



Original Research Article

Isolation of non-fermenting Gram negative bacteria in respiratory tract infections

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ABSTRACT

Introduction: Respiratory tract infections are the most common diseases that are associated with mortality and morbidity. They are often misdiagnosed due to its nonspecific presentation in clinical settings. The risk factors of RTI vary geographically and timely(1). So it is important to evaluate more for RTI causative agents.

The non-fermentative Gram negative bacilli (NFGNB) are often associated with RTI. The NFGNB are a group of several different aerobic, non-sporing, Gram negative bacilli that either unable to utilize carbohydrates as a source of energy or withhold them through metabolic pathways other than the fermentation.

Material and Methods: The study was undertaken to identify various non- fermenters by additional conventional tests, isolated from patients having respiratory tract infection in department of Microbiology, SVPPGIP and SCBMCH Medical college, a Tertiary Care Center, Odisha. Our study also line up the significant role of NFGNB as respiratory pathogens.

391 (14.6 %) non-fermenters were isolated from 2678 clinical specimens. Out of which NFGNB(391 samples), a total of 143 (36.57%) were from various respiratory samples were included in this study and were isolated on MacConkey and blood agar plates incubated at 35°C for 24-48 hours.

Results and Conclusion: Patients mostly belonged to age group of 65 to 80 years and were 96(67%)male. *Acinetobacter calcoaceticus baumannii* complex 67(46.8%) was the commonest isolate followed by *Pseudomonas aeruginosa* 56(39.1%).

While other NFGNB like *Elizabethkingia*, *Stenotrophomonas*, *Sphingomonas*, *Burkholderia* and *Chryso bacterium* were isolated in few respiratory samples.

The most effective antibiotics were Tigecycline and Polymyxin B/ Colistin, however majority of them showed multidrug resistance.

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1. Introduction

Respiratory tract infections (RTI) are among most-common infectious diseases affecting population worldwide causing significant morbidity and mortality for all age groups.¹ RTI are often misdiagnosed, mistreated and underestimated due to its nonspecific presentation. Etiological agents of RTI vary geographically and timely. The problem is much greater in developing countries.¹ So there is further need to

evaluate RTI causative agents.

Non-fermenting Gram negative bacilli (NFGNB) are assorted group of different aerobic, non-sporing bacilli that either incapable of utilizing glucose as a source of energy or utilizing it oxidatively rather than fermentatively.²

Approximately 15% of isolates from clinical samples are NFGNB.³

This group includes diverse genera like *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Burkholderia*, *Flavobacterium*, *Chryso bacterium*, *Elizabethkingia*, *Weeksella*, *Spingobacterium*, *Moraxella*, *Psychrobacter*,

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Neisseria, Bordetella, Ralstonia etc.²

The clinicians are concerned due to increased evident of non-fermenters isolated from various respiratory specimens and also found associated with the diseases like pneumonia, cystic fibrosis and respiratory tract infections (RTI) especially in immunocompromised patients. With increasingly higher proportion of hospitalised patients having underlying illness, NFs are recovered with higher frequency from clinical specimen, upto 9% must be considered as important agents of many infectious disease.

For this reason, accurate identification and isolation of NFGNB is important for effective patient management.⁴

However, the identification and isolation of the non-fermenting bacteria has been somewhat difficult for microbiologists working in clinical laboratories due to their relative low rate of recovery.

NFGNB are presumptively identified by colonial morphology, Gram stain, oxidase activity and pigment production.⁵ The oxidase reaction is an important discriminatory test. They may be oxidase positive, glucose non-fermenting, Gram negative bacilli such as *Pseudomonas aeruginosa* and oxidase negative, non-fermenting, Gram negative bacilli such as *Acinetobacter baumannii* complex.⁶ In the diagnostic clinical microbiology laboratory, identification of non-fermenters relies mainly on phenotypic characteristics. Very few laboratories in India identify these organisms as a routine because non-fermenters are slow growing and require the use of special culture media and biochemical test for their identification.⁷ A variety of automated identification systems, such as VITEK-2 (bioMérieux) is being used for identification and for antibiotic sensitivity tests of these bacteria in some of the laboratories.

Non-fermenters may differ in their pathogenic potential and transmissibility, and many are multidrug resistant.⁶ Non-fermenting bacteria associated with different infections are becoming increasingly resistant to commonly used antimicrobial agents. Nosocomially acquired isolates especially from ICUs tend to be more resistant to antimicrobials.⁷ Development of resistance by non-fermenters is multifactorial, may involved factors like mutations in genes encoding porins, efflux pump mechanisms, Penicillin binding proteins, chromosomal beta lactamase.⁸ They have developed resistance to both Imipenem 17.32% and Meropenem 22.16%.⁹ They frequently show resistance to multiple classes of antimicrobials like Beta-lactam group, Aminoglycosides, Flouroquinolones and development of resistance during monotherapy is well documented.¹⁰

So, Its important to assess their present antibiotic sensitivity and resistance pattern for proper management.

2. Material and Method

Our study was conducted in the Department of Microbiology, SCBMCH and SVPPGIP, Odisha over a period of eighteen months from March 2019 to august 2020. The present study was conducted with the objective to identify and isolate NFGNB from various respiratory specimens and also aim to evaluate the pathogenic potential of NFGNB in various respiratory tract infections in our hospital.

The study was conducted on 143 respiratory samples including sputum, throat swabs, pleural effusions, bronchoalveolar lavage, endotracheal and tracheal secretions etc. In our study demographic data of 143 patients were compared like age, sex, respiratory specimen yielding NFGNB and their association with the clinical infections, to rule out any biases. Identification, isolation and antibiotic sensitivity testing of NFGNB were also assessed and compared.

Appropriately collected and transported samples yielding growth of Gram negative bacilli on MacConkey agar, blood agar and chocolate agar (CA) 35°C at after 24-48 hours of incubation which turned out to be non fermenters in OF with glucose were included in the study.

The growth on Mac conkey plates, morphology, size, haemolytic activity, pigmentation, additional biochemical tests provides valuable information for identification Gram stain preparations from the samples were microscopically examined to assess contamination with upper respiratory tract secretions during collection. The sample was considered to be minimally contaminated if presence of 25 pus cells and <10 epithelial cells / Lpf was there. Significance was laid to inflammatory cell and predominance of bacteria in the pus cells in the gram stain preparation.¹¹

Antimicrobial Susceptibility Test (AST) was done via Kirby-Bauer method which was carried out on Mueller Hilton casein starch agar.

The specimen which was either contaminate or contains merely saliva was excluded from this study.

3. Result

NFGNB (391 samples) (14.6 %) were isolated from 2678 clinical specimens. Out of which NFGNB (391 samples), a total of 143 (36.57%) were from various respiratory samples.

Patients mostly belonged to age group of 65 to 80 years. This study showed males were more common 96(67%) as compared to females 47(33%).

All the isolates were identified by additional routine biochemical method. Any growth that was identified as non fermenters by additional routine method were processed.

In our study most common isolate was *A.baumannii* which found to have non-lactose fermenting growth

on MacConkey, negative oxidase with positive citrate utilisation and positive acid production from glucose in HL media. 67 (46.8%) isolates of *A.baumannii* group could be accurately identified by conventional tests.

Paeruginosa found to have non lactose fermenting growth on MacConkey agar and pigment production on nutrient agar, motile with positive oxidase and citrate and characteristic pigment production in King's A & B 56 (39.1%) isolates of *Pseudomonas aeruginosa* could be accurately identified by conventional tests. Other isolates of *Pseudomonas* spp could also be identified by conventional tests.

In our study *Acinetobacter calcoacetecus baumannii* complex 67(46.8%) was the commonest isolate followed by *Pseudomonas aeruginosa* 56 (39.1%).

While other non-fermenters like *Elizabethkingia* (6 cases) , *Stenotrophomonas* (4 cases), *Sphingomonas* (3 cases), *Burkholderia* (2 cases) and *Chryseobacterium* (1 cases) were isolated in few respiratory samples.(Table 1).

Table 1: Bacterial species isolated under respiratory tract infections

Organism Isolated	RTI	Percentage
<i>Acinetobacter baumannii</i> complex	67	46.8%
<i>Acinetobacter lwoffii</i>	2	1.39%
<i>Burkholderia cepacia</i>	2	1.39%
<i>Chryseobacterium indologenes</i>	1	0.69%
<i>Elizabethkingia meningoseptica</i>	6	4.19%
<i>Pseudomonas aeruginosa</i>	56	39.1%
<i>Pseudomonas fluorescens</i>	1	0.69%
<i>Pseudomonas luteola</i>	1	0.69%
<i>Sphingomonas paucimobilis</i>	3	2.09%
<i>Stenotrophomonas maltophilia</i>	4	2.79%
Total	143	100%

Acinetobacter baumannii was found to be the most common isolate among all the NFGNB isolates. *Acinetobacter baumannii* isolated from 67 cases of respiratory tract infection. *Acinetobacter lowfi* was isolated from 2 cases of respiratory tract.

Pseudomonas aeruginosa was isolated from 56 cases of RTI. Single *P.fluorescence* & *P.luteola* were isolated from respiratory infections.

The most effective antibiotics for non-fermenters were Tigecycline and Polymyxin B/Colistin in our study. In respiratory samples, NFs isolated have shown resistance Penicillin group of drugs, Gentamicin, Ciprofloxacin. In our study Aminoglycosides that are considered as good option for life-threatening respiratory infections have shown high resistance. NFGNB displays a wide and variable spectrum of antibiotic sensitivity. There is no antibiotic to which all strains of NFs are susceptible.

4. Discussion

NFGNB have been incriminated in respiratory infections such as cystic fibrosis, pneumonia, pleural infection, post-operative infection, LRTI, and other RTI. Respiratory tract infections are one of the major cause of morbidity and mortality for all age groups.¹ Patients with prolonged stay in hospital especially in ICUs, on ventilators or immunocompromised were more prone to NFGNB infections.

NFGNB'S were earlier considered as contaminants and their possibility as a pathogen was considerably ignored by the clinician. But nowadays NFGNB's are commonest nosocomial and notorious respiratory pathogens whose identification and antibiotic susceptibility testing is essential. This study was therefore formulated to identify and to isolate the NFGNB in RTI and to assessed their pathogenic potential.

In this study isolation rate of NFGNB was 14.6% and were isolated from 2678 clinical specimens. Out of which NFGNB (391 samples), a total of 143 (36.57%) were from various respiratory samples.

Our results are in accordance with the work done by Mehta et al who concluded similar rate of 15% of isolation.¹² In another study done by Mahajan et al NF's isolation rate of 12.40% was found which is similar to our study.

Where as, unlike our study Benachinmardi et al reported low isolation rate of 3.58% (54) and malini et al reported isolation rate of 4.5%.¹³

On analyzing, in our study *A.baumannii* (46.8%) were found to be most common isolate followed by *P.aeruginosa* (39.1%) from RT infections.This is in agreement with the work of Bergogne et al,¹⁴ who have also reported a similar isolation rate.

In a study conducted by Mahajan et al found contrast results with *P.aeruginosa* being the most common isolate (54.54%), followed by *A.baumannii* (41.08%).¹⁵ This is also not in agreement with the work of Rajan et al¹⁶ and Patel et al.¹⁷

A.baumannii was the main etiological agent responsible for 46.8% RT infections in our study.This is in agreement with the work of Bergogne et al,¹⁴ who have also reported a similar isolation rate. However Raina et al¹⁸ have reported a much higher isolation rate of *Acinetobacter* from various specimens.

The isolation rate of *P.aeruginosa* was found to be 39.1 % in our study. However, variable isolation rates of *P.aeruginosa* have been reported by others - Yashodhara et al 66.95%,¹⁹ Rajan et al 89.9%,¹⁶ Cristane et al 72.5%,²⁰ Mahajan et al (54.54%).¹⁵

The most common isolates were *Acinetobacter baumannii* (46.8%) followed by *P.aeruginosa* (39.1%).We were able to identify some rare NFGNB like *Elizabethkingia meningoseptica*, *Stenotrophomonas maltophilia*,

Sphingomonas paucimobilis, Burkholderia cepacia group and Chryseobacterium indologenes in RTI.

The most effective antibiotics were Tigecycline and Polymyxin B/Colistin in our study. Most of the NFs isolated were resistant to Penicillin group of drugs, Gentamicin, Ciprofloxacin. The sensitivity pattern changes from place to place and human to human.

5. Conclusion

This study reveals that a variety of NFGNB isolated and identified by additional conventional test have pathogenic potential and are responsible for RTI. The most common isolates were *Acinetobacter baumannii* followed by *P.aeruginosa* followed by other NFs especially in immunocompromised patients.

The most sensitive antibiotics were Tigecycline and Polymyxin B/ Colistin. However NFs showed increased resistance to routinely used antibiotics. And this antibiotics resistance has become a great public health issue.

6. Source of Funding

None.

7. Conflict of Interest

None.

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