



Microbiology

PREVALANCE OF NONFERMENTING GRAM-NEGATIVE BACILLI AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL IN COIMBATORE

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ABSTRACT **AIM:** Non fermenting gram-negative bacilli (NFGNB) have emerged as important opportunistic and nosocomial pathogens. They exhibit resistance not only to beta lactam but also to carbapenems. This study was undertaken to identify the non fermenters isolated from various clinical samples, to assess their clinical significance and to know their anti-microbial sensitivity pattern. **Materials and Methods :** The nonfermenters were identified using a standard protocol that included tests for Gram staining, motility, Catalase test, Oxidase production, oxidation-fermentation test for various sugars, gelatin liquefaction and growth on 10% lactose agar. The susceptibility testing was performed with the help of the Kirby-Bauer disc diffusion method. Metabeta lactamases were identified by Modified Hodge test, Combined disc test & Double disc synergy test **RESULTS:** A total of 100 NFGNB were isolated from 200 clinical specimens. Pseudomonas aeruginosa was the most common non fermenter accounting for 60% followed by Acinetobacter Baumannii (33%) and Other significant NFGNB isolated were: Acinetobacter lwoffii (3%) and Stenotrophomonas maltophilia (3%) and Alcaligenes faecalis (1%). P. aeruginosa showed good sensitivity to Meropenem (94%), Cefoperazone (70%) and Amikacin (69%), A. baumannii showed 100% sensitivity to Meropenem. **Conclusion:** P.aeruginosa and A.baumannii were the common NFGNB isolated in our study. P.aeruginosa showed good sensitivity to Meropenem, Amikacin, and Cefoperazone while A.baumannii showed good sensitivity to Meropenem.

KEYWORDS : Pseudomonas, Acinetobacter, NFGNB, MHT, DDST

Introduction

Nonfermenters (also known as non-fermenting gram-negative bacilli (NFGNB) are a group of aerobic, non sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. [1] They occur as saprophytes in the environment and some are also found as commensals in the human gut. [2,3].

NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory. [4] In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important health-care associated pathogens. They have been associated with infections like Septicaemia, Meningitis, Pneumonia and Ventilator associated pneumonia and are known to produce Extended spectrum β -lactamases and Metallo β -lactamases. [3,4]

Hence this study was undertaken to identify the various nonfermenters isolated from patients admitted to our hospital, a tertiary care centre in Coimbatore. This study was also done to assess their clinical significance and antimicrobial susceptibility pattern.

MATERIALS AND METHODS

A total of 200 clinical specimens were received in the laboratory during November 2015 and July 2016. All samples were collected under strict aseptic precautions under standard Microbiology procedures and processed. These samples were plated on Nutrient agar, Blood agar, MacConkey's agar and incubated at 37°C for 18-24 hours. The organisms isolated were identified using the appropriate biochemical tests. [1]

All the organisms that grew on Triple Sugar Iron agar and produced an alkaline reaction were provisionally considered to be NFGNB and identified further by using a standard protocol for identification. [1] The characters assessed included morphology on Gram's stain, motility, pigment production, oxidase production, catalase production, OF test (Hugh-Leifson's medium) for Glucose, Lactose, Sucrose, Maltose, Mannitol, growth on 10% lactose agar, Lysine decarboxylase test and Gelatin liquefaction test.

The clinical significance of the isolated NFGNB was assessed retrospectively by analysing the case sheets for a combination of relevant laboratory and clinical criteria. The laboratory criteria

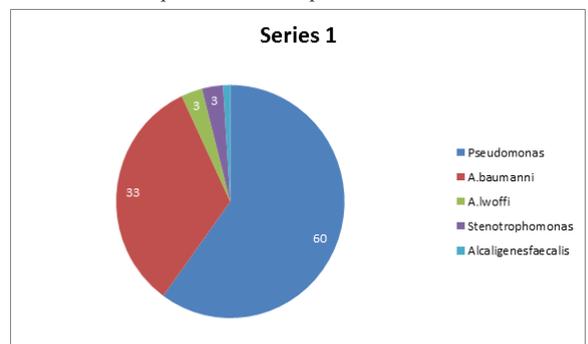
included: The presence of pus cells along with gram-negative bacilli in the stained smear from the sample, isolation of the same organism from a repeat sample, leucocytosis, and relevant radiological evidence (in cases of VAP). [5]

The clinical criteria included the presence of risk factors, such as, Bronchiectasis, Cystic fibrosis, underlying pneumonia, any malignancy, Patients on mechanical ventilation, and other immunosuppressive conditions. [6,7] The sensitivity test was performed with the help of the Kirby-Bauer disc diffusion method using commercially available discs (Hi-media). The different antimicrobials tested were Meropenem, Amikacin, Ciprofloxacin, Ceftazidime, Cefepime, Cefoperazone, Ceftriaxone and Cotrimoxazole. The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. [8] E.coli ATCC 25922 and P.aeruginosa ATCC 27853 were used as the control strains.

Phenotypic methods

RESULTS

Pseudomonas aeruginosa was the most common isolate, accounting for 120 (60%), followed by Acinetobacter baumannii 66 (33%). Acinetobacter lwoffii (3%), Stenotrophomonas maltophilia (3%), and Alcaligenes faecalis (1%) were rarely isolated NFGNB, together accounting for 7% of the isolates. The majority of the nonfermenters were isolated from pus and urine samples.



Analysis of the case records of patients with these infections revealed that most isolates of NFGNB were isolated from pus samples. These NFGNB were isolated from cases of burn wounds, non healing ulcers, bedsores, fracture wounds following road traffic accidents (RTA) and postoperative wound infection.

P.aeruginosa was the major urinary pathogen. This included cases of ureteric calculi, urinary incontinence, pelvic fracture with ruptured urethra, stricture urethra, benign prostatic hyperplasia (BPH), and prolonged catheterization.

A.baumannii was the major respiratory pathogen. It was obtained from patients with aspiration pneumonia following organo-phosphorous compound poisoning, ventilator associated pneumonia (VAP) following laparotomy, secondary infection in an asthmatic patient and pyopneumothorax.

The sensitivity pattern of the NFGNB isolated is presented. Most of the isolates of *P.aeruginosa* were sensitive to Meropenem (94.2%), Cefoperazone (70.5%), Amikacin (69%).

A.baumannii showed 100% sensitivity to Meropenem followed by 70% to Piperacillin, 53.5% to amikacin. *A.baumannii* showed a higher rate of resistance than *A.lwoffii*.

All the isolates of *Pseudomonas* & *Acinetobacter* sp were subjected to MBL detection by Modified Hodge test, Combined Disc test, Double disc synergy test. Around 10 isolates of *Pseudomonas* and 3 isolates of *Acinetobacter* sp were positive.

All the isolates of *S.maltophilia* (100%) were sensitive to cotrimoxazole and ciprofloxacin and were resistant to other antibiotics. The single isolate of *A.faecalis* was sensitive to Imipenem and Piperacillin only.

DISCUSSION:

NFGNB that were considered to be contaminants in the past have now emerged as important healthcare-associated pathogens. [9]. *P.aeruginosa* and *Acinetobacter* species are known to be the common nosocomial pathogens. [3,9] Similar observations have also been made by us. NFGNB belonging to *Pseudomonas* species: *P.aeruginosa* along with the *Acinetobacter* species accounted for 87% of the isolates.

In our study most of the isolates of NFGNB were from pus samples, similar to the observation made by others. [10,11] NFGNB were commonly involved in wound infections following RTA and in chronic non healing ulcers. Antibiotics such as Amikacin, Gentamicin and Ciprofloxacin were used to treat these infections, which most often were caused by the two pathogens: *P.aeruginosa* and *A.baumannii*.

The clinical conditions in which NFGNB were isolated in our study included SSI, UTI, VAP, and septicemia. *P.aeruginosa* and *A.baumannii* were common organisms incriminated in SSI and UTI. VAP was due to *A.baumannii*.

Resistance patterns among nosocomial bacterial pathogens may vary from country to country and also within the same country, over time. [13] *P.aeruginosa* isolates in our study were highly susceptible to Meropenem (94%), Cefoperazone (71%), and Amikacin (69%).

This contrasts with the antibiotic sensitivity pattern of isolates from Bangalore [14] and Chandigarh. [15]. In a study from Chandigarh 42% of *P.aeruginosa* isolates were found to be resistant to Meropenem. [15] Similarly *Acinetobacter* species showed higher rate of resistance to ciprofloxacin, Amikacin, Cefazidime and Piperacillin in a study at Bangalore when compared to the present study. [16]

We attribute these differences in the patient population studied by us. Most of our patients were from surgical wards and not from ICU settings. Furthermore, our patients came from rural areas without much exposure to antibiotics.

To conclude *P.aeruginosa* and *A.baumannii* are the most common NFGNB isolated in our study. Their role as healthcare-associated pathogens is well-established and they have caused UTI, septicemia, SSI, and VAP. *P.aeruginosa* has shown good sensitivity to Meropenem, Amikacin, and Cefoperazone. *A.baumannii* shows good sensitivity to Meropenem, and Piperacillin.

The different species of NFGNB have shown a varied sensitivity pattern in our study. Therefore, identification of NFGNB, and monitoring their susceptibility patterns, are important for the proper management of the infections caused by them. Our study highlights the fact that it is essential to establish the clinical relevance of the isolated NFGNB, before they are considered as pathogens. This will avoid unnecessary usage of antibiotics and emergence of drug-resistant strains.

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