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Tits of *Moraxella*, bits of resistance – titbits of LRTI from rural Bengal

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ABSTRACT

Introduction: *Moraxella catarrhalis, a nonfermenter, has long been accepted as a commensal of upper respiratory tract. Then it was confirmed to be a pathogen causing upper respiratory tract infection in children and the elderly. Now it is well known to be causing lower respiratory tract infections (LRTI) as well. Ours was an epidemiological to find out the prevalence of this bacteria among patients having features of LRTI.*

Aims & objectives: *(i) Prevalence of Moraxella catarrhalis causing LRTI patients in rural Bengal. (ii) Antibiotic susceptibility pattern of M. catarrhalis.*

Materials and Methods: *An Institution and community based cross-sectional observational study was carried out over a period of 2 years in the district of rural South 24 parganas , West Bengal. Moraxella catarrhalis was identified from sputum samples by a battery of biochemical tests, and antibiotic susceptibility patterns were determined.*

Results: *(i) Total no. of Moraxella isolates were 83 (8.76%) out of total 947 sputum samples. (ii) Most isolates were recovered from 15 – 59 yrs. age group. (iii) Highest resistance was found to Penecillin and Amoxycillin, followed by Cotrimoxazole and Erythromycin .*

Discussion: *We found a whopping 95% resistance to penicillin, which is alarming, if not unexpected. A silent colonizer once, now armed with BRO-1 and BRO-2 beta lactamases up its sleeve, this bacteria is a pathogen now in its own might. Moreover, even mixed growth should be reported, as it can potentiate antibiotic resistance in co-pathogens which have the capacity to cause respiratory tract infections.*

INTRODUCTION

During the past two decades, a bacteria has changed its name more than once, and it has also started changing its role from commensal, to commensal with a query, to pathogen. Prior to 1990, *Moraxella catarrhalis* was taken as a part of normal upper respiratory tract flora. Since then, it has established itself from an emerging to established pathogen and is now recognized as a causative agent of upper respiratory tract infection in children and elderly persons.[1,2,3,4] It is a well-known fact that it causes lower respiratory tract infection, particularly among COPD affected adults.[1,2,4] In the immunocompromised individuals it causes a variety of infections such as pneumonia, endocarditis, septicaemia, and meningitis.[5,6] In addition, this bacteria has caused outbreaks of respiratory disease in hospitals, and is now a licensed nosocomial pathogen in its own right.[7,8]

The increasing prevalence of beta-lactam resistance among them , then becomes another cause for concern in this changing clinical scenario. It is also an established fact that *M. catarrhalis* is composed of 2 lineages - one among them expanded in humans around 5 million years back, and this lineage is associated with virulence factors, seroresistance and adherence to epithelial cells.[9]

Interestingly, this member of the Order Pseudomonadales, Family Moraxellaceae is also mimicking its close taxonomical relative *Acinetobacter baumannii* by turning from Dr Jekyll to Mr Hyde as far as pathogenic potential is concerned.

All these intriguing and interesting facts are of concern to the medical fraternity worldwide. We, the microbiologists of an apex teaching institution, taking all these informations as the backdrop, tried to paint an epidemiological picture of prevalence of

Moraxella catarrhalis in rural West Bengal, along with its antibiotic sensitivity pattern.

MATERIALS AND METHODS

STUDY DESIGN: Institution and community based cross-sectional observational study.

STUDY PERIOD: 2 years.

STUDY AREA: Budge-Budge II block and L B Dutta Hospital situated in the same blosk in the district of rural South 24 parganas , West Bengal. There are 65 villages in the block with a total population of about 1,90,000.

STUDY POPULATION: People of all age and sex suffering from Acute lower Respiratory tract infection.

SAMPLING DESIGN AND SAMPLE SIZE: The multistage random sampling technique was adopted in the study. A total of 947 sputum samples from subjects with symptoms of LRTI were included.

PATIENT SELECTION: Out of 947 subjects , 208 (21.9%) were selected from OPD of L B Dutta Hospital. Rest (739) were selected at community level through 30 cluster sampling method i.e 39 – 40 study participants from each cluster.

INCLUSION CRITERIA: Only Acute LRTI as clinically manifested by cough (< 14 days duration) with or without fever / fast breathing / chest indrawing / breathing difficulty like nasal flaring / non-recurrent wheezing and / cough & expectoration or x-ray findings of ART / pneumonia.

COLLECTION AND TRANSPORT OF SAMPLES:

(i) Sample collection: Sputum samples were collected in a disposable, wide mouthed, screw capped plastic container. The patient was instructed to spit the coughed material directly into it without spilling over the rim. The cap of the container was screwed tightly. Thick portion of the sputum was taken in acotton swab and put into Amies transport medium. This specimen was then taken into the vaccine box at room temperature and transported to the laboratory within 2 – 3 hrs.

(ii) Processing of samples: Samples were processed on rhe same day. Sputum samples were selected by gram staining (following Bartletts grading). Selected amples were homogenized by shaking with sterile water and glass beads for 20 – 30 minutes and were inoculated in Sheep blood agar, Chocolate agar (with 5 – 10% CO2), and MacConkeys agar.

(iii) Microbiological identification of *M. catarrhalis*: It produces non-hemolytic, round, opaque colonies on blood agar. Colonies can be slid across the agar surface without disruption (termed the "hockey puck sign). After 48 hrs colonies tend to become larger and take on a pinkish hue. We performed a battery of biochemical tests. M. catarrhalis produce oxidase, catalase, DNAse (detected using DNAse test agar with methyl green), reduce nitrate to nitrite, and hydrolyse tributyrin.[10]

(iv) Antimicrobial susceptibility testing: this was done by Kirby-Bauer method. Antibiotic disc used were Penicillin, Amoxycillin, Coamoxyclav, Ceftriaxone, Cefotaxime, Cefuroxime, Erythromycin, Doxycycline, Chloramphenicol, Ciprofloxacin, Levofloxacin and Cotrimoxazole.

RESULTS

(i) Total no. of Moraxella isolates from Phase I,II,III, & IV are 83 (8.76%) out of total 947 sputum samples.

(ii) Maximum no. of isolates were recovered from 15 – 59 yrs. Age group, and none were found from children less than 5 yrs old.

(iii) Highest resistance was found to Penecillin and Amoxycillin, followed by Cotrimoxazole and Erythromycin.

TABLE – I

TABLE – II

DISCUSSION

According to standard textbook literature, *M catarrhalis* is responsible for approximately 10% of lower respiratory tract infections. In our study we found a similar percentage (8.7%). However, this bacteria, exclusively recovered from humans, is a colonizer of upper respiratory tract, although the prevalence of colonization varies with age. It is a well accepted fact that colonization is common in children. But there again, colonization rates vary widely among different studies. A study in Buffalo, New York showed a 66% colonization among 1 yr olds, whereas a similar study done in Goteberg, Sweden showed the colonization rate to be half of that level. In another study among rural Aboriginals near Darwin, Australia, a 100% colonization rate among 3 months olds was found.[11] The marked difference among these findings stand unexplained as yet. It seems that several factors play a role, including living conditions, hygiene, environmental factors etc. Interestingly, in our study, we did not not find *M catarrhalis* even as a pathogen from below 5yr old children.

Importance of Moraxella has also come to the forefront because of pneumococcal conjugate vaccines. Because of widespread use of this vaccine, nasopharyngeal colonization by vaccine serotypes of pneumococcus has now been replaced with nonvaccine serotypes of *S pneumoniae*, nontypeable *H influenzae*, and our very own *Moraxella catarrhalis*.[12] We have reported only when pure isolates of Moraxella have been recovered from sputum samples, considering them to be pathogens, not colonisers.

Thus, Moraxella is gaining strength, increasing our concern. The fact which is adding to the worries of the medical fraternity is its beta – lactamases, BRO-1 and BRO-2. Before 1970, no Moraxella isolate was reported to produce beta-lactamse., and the first such report of beta-lactamase positive strain came in 1976. By 1990, 80% of isolates from USA and 90% of isolates from UK were positive for beta-lactamase. Recent studies from Australia,

Europe and USA have reports beta-lactamase production in over 90% of isolates.[13,14,15] In our study, we found penicillin resistance in the tune of 95%. It is alarming, because (i) M catarrhalis is no more a harmless commensal, (ii) although these enzymes are encoded by chromosomal genes, they can be easily transferred by conjugation, and (iii) beta-lactamase of Moraxella not only protects the bacteria but also inactivate penicillin therapy of associated infections, if any, by dangerous airway pathogens like *S pneumoniae* or H influenza – a phenomenon referred to as Indirect pathogenicity of *M catarrhalis*.In these cases, treatment failures have been reported and shows the significance of reporting not only pure but also mixed cultures. Thankfully, we did not find *S pneumoniae or H influenzae* along with Moraxella, which may jeopardise the treatment and hence, we refrained from reporting any mixed culture.

Hence this bacteria with an interesting and checkered taxonomic trail behind, with a 100 yrs history in medical literature, and suspected by Sir William Osler to be the cause of his own terminal pneumonia – is very much in the run with other pathogens. One should be careful enough not to overlook or ignore this bacteria while reporting, and epidemiological studies in details supported by molecular techniques as far as resistance is concerned, are in our future plans in the immediate vicinity.

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TABLE – I

Resistance pattern of Moraxella isolates

	P	AMX	AMC	CTR	CTX	CXM	E	DO	C	CIP	LE	COT
NO. OF RESISTANT ISOLATES	79	79	12	3	5	14	41	22	7	32	21	74
% RESISTANCE	95.1	95.1	14.2	3.57	5.95	16.6	48.8	26.1	8.33	38	25	88

TABLE – II

Age wise distribution pattern of Moraxella isolates

AGE	NO. OF ISOLATES
<5 yrs.	0
5 – 14 yrs.	8
15 – 59 yrs.	60
➢ 60 yrs.	15