

Characteristics, virulence factors and antibiogram of *Acinetobacter* spp. and *Moraxella catarrhalis* isolates from a tertiary care hospital in India

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Abstract

Non-fermenting Gram negative bacteria are very common causes of infection in the hospital. They are often resistant to multiple antibiotics and very difficult to treat. We here present a compilation of data regarding isolates of *Moraxella catarrhalis* and *Acinetobacter* spp. which were isolated from different sources of the hospital. They were isolated and identified by conventional biochemical tests. They were found to be mostly resistant to Cotrimoxazole and beta-lactams. Lipase was the principal virulence factor in both the bacteria. In cases of both, females were found to suffer more than males. This data can be helpful for devising pre-emptive antibiotic strategy and also for designing anti-virulence strategies.

Keywords: Acinetobacter, Moraxella, Virulence.

Introduction

Acinetobacter spp. are non-fermenting Gram negative bacteria which are frequent causes of nosocomial infections.⁽¹⁾ Earlier thought as commensals and saprophytic bacteria, they are now recognized as important causes of nosocomial infections.⁽¹⁾ *Moraxella catarrhalis* is also a prominent cause of respiratory tract and other infections.⁽²⁾ Infections by *Acinetobacter* spp. gain severe status in immunocompromised patients and those admitted in Intensive Care Units (ICU).⁽³⁾ This bacterium possesses, in its arsenal, an array of virulence factors like lipase, gelatinase, esterase and protease.⁽³⁾ Its ability to withstand moisture and dryness, and high level of antibiotic resistance, is a matter of great concern for both clinicians and laboratory personnel.⁽⁴⁾ Data regarding drug sensitivity and virulence factors of *Acinetobacter* spp. And *Moraxella catarrhalis* is scarce in India, and information regarding species distribution of *Acinetobacter* spp. In India are also rare. Often pre-emptive antibiotics are administered in case of infections by these pathogens. So our study was aimed at addressing these issues.

Materials and Method

This was a laboratory based observational study, carried out in the Department of Microbiology of the institute from August 2016 to April 2017 (8 months). Samples routinely received in the Microbiology section from clinical departments, like urine, pus, sputum, etc. were inoculated on routine culture media and identified by staining, catalase, oxidase and routine biochemical tests. *Acinetobacter* spp. were identified by the following biochemical tests:

Non lactose fermenting colonies (dewdrop colonies) on MacConkey agar.

Oxidase, 1% TMPPD (paper strip): negative.

Indole negative.

Non-motile on semi-solid agar stab.

Citrate variable (utilised mostly in case of *A. baumannii* and not utilised in case of *A. lwoffii*).

Urease variable (hydrolysed in case of *A. baumannii* and not hydrolysed in case of *A. lwoffii*).

TSI: k/k, no gas, no H₂S.

Moraxella catarrhalis isolates were identified by the following biochemical tests:-

Gram negative coccobacilli, Non lactose fermenting dry colonies (hockey-puck colonies) on Blood agar, no growth on macConkey agar.

Oxidase test with 1% TMPPD(paper strip): positive.

Indole negative.

Non-motile on semi-solid agar stab.

Colistin resistant.

Following identification antibiotic susceptibility of the isolates were carried out on Mueller Hinton agar for the following antibiotic disks: Azithromycin(15 mcg drugs), Cefixime(30 mcg disk), Cotrimoxazole(25 mcg drugs), Piperacillin-Tazobactam(110 mcg drugs), Nitrofurantoin(300 mcg drugs) (only in case of urinary isolates), Amikacin(30 mcg), Fluroquinolones (Levofloxacin 5 mcg), Ampicillin(30 mcg), by Kirby Bauer Disk diffusion test as per CLSI protocol.⁽⁵⁾ *E. coli* ATCC 25922 strain was used as susceptible control here. Lecithinase, lipase and protease activities were detected on Egg yolk agar (formula: Molten Nutrient agar 90 ml + beaten yolk of fresh hen's unfertilised egg 10 ml). Lecithinase activity was denoted by zone of opalescence around the colonies while lecithinase and protease were signified by appearance of zone of clearing around the colonies, and eparly sheen on surface of colonies, respectively.

Results

About 37 isolates of *Acinetobacter* spp. and 22 isolates of *Moraxella catarrhalis* were grown in this period. Among this 37 isolates of *Acinetobacter* spp., 31 were *A. lwoffii* and 3 were *A. baumannii*. Among all

non-fermenter isolated from samples in this period, these two bacteria together constituted 71% isolates. Thus in our hospital, majority of non-fermenters were either *Acinetobacter* spp. or *Moraxella catarrhalis*.

Among *Acinetobacter* spp., 5 were multidrug resistant isolates (resistant to 3 or more classes of antibiotics). All MDR isolates belonged to *A. lwoffii* species. Male to female ratio was 3:5 in case of *A. lwoffii* and 1:2 in *A. baumannii*. Among all samples, 19 (61.2%) were urine samples, 7 (22.5%) were pus samples, 3 (9.6%) blood, 2 (6.4%) sputum. In case of *A. lwoffii*, resistance to Azithromycin was seen in 23.5% isolates, to cefixime in 32.35% isolates, to Ampicillin in 17.34%, Levofloxacin in 14.7%, Nitrofurantoin in 21.05% strains. Virulence factors studied, showed that lipase was found in 77.41% strains, Lecithinase in 38.7% and protease in 22.58% isolates. In *A. baumannii*, all were susceptible to all these drugs. Lecithinase was found in 33% isolates and all were lipase positive.

Among the 22 *M. Catarrhalis* isolates grown, *Moraxella* isolates, 6 were multidrug resistant (MDR); male: female ratio was 3:4. They were mostly recovered from urine (44.4% samples), sputum (11.11% samples), blood (5.55% samples), pleural fluid (5.55% samples), pus (33% samples), tissue (5.55% samples). Resistance to azithromycin was observed in 22.7% isolates, cefixime in 18.18%, Cotrimoxazole in 40.9% strains), Piperacillin-Tazobactam in 4.54% isolates, Nitrofurantoin 0 (in urinary isolates only), Amikacin in 18.18% isolates, Amoxiclav in 45.4% isolates, Fluoroquinolones in 36.36% isolates, and Ampicillin in 27.7%). Lecithinase was found in 31.8% strains, lipase in 54.5%, protease in 13.63% isolates. The virulence factors have been tabulated in Table 1.

Table 1: Study of virulence factors

Bacterium	Lecithinase (%-age isolates)	Lipase (%-age isolates)	Protease (%-age isolates)
<i>Acinetobacter lwoffii</i>	38.7	77.41	22.58
<i>Moraxella catarrhalis</i>	31.80%	54.5	13.63%

Discussion

Acinetobacter spp. are highly ubiquitous in environment and often drug resistant, and our data is in contradiction with western ones where *A. baumannii* is mostly drug resistant.⁽⁶⁾ There is very little data in literature regarding species distribution of *Acinetobacter* in samples in India. One study reports that *A. calcoaceticus* and *A. hemolyticus* were most common in skin of healthy tribals in India.⁽⁷⁾ Serum resistance and aerobactin were common virulence traits found in *Acinetobacter* spp. As studied in Iran.⁽⁸⁾ Regarding virulence of *M. catarrhalis*, one study says

that lipase and biofilm formation were the most common virulence traits of the bacterium.⁽⁹⁾ As per other Indian researchers, beta-lactam resistance is very high (in the order of 70%) in *M. catarrhalis*, but our study found that it is low.⁽¹⁰⁾ Although the number of isolates in our study is less, it can still be safely concluded that for suspected infections caused by non-fermenting bacteria like *Acinetobacter* spp. and *Moraxella* spp., Cotrimoxazole is certainly not the antibacterial of choice and Amoxiclav and Cefixime should also be used with caution. Another interesting observation was that *Moraxella catarrhalis* were isolated not only from sputum but also from samples like urine and blood. In fact, they were most commonly retrieved from urine and pus. In a report from Western India, Shyamkuwar *et al* report isolation of *M. catarrhalis* mostly from sputum and throat swab and very rarely from urine and pus.⁽¹¹⁾ This study is the first in this region of the country evaluating the virulence factors and antibiogram of these bacteria, also. These are quite interesting areas of future research.

Conclusion

Acinetobacter spp. And *Moraxella catarrhalis* are very commonly found in the hospital and can infect many organs, and are fast becoming multi drug-resistant. Proper knowledge of their antibiogram and virulence traits is essential to devise a presumptive antibiotic plan and anti-virulence techniques, respectively.

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