATCC 43627) and *M. catarrhalis* isolates No. 1-2. Dots represent identical amino acids.

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CONFLICTS OF INTEREST

The authors have no professional conflicts of interest to declare.

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