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Prevalence and *In Vitro* Antimicrobial Susceptibility Pattern of Non-Lactose Fermenting Gram Negative Bacteria Isolated in a Tertiary Care Hospital in Kathmandu, Nepal

Manisha Sharma[1], Narayan Dutt Pant[1,2*]

[1]Department of Microbiology, Kathmandu Medical College, Kathmandu, Nepal

[2]Department of Microbiology, Grande International Hospital, Dhapasi, Kathmandu, Nepal

Abstract

Background: Non-lactose fermenting gram negative bacteria are emerging as important human pathogens. This group of bacteria was earlier considered to be colonizers but are now frequently isolated from different clinical specimens and are responsible for a wide range of human infections especially in immune compromised hosts. The main purpose of our study was to determine the prevalence and role of non-lactose fermenting Gram negative bacteria in causing human infections with their antimicrobial susceptibility pattern among the patients coming to a tertiary care hospital in Kathmandu, Nepal.
Methods: A retrospective study was conducted by analyzing the data on non-lactose fermenting Gram negative bacteria isolated from a total of 12970 different clinical samples obtained from the admitted patients attending a tertiary care hospital in Kathmandu, Nepal from March 2013 to March 2015. The clinical samples were processed following standard microbiological procedures and the colonies grown were identified with the help of colony characteristics, Gram staining tests. Antimicrobial susceptibility testing was performed using Kirby-Bauer disc and biochemical diffusion technique.
Results: The non-lactose fermenting gram negative bacilli were isolated from 3.5% of the samples. The most common non-lactose fermenting gram negative bacillus isolated was *Acinetobacter baumannii* followed by *Pseudomonas aeruginosa*. Respiratory samples recorded the highest number of non-lactose fermenting Gram negative bacilli, followed by urine samples. Among the commonly used antibiotics, the highest rate of susceptibility was found towards imipenem.
Conclusion: *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have emerged as the most common non-lactose fermenting gram negative bacteria causing human infections. Amongst the commonly used antibiotics, imipenem retained activity against such bacteria.

Keywords: Non-lactose fermenting gram negative bacteria, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Antibiotic susceptibility.

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Introduction

Non-lactose fermenting Gram negative bacteria are emerging as important human pathogens. Previously they were considered as colonizers or contaminants even when isolated from clinical specimen [1,3]. Non-lactose fermenting gram negative bacteria are evolving as important healthcare-associated pathogens and are responsible for infections like pneumonia, septicemia, meningitis, surgical wound infections, and urinary tract infections in hospitalized patients [2]. Non-lactose fermenters account for approximately 15% of all bacteria isolated from clinical specimens [2]. They are intrinsically resistant to many antibiotics and may produce extended spectrum ß-lactamases and metallo ß-lactamases [2] which are due to why the management of the infections caused by these bacteria has become a great problem. Furthermore, abuse of antibiotics has added to the development of drug resistance among non-lactose fermenting bacilli [4].

Amongst all the non-lactose fermenters, including *Alkaligenes* spp. *Acinetobacter* spp, *Burkholderia cepacia*, *P. aeruginosa* and *Acinetobacter* species have established themselves as the most common nosocomial pathogens [5,6]. Risk factors associated with emergence of non-lactose fermenting gram negative bacteria as nosocomial pathogens are immune-suppression, neutropenia, mechanical ventilation and cystic fibrosis, indwelling catheters and invasive procedures [4]. The antibiotic susceptibility patterns of bacterial isolates responsible for nosocomial infections may exhibit temporal and geographical variations [7]. Due to high innate resistance shown by non-lactose fermenting gram negative bacteria to commonly used antibiotics, proper identification and determination of antibiotic susceptibility pattern is very

important for proper management of infections caused by these pathogens [4].

Sufficient data on clinical isolates and their antibiotic susceptibility patterns in Nepal is not available. Therefore, we undertook this study to determine the prevalence and role of non-lactose fermenting gram negative bacteria in human infections and their antimicrobial susceptibility pattern.

Materials and Methods

A retrospective study was conducted by analysing the data on non–lactose fermenting gram negative bacteria isolated from total of 12970 different clinical samples obtained from the admitted patients attending a tertiary care hospital in Kathmandu, Nepal from March 2013 to March 2015. The clinical specimens collected included urine, sputum and other respiratory samples, blood, pus/wound and from other body fluids. The clinical samples received were processed following standard microbiological procedures [8]. The colonies grown were identified with the help of colony characteristics, Gram staining and biochemical tests [9]. Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion technique according to clinical and laboratory standards institute guidelines [10]. *E. coli* ATCC 25922 was used for quality control. Statistical analysis was performed by using statistical package for the social sciences version 20.0.

Results

Of the total 12970 different clinical specimens investigated, non-lactose fermenting gram negative bacilli were isolated from 454 (3.5%) samples. The most common non lactose fermenting gram negative bacillus isolated was *Acinetobacter baumannii* 229 (50.4%) from the different clinical specimens, followed by *Pseudomonas aeruginosa* with 173 (38.1%) isolations. The least bacilli isolated are *Morganella morganii* 2 (0.4%) and *Moraxella* spp. 1 (0.2%). Highest numbers of non-lactose fermenting gram negative bacilli were isolated from sputum and other respiratory samples 215, followed by urine samples 101. The least bacilli were isolated from the body fluids 7 (Table 1).

Table 1. *Different clinical specimens showing growth of non-lactose fermenting gram negative bacilli*

	Specimens					**Total (%)**
Clinical isolates	Urine	Respiratory samples	Blood	Pus/Wound swab	Fluids	
Acinetobacter baumannii	18	129	51	25	6	229 (50.4)
Pseudomonas aeruginosa	47	84	7	35	0	173 (38.1)
Proteus vulgaris	16	1	0	6	0	23 (5.1)
Proteus mirabilis	15	0	0	6	0	21 (4.6)
Providencia spp	3	0	0	1	1	5 (1.1)
Moraxella spp.	0	1	0	0	0	1 (0.2)
Morganella morganii	2	0	0	0	0	2 (0.4)
Total	101	215	58	73	7	454

All the strains of bacteria those do not show intrinsic resistance were found to be susceptible toward tigecycline, colistin and polymixin B. Apart from these antimicrobials, the highest rate of susceptibility among commonly used antibiotics was found toward imipenem,76.8% (Table 2).

Table 2. *Antimicrobial susceptibility pattern of non-lactose fermenting gram negative bacilli toward commonly used antibiotics*

Antibiotics	**Susceptibility (%)**
Amikacin	48.0
Amoxycillin/clavulanic acid	6.5
Ampicillin	7.4
Azithromycin	43.5
Cefixime	24.4
Ceftriaxone	22
Ciprofloxacin	27.1
Cotrimoxazole	21.3
Erythromycin	12.5
Gentamicin	40
Imipenem	76.8
Levofloxacin	57.4
Meropenem	56.7
Ofloxacin	35
Piperacillin/tazobactam	50.2

Discussion

Previously non-lactose fermenting gram negative bacilli were considered to be contaminants [2] and were disregarded even when grown in the clinical samples. But nowadays these

bacteria, esp. *P. aeruginosa* and *Acinetobacter* species have established themselves as the most common nosocomial pathogens [2,5,6]. In our study too *Acinetobacter baumannii* was the most common isolate followed by *Pseudomonas aeruginosa*. However, Malini et al. [2], Bhargava et al. [1] and Benachinmardi et al. [4] found the most common isolate to be *Pseudomonas aeruginosa* followed by *Acinetobacter* spp.

Gram-negative bacilli mainly *P. aeruginosa* and *A. baumannii* are the predominant cause of hospital-acquired pneumonia [11]. This agreed with our findings too, the largest number of *Acinetobacter baumannii* were isolated from sputum and other respiratory samples followed by blood. Similarly, highest numbers of *Pseudomonas aeruginosa* were isolated from respiratory samples followed by urine. Bhargava et al. [1] noted the highest numbers of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to be isolated from pus/exudates and body fluids followed by urine. Furthermore, Malini et al. [2] reported the isolation of highest numbers of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from pus. Benachinmardi et al. [4] isolated highest numbers of *Acinetobacter baumannii* from respiratory samples and highest numbers of *Pseudomonas aeruginosa* from pus samples.

Non-lactose fermenting gram negative bacilli are intrinsically resistant to many antibiotics and may produce extended spectrum ß-lactamases and metallo ß-lactamases [2]. In our study, *Acinetobacter baumannii* was highly susceptible to polymixin B, tigecycline and colistin followed by imipenem (73.1%). Similarly, highest rate of susceptibility of *Pseudomonas aeruginosa* was toward polymixin B and colistin followed by imipenem (75.5%). In a study by Malini et al. [2] highest rate of susceptibility of *P. aeruginosa* (94.2%) and *Acinetobacter baumannii* (100%) was found toward imipenem. Bhargava et al. [1] also reported similar findings with *P. aeruginosa* (82%) and *A. baumannii* (70%). However, Benachinmardi et al. [4] showed the susceptibility of the *P. aeruginosa* to be highest to amikacin (83.3%) followed by imipenem (80%) and piperacillin-tazobactam (73.3%). So, on the basis of our findings and results reported by other studies, imipenem may be used as the drug of choice for treatment of infections caused by non -lactose fermenting gram negative bacilli. Further, increasing rates of isolation of multidrug resistant Pseudomonas have compelled clinicians to use polymyxins for treatment of the infections caused by such bacteria despite their nephrotoxicity [12]. Similarly, US Food and Drug Administration have approved tigecycline for treatment of many infections caused by drug resistant bacteria [12].

Conclusion

Acinetobacter baumannii and *Pseudomonas aeruginosa* are the most common non-lactose fermenting gram negative bacteria causing human infections. Among all the commonly used antibiotics, we recommend use of imipenem for the preliminary treatment of the infections caused by such bacteria in our settings. Further, in case of serious infections caused by highly drug resistant non-lactose fermenting gram negative bacteria, use of polymixin B, tigecycline and colistin may prove to be beneficial provided the bacteria causing infections do not show intrinsic resistance to these drugs.

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***Correspondence to**

Narayan Dutt Pant

Department of Microbiology

Grande International Hospital, Dhapasi, Kathmandu

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