

FREQUENCY OF *MORAXELLA CATARRHALIS* FROM PATIENTS WITH LOWER RESPIRATORY TRACT INFECTION IN KHARTOUM STATE, SUDAN

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ABSTRACT

Lower respiratory tract infections have been the focus of much attention for almost two decades. Their importance lies in their frequency as a leading cause of morbidity and mortality in all age groups despite all the advances made by medical science. Many reports have been done in many countries reflecting the emergence of *M. catarrhalis* as important pathogen in the last two decades, together with the increasing prevalence of β -lactamase-producing strains, has renewed interest in this bacterial species. The aim of this study was to determine the frequency of *Moraxella catarrhalis* in sputum specimens among patients with lower respiratory tract infection. A total of 200 sputum specimens collected from Khartoum state in Alshaab hospital

during January to June 2011. Bacteriological analysis of sputum specimens were performed for *Moraxella catarrhalis* including inoculation on Sheep blood agar and on chocolate agar, the identity of the isolates was confirmed by DNase test, Tributyrin test and reduction of nitrate to nitrite. Susceptibility testing and β -lactamase production were carried out for each isolate. Out of 200 sputum specimens; (2) (1%) *Moraxella catarrhalis* was recovered from patients with age more than 50 years. The two isolates produced β -lactamase and resistance to ampicillin. They were susceptible to Amoxycylav, Azithromycin, Ceftazidime Ceftriaxon, Cephalexin, Cephotaxime, Chloramphenicol, Ciprofloxacin, Cotrimoxazole and Erythromycin. This study showed that *M. catarrhalis* considered to be one of important respiratory tract pathogen in adults particularly with age more than 50 years

old. Since most strains produce β -lactamase, antibiotic therapy should be guided by *in-vitro* susceptibility tests.

KEYWORDS: *Moraxella catarrhalis*, respiratory infection, β -lactamase, Sputum.

INTRODUCTION

Moraxella catarrhalis is a Gram-negative non encapsulated diplococcal bacterium belonging to the family *Moraxellaceae* the organism was first described in 1896, frequently found as a commensal of the upper respiratory tract in 40–50% of normal school children and in 1 to 5% of healthy adults (Stevanovic *et al.*, 2010). Over the last 20 to 30 years, the bacterium has emerged as a genuine pathogen and is now considered an important cause of both the upper and the lower respiratory tract infections. It is the third most common pathogen isolated in childhood acute maxillary sinusitis and otitis media after only non-typeable *Haemophilus influenzae* and *Streptococcus pneumoniae*, it is responsible for up to 20% of the cases and More than 70% of children under 2 years of age experience at least one episode of acute otitis media (Brooks *et al.*, 2007).

Moreover, *M. catarrhalis* is an important pathogen particularly in adults with chronic obstructive pulmonary disease, (COPD) it was thought to be responsible of 10% of exacerbations of COPD accounting for approximately 2-4 million episodes in the U.S. each year resulting in increased morbidity and mortality of these patients. In immunocompromised host, the bacterium can cause a variety of severe infections including pneumonia, endocarditis, septicaemia, and meningitis; in addition, hospital outbreaks of respiratory disease due to *M. catarrhalis* have been described establishing the bacterium as a nosocomial pathogen. Because most strains of *M. catarrhalis* from clinically significant infections produce β -lactamase which now account for approximately 90 to 95% of isolates (Murphy *et al.*, 2009). Further, it has been suggested that the production of β -lactamases by *M. catarrhalis* could protect colonizing pathogens from the effects of β -lactam antibiotic treatment. Many treatment failures with ampicillin or amoxycillin are due to the production of this enzyme and there is a significant cost in treating disease related to *M. catarrhalis*, complicated in part by a rise in antibiotic-resistant strains (Ahmad and Tahir, 1994).

Common characteristics of the *Moraxella* genus include: a lack of colony pigmentation, Gram-negative staining coccoid/bacillus morphology with a tendency to resist decolorisation; positive with oxidase reagent and tetra-/dimethyl-*p*-phenylenediamine; and a GC content of

between 40 and 47.5 mol%. Optimum growth conditions are achieved on blood agar plates under aerobic conditions at a temperature of approximately 33-37 °C. In general, distinguishing between the different *Moraxella species* tends to be difficult, not least because of the asaccharolytic nature of the genus, though some publications have indicated that 16S rRNA sequence polymorphisms may be a useful adjunct to biochemical testing (Enright *et al.*, 1994; Brenner *et al.*, 2005; and Hays, 2006). The surface antigen of *M.catarrhalis*, including Lipooligosaccharide (LOS) is an important virulence factor. LOS, a predominant surface-exposed component of the outer membrane, has been implicated as a virulence factor in the pathogenesis of *M.catarrhalis* (Verduine *et al.*, 2002).

M. catarrhalis is now considered as an important pathogen in respiratory tract infections, both in children and adult with underlying COPD. Occasionally, the bacterium causes systemic disease, e.g., meningitis and sepsis (Doern *et al.*, 1981; Catlin, 1990; Collazos *et al.*, 1992; and Abuhammour *et al.*, 1999). Bacteremia due to *M. catarrhalis* should be considered especially in febrile children with an underlying immune dysfunction and an upper respiratory tract infection (Abuhammour *et al.*, 1999).

Despite the many reports have been done in many countries reflecting the emergence of *M. catarrhalis* as important pathogen in the last two decades, together with the increasing prevalence of β -lactamase-producing strains, has renewed interest in this bacterial species, there has been no previous report on the isolation of *M. catarrhalis* from clinical specimens in Sudan.

MATERIALS AND METHODS

A total of 200 sputum specimens collected from Khartoum state in Alshaab hospital during January to June 2011. Bacteriological analysis of sputum specimens were performed for *Moraxella catarrhalis* including inoculation on Sheep blood agar and on chocolate agar. The identity of the isolates was confirmed by DNase test, Tributyrin test and reduction of nitrate to nitrite. Susceptibility testing and β -lactamase production were carried out for each isolate. Also control positive strains of *M. catarrhalis* were used in this study (*Moraxella catarrhalis* ATCC 23246, *M. catarrhalis* ATCC 25240 and *M.catarrhalis* ATCC 25238).

RESULTS

In this study a total of 200 sputum specimens collected from patients suspected of having pulmonary infection during January to June 2011. There were (126) males and (74) females

with ages ranging from (19 to 86) years old. The mean age was (41.9) years. All patients had cough with purulent sputum as their major complaint and all were outpatients, they treated with some antibiotics and came to hospital because they still suffering from theirs complain. (2) (1%) *Moraxella catarrhalis* were isolated out of 200 sputum culture. The two isolates of *Moraxella catarrhalis* were in (2) males admitted to alshaab hospital complaining of chronic cough with purulent sputum, sputum for Ziehl-Neelsen stain requested and it was negative, after direct gram stain has been done, abundant gram negative diplococci with pus cells were detected. There was no isolation obtained in females, the frequency of of *Moraxella catarrhalis* isolation is 1% as shown in (table. 1).

Moraxella catarrhalis was observed to grow well on chocolate agar. The incubation period ranged from 24 to 48 hour and the colony appeared small after 24 hour and the size increased with incubation time. They grew better at both in presence and absent of 5 to 10% Carbon dioxide. Typical colonies were usually grey to white, opaque and smooth and slide across the surface of the agar when nudged with the end of a bacteriological loop (hockey puck on ice). The growth of *M. catarrhalis* on sheep blood agar is similar to that of chocolate agar. Typical colonies were usually gray to white, opaque and smooth and slide across the surface of the agar when nudged with the end of a bacteriological loop (hockey puck on ice).

M. catarrhalis was positive for Oxidase, Catalase, Deoxyribonuclease, Nitrate reduction and Tributyrin test. The identification of *M. catarrhalis* was based on tables of Bergey's manual of systematic bacteriology. The result of biochemical tests is summarized in table. (2).

Results of β -lactamase demonstration

Beta-latamase discs were inoculated by the isolates and the reference strains were read immediately. All clinical isolates of *Moraxella catarrhalis* were strong β -lactamase producers (discs with red spots) while the reference strains were non- β -lactamase producers (discs without red spots).

Results of antibiotics susceptibility tests

All isolates were noted to be sensitive to Amoxyclav, azithromycin, ceftazidime ceftriaxon, cephalixin, cephotaxime, chloramphenicol, ciprofloxacin cotrimoxazole and erythromycin but they were resistant to ampicillin while reference strains were sensitive to all including ampicillin, table .(3).

Table. 1: Distribution of study samples and isolation rates.

	Frequency	Percent %	No. of Positive isolates	Total
Male	126	63	2	126
Female	74	37	0	74
Total	200	100	2	200

Table. 2: Results of biochemical tests of *Moraxella catarrhalis* isolates and control strains.

<i>M.catarrhalis</i> strains	ATCC 23246	ATCC 25240	ATCC 25238	<i>M.catarrhalis</i> Isolate
Biochemical tests				
Oxidase	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve
Deoxyribonuclease	+ve	+ve	+ve	+ve
Nitrate reduction	+ve	+ve	+ve	+ve
Tributyrin test	+ve	+ve	+ve	+ve

Table .3: Antibiotics susceptibility pattern of *Moraxella catarrhalis*.

<i>M.catarrhalis</i> strains	ATCC 23246	ATCC 25240	ATCC 25238	<i>M.catarrhalis</i> isolate no. 1	<i>M.catarrhalis</i> isolate no. 2
Antibiotic discs					
Ampicillin	S	S	S	R	R
Amoxyclav	S	S	S	S	S
Azithromycin	S	S	S	S	S
Ceftazidime	S	S	S	S	S
Ceftriaxon	S	S	S	S	S
Cephalexin	S	S	S	S	S
Cephotaxime	S	S	S	S	S
Chloramphenicol	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S
Cotrimoxazole	S	S	S	S	S
Erythromycin	S	S	S	S	S

*S= Sensitive, R= Resistant

DISCUSSION

Lower respiratory tract infections have been the focus of much attention for almost two decades. Their importance lies in their frequency as a leading cause of morbidity and mortality in all age groups despite all the advances made by medical science. Many reports have been done in many countries reflecting the emergence of *Moraxella catarrhalis* as an important pathogen of lower respiratory tract in the last two decades, together with the increasing prevalence of β -lactamase-producing strains, has renewed interest in this bacterial species.

This research was performed to study the frequency of *M. catarrhalis* among patients with lower respiratory tract infection, their antibiotics susceptibility pattern and β -lactamase activity.

Out of 200 sputum specimens two isolates of *Moraxella catarrhalis* were recovered with an incidence of 1%. They were isolated from patients aged 58 and 67 years old in Khartoum state suffering from chronic cough with purulent sputum production. This findings was similar to study done in Malaysia by Ahmad and Tahir (1994) where *Moraxella catarrhalis* was isolated from 15 (0.89%) out of 1678 specimens. However, it was not coinciding with that of Tamang *et al* (2005) who reported 6.9% recovery of the total respiratory isolates. The low isolation rate of *Moraxella catarrhalis* in the present study may be attributed to many reasons firstly the sputum samples were collected from patient's previously administered antibiotics either by clinicians or randomly by themselves and their complaints still present or even increased worse.

Secondly according to many reports, *Moraxella catarrhalis* incidence is more in individuals aged above 60 years old (Boyle *et al.*, 1991; and Chin *et al.*, 1993). Study of respiratory isolates from a hospital in Texas found that 81% of patients with *M. catarrhalis* were over 55 years (Chang *et al.*, 2001). The short-term mortality in some patient categories is as high as 45% and most patients are elderly (older than 65 years) (Verduine *et al.*, 2002). The age of patient having *M. catarrhalis* in this study was 58 and 67; this was coinciding with above mentioned reports. Studies have shown that beta-lactamase producing *M. catarrhalis* have increasingly been isolated as clinically significant isolates. Prior to 1977 only 4% of *M. catarrhalis* were resistant to penicillin but in 1985 others studies showed that 86.7% of a sample of 53 strains elaborated beta-lactamase and this trend seems to be increasing (Verduin *et al.*, 2002).

The two isolates that recovered here were strong β -lactamase producers which explained their resistance to ampicillin.

It is important to test for beta-lactamase in all significant isolates as it will help the physicians to choose the right antibiotic treatment. In any case, the choice of treatment should ideally be based upon the type of disease and condition of the patient.

CONCLUSION

In conclusion, *Moraxella catarrhalis* was found to constitute about 1% of the respiratory tract infected patients among the study population the two isolate found to be β -lactamase producers which explained their resistance to ampicillin. Therefore, it is necessary to look for beta-lactamase production in all isolates as it will affect the type of antibiotic chosen for therapy. Further research is needed to study the epidemiology and the extent of *Moraxella catarrhalis* infections in Sudan. Using of molecular techniques for the detection of *Moraxella catarrhalis* and BRO genes which are responsible of β -lactamase production.

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