



## Original Research Article

# Prevalence of Non-Fermentative Gram Negative Bacilli Infection in Tertiary Care Hospital in Birgunj, Nepal

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## ABSTRACT

Non-fermentative gram negative bacilli have emerged as an impending pathogenic entity with the ability to show resistance for commonly used antimicrobials. With this background the present study was undertaken to detect the clinical distribution and antibiogram profile of non-fermenting gram negative bacilli isolated from the clinical samples such as pus, urine, blood etc. Different non-fermentative gram negative bacilli were isolated from 1945 clinical samples obtained from various clinical departments of National Medical College and Teaching Hospital of Birgunj, Nepal during January 2014 to January 2015. The isolates were identified using the standard basic tests including motility, catalase, oxidase production, indole, triple sugar iron agar test, citrate utilization, urease production, oxidation-fermentation and phenylalanine deaminase tests. Antibiotic sensitivity testing of isolated gram-negative bacilli was performed by the Kirby Bauer disc diffusion method using Mueller Hinton Agar plates following the CLSI guidelines. 1215 samples were found to be positive for bacterial culture, while 360 (29.62%) samples showed growth of non-fermentative gram negative bacilli. The most common isolates were *Pseudomonas aeruginosa* accounting for 205 (56.94%) followed by *Acinetobacter baumannii* 75 (20.83%). Antibiotic sensitivity profile of these isolated organisms indicates higher sensitivity towards Imipenem, Cefipime-Sulbactam and Ceftazidime-Sulbactam with certain degrees of resistance towards Amikacin, Piperacillin and Co-trimoxazole. The study findings could be useful for the clinicians towards promoting rational use of antibiotics and contributing to abate unnecessary development of resistance.

## Keywords

Non lactose fermenter, Gram negative bacilli, Antibiotic sensitivity test, Imipenem

## Introduction

The pathogenic potential of non-fermentative gram negative bacilli (NFGNB) has been established beyond

doubt because of their repeated isolation from clinical specimens and association with the disease. These diverse groups of

microorganisms have familiar traits of clinical importance to justify their inclusion in a single group. NFGNB can cause a wide variety of infections and account for approximately 15% of all gram negative bacilli cultured from clinical samples (Siou *et al.*, 2009). Non-fermenters vary in their pathogenic prospective, transmissibility and many have developed resistance to antibiotics.

Accurate identification of non-fermenters is important for proper patient management. Infection caused by non-fermenters can be endogenous or exogenous in origin, which depends on several factors such as: irregular use of broad spectrum antibiotics, use of immunosuppressants, prolonged surgical procedure and inadequate instrumentation (Cristiane and Jose, 1998).

In recent years, the problem is further compounded by the emergence of resistance to antimicrobial agents which are widely used against the non-fermenters, making them as an important healthcare associated pathogen. Non-fermenting gram negative bacilli are intrinsically resistant to many antibiotics and are known to produce extended spectrum  $\beta$ -lactamases and metallo  $\beta$ -lactamases (Malini *et al.*, 2009). Non-fermenters have been incriminated in infections such as pneumonia, septicaemia, urinary tract infection, surgical site infection and have the potential to spread from patient to patient via fomites or the hands of medical personnel (Quinn, 1998).

With this concept, in our present study, an effort has been made to isolate, identify and characterize various non-fermenters with clinical condition of the patient. Study was also done to assess the antimicrobial susceptibility and to identify various healthcare related problems.

## **Materials and Methods**

A total of 1945 clinical specimens were received in Microbiology Laboratory of National Medical College, Birgunj, for culture and sensitivity from January 2014 to January 2015. Among the 1945 clinical samples, 500 were urine samples, 410 pus swabs, 520 blood cultures, 180 tracheal aspirates, 135 throat swab and 200 were body fluids. All the received specimens were inoculated in blood agar, Mac Conkey agar, CLED agar (Cysteine lactose electrolyte deficient Agar) and nutrient agar. The plates were incubated under aerobic condition at 37°C for 24–48 hours, followed by their cultural characteristics.

NFGNB were identified by colony characteristics and biochemical reactions as described in Bailey and Scotts diagnostic microbiology (Gautam *et al.*, 2009). Morphology and motility of the organisms were determined by Gram's stain and Hanging drop preparation method respectively. Every single gram negative bacilli/coccobacilli, oxidase positive or negative were inoculated on Triple sugar iron agar medium (TSI). Organisms showing alkaline reactions were initially considered to be non-fermenter gram negative bacilli. The isolates were identified up to the species level based on motility, pigment production, enzyme production (e.g. catalase, urease, nitrate reductase) and various biochemical tests including Hugh and Leifson's medium to find out whether a particular organism was oxidizer or non-oxidizer, indole test citrate utilization, production of hydrogen sulphide and nitrate or nitrite reduction (Sidhu *et al.*, 2010). Provisional identification of the unknown isolates of being a non-fermenter is lack of evidence of glucose fermenter and positive cytochrome oxidase reaction (Koneman *et al.*, 2004).

The scheme for identification of NFGNB is depicted in figure 1.

Antimicrobial sensitivity testing was carried out for all the isolates with the help of the Kirby-Bauer disc diffusion method using commercially available discs on Muller-Hinton agar (MHA). The results were interpreted as per the Clinical and Laboratory Standard Institute (CLSI-2014) guidelines (CLSI, 2014).

## Results and Discussion

After analyzing 1945 clinical samples, 1215 (62.46%) samples were found positive for bacterial culture and 51 (2.62%) in favour of *Candida*, and the remaining samples were culture negative or normal flora. Among the total isolates, 283 (23.80%) belonged to Gram positive and 881 (72.51%) represented Gram negative organisms. Amongst the clinical specimens presenting positive for bacterial culture, 360 (29.62%) were found to be positive for non-fermenter gram negative bacilli. Non-fermenter gram negative bacilli's (NFGNB) were isolated from pus, urine, blood, tracheal aspirate, throat swabs and body fluids. The study found higher prevalence of NFGNB from the pus exudates and the body fluids (41.66%), followed by the urine samples (33.05%), blood (15.83%), tracheal aspirate (7.77%), and throat swab (1.66 %) respectively. The chunk of the NFGNB was isolated from pus, body fluids and urine samples, which hold for 74.72% of the total isolates. The distribution of NFGNB among all the specimens is shown in table 1.

The organisms most commonly isolated, were *Pseudomonas aeruginosa* (56.94%) followed by *Acinetobacter baumannii* (20.83%), *Pseudomonas sp.* (8.33%), *Proteus sp.* (6.66%), *Acinetobacter sp.* (3.61%), *Moraxella sp.* (2.22%) and

*Providencia sp.* (1.3%). Among the all pus exudates and body fluids, 150 samples yielded NFGNB. The most pathogenic isolates from the pus exudates and the body fluids were *P. aeruginosa* followed by *sp.*, *A. baumannii* and eight isolates of *Proteus sp.* Among the 119 urine samples showing NFGNB, *P. aeruginosa* was the major pathogen isolated followed by *A. baumannii*, *Pseudomonas sp.*, and *Proteus sp.* The culture of blood samples yielded 57 NFGNB, of which, *P. aeruginosa* was the main offender followed by *Acinetobacter baumannii* and *Pseudomonas sp.* The patients bearing infection in throat showed the presence of *Pseudomonas aeruginosa* and three isolates of *Moraxella* as the pathogenic entity. The findings from majority of the clinical specimens clearly illustrate *P. aeruginosa* as the most common pathogen followed by *A. baumannii*.

The antibacterial sensitivity pattern of isolated NFGNB as a pathogen showed that 82% of *P. aeruginosa* were sensitive to Imipenem, 79% were sensitive to Cefipime-Sulbactam, 69% were sensitive to Ceftazidime-Sulbactam, 34% were sensitive to Amikacin, 46% for Piperacillin and 41% for Co-trimoxazole. While the second most isolated NFGNB, *A. baumannii* showed highest sensitivity (75%) towards Cefipime-Sulbactam followed by Imipenem (70%). Similarly *Pseudomonas sp.* showed maximum sensitivity towards Imipenem and the least towards Co-trimoxazole (Table 2). Isolated *Acinetobacter sp.* showed total resistant towards Co-trimoxazole and maximum susceptibility against Ceftazidime-Sulbactam and Imipenem. While the other rare isolates such as *Moraxella sp.* and *Providencia sp.* were highly susceptible to the tested antibiotics. Antimicrobial sensitivity pattern of all the non-fermenter gram negative bacilli is shown in table 2.

Non-fermenters gram negative bacilli are omnipresent in nature. They were considered to be a contaminant in the past but in the contemporary world, it emerges as an important healthcare pathogen. In the current study, predominantly isolated NFGNB was *Pseudomonas aeruginosa* followed by *Acinetobacter baumannii*, which comply with the results of Malini *et al.* (2009) and Vijaya *et al.* (2000). Different researchers have reported inconsistent results in their works. Rao and Shivananda, (1993) reported higher (66.88%) positivity rate of non-fermenters, while Chang and

Huang isolated 31.62% of non-fermenters (Chang and Huang, 2000). In our recent clinical study, we also reported 29.62% of non-fermenters. These variations might be due to the hospital infection control practices of the institutes (Sidhu *et al.*, 2010). Amongst the different clinical samples received and processed for the isolation of pathogenic bacteria responsible for causing the disease, the pus exudates and body fluid samples showed the highest number of non-fermenting gram negative bacilli isolates. This observation goes along with the study of Malini *et al.* (2009).

**Table.1** Distribution of different non-fermenter gram negative bacilli among different specimens

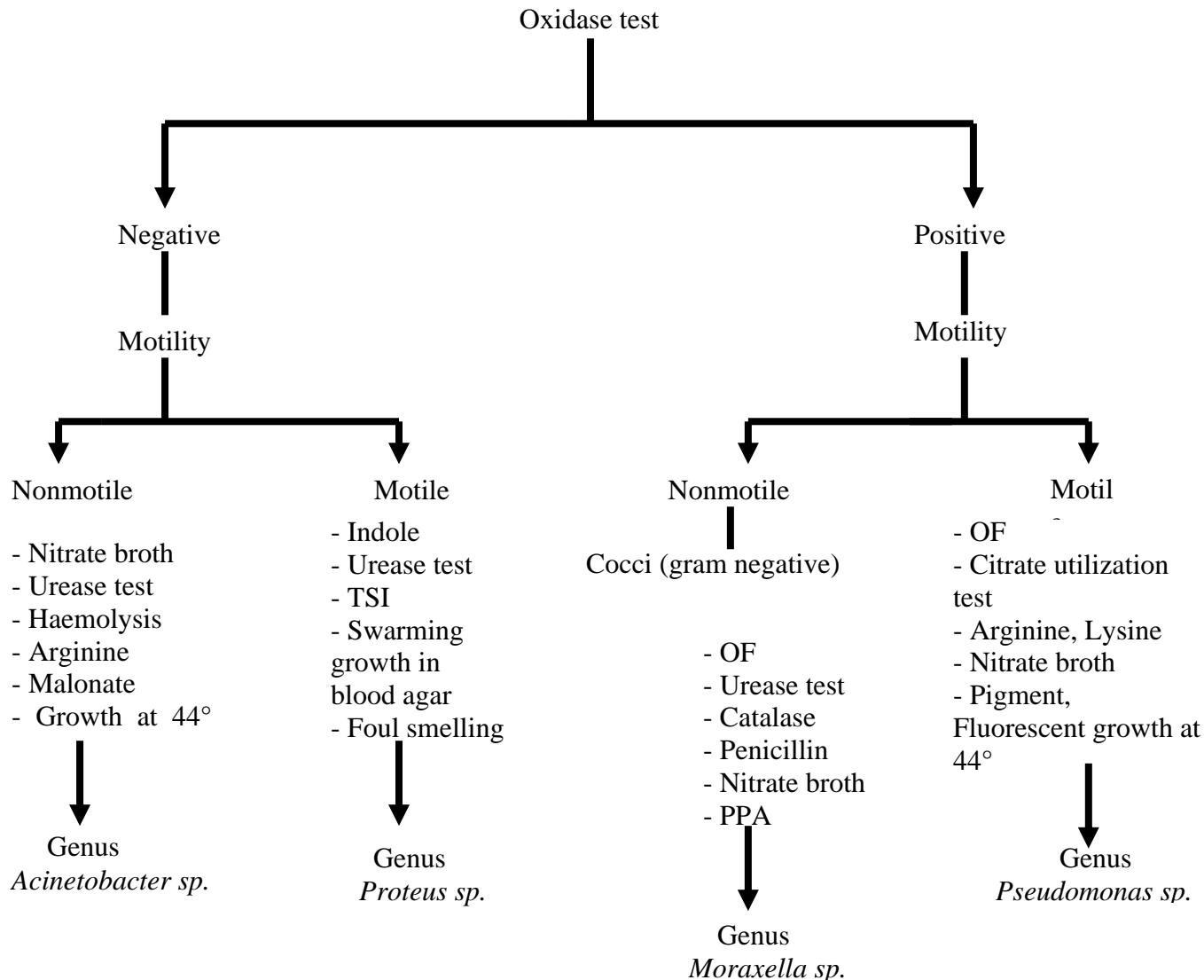
Samples → Species ↓	Pus exudates and body fluids	Urine	Blood	Tracheal aspirate	Throat swabs	Total N (%)
<i>Pseudomonas aeruginosa</i>	90	58	35	19	03	205 (56.94%)
<i>Pseudomonas sp.</i>	13	11	04	02	0	30 (8.33%)
<i>Acinetobacter baumannii</i>	31	26	12	06	0	75 (20.83%)
<i>Acinetobacter sp.</i>	03	08	02	0	0	13 (3.6%)
<i>Proteus sp.</i>	08	11	04	01	0	24 (6.66%)
<i>Moraxella sp.</i>	03	02	0	0	03	8 (2.22%)
<i>Providencia sp.</i>	02	03	0	0	0	5 (1.38%)

**Table.2** Antimicrobial sensitivity pattern of non-fermenting gram negative bacilli

Antimicrobial agents	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>Pseudo-monas sp.</i>	<i>Proteus sp.</i>	<i>Acineto bacter sp.</i>	<i>Moraxel-la sp.</i>	<i>Providencia sp.</i>
Imipenem	82	70	83	79	61	100	100
Amikacin	34	37	63	83	69	87	100
Ceftazidime-sulbactam	69	53	73	71	84	100	100
Cefipime-sulbactam	79	75	86	75	54	75	100
Piperacillin	46	53	60	71	38	—	67
Co-trimoxazole	41	44	46	42	—	—	33

The data shown in the table is the percentage for sensitive organisms

Fig.1 Identification of non-fermenting gram negative bacilli



- OF = Oxidative Fermentative test for glucose, maltose, mannitol, sucrose, lactose  
 - PPA = Phenyl alanine deaminase

Antibiogram of the isolated NFGNB from different clinical samples was established using different antibiotic discs by disc diffusion method. Antibiotics like Imipenem, Cefipime-Sulbactam and Ceftazidime-Sulbactam were found the most potent against the isolated non-fermenter gram negative bacilli which is similar to the observation made by Malini *et al.* (2009). According to the work of Taneja *et al.*, in Chandigarh, 42% of *P. aeruginosa* isolates

were resistant to Imipenem (Taneja *et al.*, 2003), whereas, it is only 18% in our study. The differences in the rate of drug resistance are due to variations in type of antimicrobials being prescribed by the clinicians.

In the present study, the outcome of interest was to isolate the NFGNB from the clinical specimens and to view their antibiotic profile. It appears that the isolated

organisms were susceptible to higher generation of antibiotics, but showed some degree of resistance to Amikacin, Piperacillin and Co-trimoxazole. Appearance of multi-drug resistant pathogens in hospital environment is increasing worldwide and limiting the therapeutic options for clinicians. The most probable reason is the wide spread use of antibiotics. Furthermore, easy availability of over-the-counter drugs without prescription is common in practice in this part of the world (Tiwari and Kaur, 2010). While describing the non-judicious use of these life saving molecules, it was well said by one of the experts in the field, “When it comes to prescribing antibiotics, most doctor’s use the canon, when a gun can be sufficient to kill the enemy” (Datta, 2004).

Similarly, *A. baumannii* showed higher degree of resistance to Amikacin, Co-trimoxazole and Piperacillin, which is in accordance with the findings of Ishikawa *et al.*, (2005) and Chitnis *et al.* (2003). The resistance pattern of *A. baumannii* demands for more attention because of its impending ability to form biofilm might also clarify its outstanding antibiotic resistance, survival properties and increased virulence.

Along with other NFGNB *P. aeruginosa* and *A. baumannii* were the most common NFGNB isolated in our study. Their position as healthcare associated pathogen is well established, as they have the ability to cause respiratory tract infection, bacteraemia, septicaemia, urinary tract infection and so on. Therefore, proper recognition of NFGNB and close monitoring of their antibiogram profile is of utmost importance in the proper management of the infections caused by them.

The present study highlighted the fact that NFGNBs have emerged as an important

pathogen and shows resistance to commonly used antimicrobials. More significantly, the isolated NFGNB’s have great ability to survive in hospital environment, so effective methods of sterilization and infection control measures should be implemented.

To minimize the drug resistance, emphasis should be given to carry out systemic surveillance of antibiotic sensitivity test and proper drug administration in accordance with the sensitivity test results. This study could be useful for the clinicians in particular of the region towards rational selection of antibiotics for optimal outcome.

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