

Study of Antibiogram and Virulence Factors of *Moraxella Catarrhalis* From a Tertiary Care Hospital

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ABSTRACT

Background: *Moraxella catarrhalis* is a known secondary pathogen in COPD. Its antibiogram has not been studied much but is important for empiric chemotherapy. Also, studying its virulence factors is important.

Objectives: This study was planned to see antibiogram, virulence factors and biofilm formation.

Methods: Over 16 months, *M. catarrhalis* was isolated from sputum and other samples by inoculating on suitable media and identified using staining and standard biochemicals. Virulence factors studied were lecithinase, lipase and protease, serum resistance assay, and biofilm formation by test tube method. Antibiogram was done by Disk diffusion method using 11 different antibiotics.

Results: A total of 60 *M. catarrhalis* isolates were recovered; 51 from sputum and 9 from other samples. Mean age and male to female ratio of patients with respiratory isolates were 38.4 and 1.8:1 respectively. In 3 cases, *M. catarrhalis* was found to coexist with *M. tuberculosis* and *S. aureus*. All respiratory isolates were susceptible to Ceftriaxone and Piperacillin-Tazobactam. Resistance to Prulifloxacin and Cotrimoxazole were considerable, and that against Amoxiclav and Levofloxacin were low. Four isolates were Multi-drug resistant,

3 from sputum and 1 from urine. Lipase was found in 38.4% isolates; lecithinase and protease were absent. Biofilm was shown by 67% isolates. All were serum resistant.

Conclusion: *M. catarrhalis* is a smart pathogen, having array of virulence factors and can cause disseminated infection. Prulifloxacin and Cotrimoxazole are not warranted for empiric therapy, at least in our area.

Keywords: *Moraxella*, LRTI, MDR.

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Article History:

Received: 26-02-2017, Revised: 16-03-2017, Accepted: 28-03-2017

Access this article online

Website: www.ijmrp.com	Quick Response code 
DOI: 10.21276/ijmrp.2017.3.2.047	

INTRODUCTION

Moraxella catarrhalis, a Gram negative coccobacillus, known as secondary pathogen in COPD.¹ It is generally susceptible to most antibiotics, but due to Beta-lactamases (BRO-beta lactamases), it is prone to develop resistance to empirically given Penicillins but usually not to cephalosporins.² Hence knowing its antibiogram is very important. Very few such studies are available in the repository. Study of virulence traits of the pathogen is also important since it can predict success of antibiotic therapy.³ Keeping these things in mind, we aimed to (i) isolate and identify *M. catarrhalis* from the various clinical samples and observe its antibiogram and (ii) see virulence factors like lecithinase, lipase, protease, serum resistance and biofilm formation. Our objectives were to identify *M. catarrhalis* by biochemicals, perform antibiogram by disk diffusion and study virulence factors on egg yolk agar (lecithinase, lipase, protease) and standard methods.

MATERIALS AND METHODS

Type of study: This was a laboratory-based observational study.

Place of study: The study was carried out in the Department of Microbiology of the institute.

Time of study: From February 2014 to June 2015 (16 months). This was a pure observational study using lab isolates and patients' identity was not revealed.

Methodology: *Moraxella catarrhalis* was isolated and identified from clinical samples routinely received in the lab., like sputum, urine and blood.

Identification: *M. catarrhalis* isolates were identified by NLF, Dry colonies that were wholly movable over surface of agar (hockey-puck colonies) on CLED/Chocolate agar.

From colonies, Gram stain was done. Gram negative coccobacilli, Oxidase positive (using 1% TMPPD disks, Himedia labs, Delhi),

which were positive for Nitrate reduction (KNO₃ broth), with no sugar fermentation (on sugar broth with phenol red), and having no growth on MacConkey agar but good growth on CLED/ NHA (Serum-free media), were taken as *Moraxella catarrhalis*, according to literature references.⁴

Isolates that were positive for all these were considered as *M. catarrhalis*.⁴

Sputum sample: Growth was considered significant only when growth pure/almost pure. Sixty (60) such isolates from various samples were studied.

Antibiotic susceptibility of the isolates were tested by Kirby-Bauer disk diffusion method against 11 different antibiotics, e.g. Ceftazidime (30 µg), Cotrimoxazole, Amikacin (30 µg), Azithromycin (15 µg), Piperacillin-Tazobactam (110 µg), Amoxiclav (30 µg), Levofloxacin (5 µg), Prulifloxacin (5 µg), Gentamicin (30 µg), Cefotaxime (30 µg), and Cefixime (5 µg) as per CLSI protocol, and for Prulifloxacin, new interpretive data.^{5,6} Susceptibility and resistance was calculated by SENTRY interpretive criteria of disk diffusion.⁷ All disks were supplied by HiMedia labs, Delhi, India. Patients were also followed up for clinical recovery to match data with in-vivo response.

Virulence Traits Tested: The following virulence factors were tested, like lecithinase, Lipase, Protease (all on Egg-yolk agar), serum resistance and biofilm formation (by test-tube method).

Positive lecithinase (phospholipase) was indicated by zone of haziness around colonies on Egg yolk agar. Lipase was indicated by pearly shine on colonies, and protease by zone of clearing observed around colonies.⁸ Biofilm formation was assessed visually using test tube method.⁹

Serum resistance test: In 2 separate test tubes, 100 µl of 1 MacFarland bacterial suspension was mixed with (a) 300 µl of peptone water, and (b) 300 µl of human serum, and incubated for 90 minutes at 37°C.

Then 10 µl of each was streaked on Chocolate agar plates and incubated overnight at 37°C.

The isolate was taken as serum sensitive, when there was >90% inhibition of colony count in culture done from tube where serum was added. When there was <90% inhibition of growth, it was serum resistant.¹⁰

RESULTS

Out of a total of 60 *M. catarrhalis* isolates which were recovered, 51 were from sputum and 9 from other samples. These other samples were synovial tissue, blood, swab and urine.

Mean age of patients growing it from sputum, was 38.4 years.

For respiratory isolates, male to female ratio was 1.8:1. (Thus male-dominant)

M. catarrhalis formed 43% of all respiratory bacterial isolates. (All were OPD isolates)

Other isolates (in decreasing order) were *Pseudomonas aeruginosa*, *K. pneumoniae*, *Staphylococcus aureus* etc.

In 3 cases, *M. catarrhalis* was found to coexist in sputum with other pathogens (*M. tuberculosis* in 2 and *S. aureus* in 1 case). All respiratory isolates were universally susceptible to Ceftazidime and Piperacillin-Tazobactam. Resistance to Prulifloxacin and Cotrimoxazole was exorbitant (78.5% and 77.7% respectively). The same figures against Amoxiclav and Levofloxacin were lower (4.7% and 10.7% respectively).

MDR (Multi-drug resistance) was found in 3 respiratory isolates (MDR: Resistance to 3 or more different groups of antibiotics as depicted by Gupta *et al.*)¹¹

Study of virulence factors: Lipase enzyme was found in 38.4% isolates; lecithinase and protease could be found in no isolates. Biofilm formation was shown by 67% isolates. All these isolates were serum resistant.

Results of antibiogram are shown in **Table 1**.

Apart from sputum, 9 other samples grew *M. catarrhalis*. (Urine: 4, Synovial fluid/tissue: 2, Wound swab: 1, Blood: 1, Ascitic fluid: 1)

All of the urinary isolates were susceptible to Piperacillin-Tazobactam. One urinary isolate was MDR.

Table 1: Resistance to some antibiotics other than Prulifloxacin and Cotrimoxazole

Antibiotic	Resistance (%)
Amikacin	25%
Azithromycin	27.8%
Cefixime	15.38%
Amoxicillin-Clavulanic Acid	4.76%
Levofloxacin	10.71%
Ceftazidime	0
Piperacillin-Tazobactam	0
Gentamicin	11.1%

DISCUSSION

M. catarrhalis, earlier called *Branhamella catarrhalis*, is a smart pathogen, gaining significance as a pathogen over few decades, and having an array of virulence factors, of which biofilm formation and lipase and serum or complement resistance are important.^{12,13}

This bacterium is an exclusively human pathogen causing Lower Respiratory Tract Infection, Otitis media and other infections.¹

Earlier thought only to be a respiratory tract commensal, since the late 1970s, it has been clearly shown that *M. catarrhalis* is an important and common human respiratory tract pathogen and it

has been shown to cause LRTI specially after COPD.¹ However, Isolation in children, is mostly regarded as colonisation.¹⁴ In other Indian studies also, *M. catarrhalis* was found to be third most common cause of airway pneumonia, and was more commonly found in male patients and in age group above 60 years.¹⁵ In another study from Karnataka, South India, also, *M. catarrhalis* respiratory isolates were highly susceptible to Amoxicillin-Clavulanic acid but mostly refractory to Ampicillin.¹⁶

According to our findings, as also from other reports, *M. catarrhalis* is often serum resistant and has a high propensity to

cause disseminated infection, as also reported by Attia *et al.*¹⁷ We here report that it can also coexist with other pathogens, and can not only cause pneumonia but are also implicated in Urinary tract infections (UTI), arthritis, wound infection and bloodstream infection. More infection in males, found in our study, was probably due to more active smoking and consequent COPD in male subjects. Another interesting observation was that Prulifloxacin and Cotrimoxazole are not warranted for empiric therapy, at least in our area. A study from Italy shows his figure in Prulifloxacin to be about 14% in acute exacerbation of COPD.¹⁸ Further studies regarding this are awaited.

REFERENCES

- Murphy TM, Parameswaran GI. *Moraxella catarrhalis*, a Human Respiratory Tract Pathogen. *Clin Infect Dis* 2009; 49:124–31.
- Wallace RJ Jr, Nash DR, Steingrube VA. Antibiotic susceptibilities and drug resistance in *Moraxella* (*Branhamella*) *catarrhalis*. *Am J Med* 1990 ;88: 46S-50S.
- Committee on New Directions in the Study of Antimicrobial Therapeutics: New Classes of Antimicrobials. Challenges for the Development of New Antimicrobials— Rethinking the Approaches: Report of a Workshop. In: National Research Council (US) Committee on New Directions in the Study of Antimicrobial Therapeutics: New Classes of Antimicrobials; National Research Council (US) Committee on New Directions in the Study of Antimicrobial Therapeutics: Immunomodulation. Treating Infectious Diseases in a Microbial World: Report of Two Workshops on Novel Antimicrobial Therapeutics. Washington (DC): National Academies Press (US); 2006. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK19843/>.
- Enright MC, McKenzie H. *Moraxella* (*Branhamella*) *catarrhalis* - clinical and molecular aspects of a rediscovered pathogen. *J Med Microbiol* 1997; 46: 360-371.
- Clinical Laboratory Standards Institute. 2006. Performance standards for antimicrobial disk susceptibility tests; Approved standard—9th ed. CLSI document M2-A9. 26:1. Clinical Laboratory Standards Institute, Wayne, PA.
- Montanari MP, Ferrante L, Tili E, Cochetti I, Rossi V, Varaldo PE. Interpretive criteria for disk diffusion susceptibility testing of ulifloxacin, the active metabolite of prulifloxacin. *J Chemother.* 2005 Apr;17(2):138-42.
- Bell JM, Turnidge JD. Development of a Disk Diffusion Method for Testing *Moraxella catarrhalis* Susceptibility Using Clinical and Laboratory Standards Institute Methods: a SENTRY Antimicrobial Surveillance Program Report. *J Clin Microbiol* 2009; 47(7): 2187–93.
- Dowell and Hawkins, 1987, Laboratory Methods in Anaerobic Bacteriology, CDC Laboratory Manual, HHS Publication No. (CDC) 87-8272, Centers for Disease Control, Atlanta, Ga.
- Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. *Braz J Infect Dis* 2011; 15(4):305-311.
- Shaw P, Shaw CK, Saileela K. Pattern of serum resistance in uropathogenic *Escherichia coli*. *International Journal of Pharma and Bio Sciences* 2012; 3(2):B43-B48.
- Gupta N, Arora N, Kundra S. *Moraxella catarrhalis* as a respiratory pathogen. *Indian J Pathol Microbiol* 2011 Oct-Dec;54(4):769-71.
- Perez Vidakovics ML, Riesbeck K. Virulence mechanisms of *Moraxella* in the pathogenesis of infection. *Curr Opin Infect Dis.* 2009 Jun;22(3):279-85.
- Verduin CM, Hol C, Fleer A, Van Dijk H, Belkum AV. *Moraxella catarrhalis*: from Emerging to Established Pathogen. *Clin Microbiol Rev* 2002;15(1):125-144.
- Robinson J. Colonization and infection of the respiratory tract: What do we know? *Paediatr Child Health.* 2004 Jan; 9(1): 21–24.
- Sirwar SB, Indupalli AS, Pal R, Zaman FA, Kar S. *Moraxella catarrhalis*: An emerging pathogen in bronchopulmonary infections. *Ann Trop Med Pub Health* 2013;6(1):76-79.
- Krishna S, Sagarika S, Jeer M, Surekha YA, Shafiyabi S, Pushpalatha H, Shruthi U. Prevalence and Antibiotic Sensitivity Pattern of *Moraxella catarrhalis* in Patients with Lower Respiratory Tract Infections in a Tertiary Health Care Centre in India. *Int J Curr Microbiol Appl Sci* 2016;5(6): 72-78.
- Attia AS, Lafontaine ER, Latimer JL, Aebi C, Syrogiannopoulos GA, Hansen EJ. The UspA2 protein of *Moraxella catarrhalis* is directly involved in the expression of serum resistance. *Infect Immun.* 2005; 73(4):2400-11.
- Pasqua F, Biscione G, Crigna G, Cazzola M. Prulifloxacin in the treatment of acute exacerbations of COPD in cigarette smokers. *Ther Adv Respir Dis* August 2008 vol. 2 no. 4 209-214.

Source of Support: Nil. **Conflict of Interest:** None Declared.

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Cite this article as: Bhattacharyya S, Singh S, Sarfraz A, Jaiswal NK, Kumar R, Kumar A, Sengupta A, Kumar D, Singh S. Study of Antibiogram and Virulence Factors of *Moraxella Catarrhalis* From a Tertiary Care Hospital. *Int J Med Res Prof.* 2017; 3(2):236-38. DOI:10.21276/ijmrp.2017.3.2.047