Nonfermenting Gram-negative Bacilli other than *Pseudomonas aeruginosa* and *Acinetobacter* Spp. Causing Respiratory Tract Infections in a Tertiary Care Center

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ABSTRACT

Background: Nonfermenting gram-negative bacilli have emerged as important healthcare-associated pathogens. It is important to correctly identify all clinically significant nonfermenting gram-negative bacilli considering the intrinsic multidrug resistance exhibited by these bacteria. **Materials and Methods:** A retrospective study was undertaken to identify the various nonfermenting gram-negative bacilli other than *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated from respiratory samples (*n* = 9363), to understand their clinical relevance and to analyze their antibiotic susceptibility pattern. **Results:** Nonfermenting gram-negative bacilli were isolated from 830 (16.4%) samples showing significant growth. Thirty-three (4%) isolates constituted nonfermenting gram-negative bacilli other than *P. aeruginosa* and *Acinetobacter* spp. *Stenotrophomonas maltophilia* (15, 45.5%) was the most common isolate followed by *Burkholderia cepacia* (4, 12.1%), *Sphingomonas paucimobilis* (3, 9.1%), and *Achromobacter xylosoxidans* (3, 9.1%). On the basis of clinicomicrobiological correlation, pathogenicity was observed in 69.7% (*n* = 23) isolates. Timely and correct treatment resulted in clinical improvement in 87.9% cases. **Conclusion:** Any nonfermenting gram-negative bacilli isolated from respiratory tract infection should not be ignored as mere contaminant, but correlated clinically for its pathogenic potential and identified using standard methods so as to institute appropriate and timely antibiotic coverage.

Key words: Intrinsic resistance, Nonfermenting gram-negative bacilli, *S. maltophilia*

INTRODUCTION

Injudicious and empirical use of antibiotics has enabled non-fermenting gram-negative bacilli (NFGNB) emerge as important healthcare-associated pathogens.[1] These organisms are ubiquitous in nature particularly in soil and water. In the hospital environment, they may be isolated from instruments such as ventilator machine humidifiers, mattresses, and other equipments as well as from the skin of healthcare workers.[2] All these organisms have the potential to spread horizontally on fomites or the hands of healthcare workers.[1-3] The identification of these non-fermenters is important because of the fact that most of them show multidrug-resistant pattern and inherent resistance to many antibiotics.[3] Majority of the earlier studies have only focused on the identification of *Pseudomonas* spp. and *Acinetobacter* spp. because of their higher isolation rates. However, in immunocompromised patients especially, NFGNB other than *Pseudomonas* spp. and *Acinetobacter* spp. can also cause the disease. So, it becomes imperative to know about the occurrence and susceptibility pattern of these non-fermenters.

This study was undertaken to identify the various NFGNB other than *P. aeruginosa* and *Acinetobacter* spp. isolated from the respiratory samples, to understand their clinical relevance, and to analyze their antibiotic susceptibility pattern.

MATERIALS AND METHODS

A retrospective study was conducted in the Clinical Microbiology Laboratory of a tertiary care center in Coastal Karnataka from December 2009 to November 2011. All the respiratory samples (sputum or bronchoalveolar lavage fluid) received during this time period were included. Sputum gram stains were read at ×100 magnification and evaluated according to the Bartlett criteria.[4] Specimens were scored 0, +1, or +2 according to the number of

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leukocytes seen per field and 0, −1, and −2 according to the number of squamous epithelial cells seen per field. Specimens with total scores of 0 or less were considered inadequate and heavily contaminated with oropharyngeal flora. Those containing greater than 25 leucocytes and fewer than 10 squamous epithelial cells per field were optimal specimens and further processed for culture.[4] All samples were inoculated on sheep blood agar, MacConkey agar and chocolate agar, and incubated at 37°C for 18-24 hours. Bronchoalveolar lavage fluid was processed by quantitative culture with positive threshold of 10^4 CFU/mL.[5] The NFGNB other than *P. aeruginosa* and *Acinetobacter* spp. isolated as the predominant organism from sputum (at least two samples per patient) or $\geq 10^4$ CFU/mL of bronchoalveolar lavage fluid and correlating with microscopic findings of the sample were considered significant and included in the study. The organisms isolated were subjected to routine biochemical reactions like oxidase test, growth on Triple Sugar Iron agar, mannitol motility test medium, indole production, hydrogen sulfide production, urea hydrolysis, and citrate utilization. The non-fermenters which could not be identified on the basis of above-mentioned biochemical reactions were subjected to Vitek 2 system for identification. The susceptibility testing was performed using Kirby-Bauer disc diffusion method using commercially available discs according to Clinical Laboratory Standards Institute guidelines.[6]

Details of patients such as the demographic parameters, clinical presentation, radiological findings, location of stay in the hospital, duration of hospital stay, and prognostic outcomes were noted in a structured proforma retrospectively from the medical record department. The clinical significance of the NFGNB isolates was assessed by correlating with clinical and radiological findings of the patient. Culture isolates obtained as scanty growth or mixed growth with more than two types of organisms from sputum samples; or $<10^4$ CFU/mL from bronchoalveolar lavages and not correlating with gram stain findings and clinical criterion of nosocomial pneumonia were considered as colonizers and not included in the analysis. Descriptive analysis of results was performed using statistical software, SPSS version 16.0. Frequencies and percentages were derived for categorical data. For continuous data, mean was obtained.

Nosocomial pneumonia was defined as a new or progressive and persistent infiltrate or consolidation or cavitation on two serial chest radiographs that occurred at least 48 h after hospital admission in the presence of at least one of the following clinical signs: Fever (temperature >38°C) with no other recognized cause, leukopenia ($<4.0 \times 10^9$ cells/L) or leukocytosis ($>12.0 \times 10^9$ cells/L), altered mental status with no other recognized cause and any two of the following: New onset of purulent sputum, change in character of sputum, increased respiratory secretions, or increased suctioning requirements, new-onset or worsening cough, or dyspnea, or tachypnea, rales or bronchial breath sounds, worsening gas exchange (e.g., oxygen desaturation ratio [PaO2-FiO2] ≤240, increased oxygen requirement, or increased ventilation demand).[7]

RESULTS

A total of 9363 respiratory specimens were received during the study period out of which 5056 (54%) cultures yielded a significant growth and 4307 (46%) cultures had growth of normal oropharyngeal flora. NFGNB were isolated in 830 (16.4%) out of 5056 sputum cultures. Out of 830 isolated NFGNB, 471 (56.7%) were *P. aeruginosa*, 326 (39.3%) were *Acinetobacter* spp., and 33 (4%) constituted others. The distribution of these nonfermenters is shown in Table 1. *Stenotrophomonas maltophilia* (15, 45.5%) was the most common among other NFGNB followed by *Burkholderia cepacia* (4, 12.1%) and others. Demographic parameters and various clinical presentations of the patients are shown in Table 2. On the basis of clinicomicrobiological correlation, among the 33 isolates, 69.7% ($n = 23$) were pathogens and 30.3% ($n = 10$) were colonizers. The pathogens were responsible for hospital-acquired pneumonia in 24.24% ($n = 8$) patients including *S. maltophilia* ($n = 6$), *B. cepacia* ($n = 1$) and *Elizabethkingia meningoseptica* ($n = 1$). Susceptibility pattern of the isolates to various antimicrobial agents is shown in Table 3.

DISCUSSION

The current study has shown the isolation of NFGNB in 16.4% of respiratory samples. The importance of isolation

Table 1: Nonfermenting gram-negative bacilli other than *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated from clinical specimens

Organism	No. (%)
Stenotrophomonas maltophilia	15 (45.5)
Burkholderia cepacia	4 (12.1)
Sphingomonas paucimobilis	3 (9.1)
Achromobacter xylosoxidans	3 (9.1)
Burkholderia pseudomallei	2 (6.1)
Chryseobacterium indologenes	2 (6.1)
Alcaligenes faecalis	2 (6.1)
Elizabethkingia meningoseptica	1 (3)
Shewanella putrifaciens	1 (3)
Total	33 (100)

Table 2: Demographic and clinical details of the patients

Parameter	% distribution (number of cases) (Total number of cases=33)
Age (mean±SD)	54.42±16.57
Gender	
Male	78.8 (26)
Female	21.2 (7)
Primary diagnosis	
Acute exacerbation of chronic obstructive pulmonary disease	33.33 (10)
Pneumonia	15.15 (5)
Pulmonary Koch's	6.06 (2)
Melioidosis	6.06 (2)
Idiopathic pulmonary fibrosis	3.03 (1)
Lung abscess	3.03 (1)
Chronic bronchopulmonary aspergillosis	3.03 (1)
Ruptured amoebic liver abscess	3.03 (1)
Cirrhosis	3.03 (1)
Acute myeloid leukemia on chemotherapy	3.03 (1)
Hospital-acquired pneumonia	24.24 (8)
Radiological findings (*n*=26, data missing: 7)	
Unilateral involvement	50 (13)
Bilateral involvement	50 (13)
Haziness	57.7 (15)
Consolidation	23.1 (6)
Cavitation	19.2 (4)
Location of patients in the hospital	
Wards	87.8 (29)
Medical	55.2 (16)
Surgical	20.7 (6)
Pulmonology	24.1 (7)
Intensive care unit	12.2 (4)
Medical	75 (3)
Surgical	25 (1)
Outcome	
Improved	87.9 (29)
Expired	9.09 (3)
Discharged against medical advice	3.03 (1)
Duration of hospital stay (median, IQ range)	9.00 (7.00-18.00)

IQ: Interquartile range; SD: Standard deviation

of non-fermenters has increased in last decade, after more and more reports are correlating them with the either infection outbreaks in hospitals, or healthcare-associated infections.[1] Earlier the identification of non-fermenters, based on biochemical tests, was cumbersome and many non-fermenters were misidentified. But, now with the availability of commercial systems like Vitek-2 or API, the identification has become easier.[8] Malini *et al.,*[9] from Kolar in India have documented the isolation of 6.8% (25 of 365) of NFGNB in respiratory samples.

S. maltophilia is considered now as a common non-fermenter to cause infection in hospital settings.[10,11] Correct identification of this NFGNB assumes importance as it shows inherent resistance to commonly used broad spectrum beta-lactam group antibiotics and even to imipenem.[12] Our study has shown the isolation of this bacterium in 45.5% cases. Earlier A'Court and Garrard[13] have reported *S. maltophilia* to account for 5% of nosocomial pneumonias. These nosocomial pneumonia are frequently associated with mechanical ventilation, tracheostomy, previous exposure to broad-spectrum antibiotics, the use of respiratory tract equipment such as nebulizers[14-21] and therapy with aerosolized polymyxin.[22] Majority of our isolates were sensitive to ciprofloxacin (93.3%) followed by trimethoprim-sulfamethoxazole (86.7%). Malini *et al.,*[9] have documented 100% sensitivity to ciprofloxacin and trimethoprim-sulfamethoxazole. This bacterium produces an unusual chromosomally encoded zinc-depended β-lactamase that confers broad resistance to carbapenems and other β-lactames.[23]

B. cepacia complex (BCC) is another NFGNB colonizing and infecting patients with chronic respiratory illness. It is known to cause disease in cystic fibrosis (CF) patients and once infected, it is very difficult to eradicate.[24] In our study

Table 3: Susceptibility profile of non-fermenters to various antimicrobials

Organisms (No.)	Antimicrobials (% sensitivity)										
	CTZ	CEF	PIP	CES	PTZ	TCL	CIP	STX	GEN	AMK	MER
Stenotrophomonas maltophilia (15)	NT	NT	NT	66.7	NT	NT	93.3	86.7	NT	NT	NT
Burkholderia cepacia (4)	100	NT	NT	NT	NT	NT	0	0	NT	NT	100
Sphingomonas paucimobilis (3)	66.7	66.7	100	33.3	66.7	0	33.3	0	0	0	33.3
Achromobacter spp. (3)	66.7	66.7	66.7	100	100	100	33.3	0	33.3	0	100
Chryseobacterium indologenes (2)	NT	NT	NT	100	50	NT	100	0	NT	50	NT
Burkholderia pseudomallei (2)	50	50	0	100	100	0	0	100	NT	NT	100
Alcaligenes faecalis (2)	0	0	50	100	50	0	50	50	0	0	0
Elizabethkingia meningoseptica (1)	NT	NT	NT	100	0	100	100	100	NT	0	NT
Shewanella putrifaciens (1)	100	100	100	100	100	100	100	0	100	100	100
Total	66.7	54.5	36.4	75.9	71.4	41.7	63.6	51.5	22.2	16.7	73.3

CTZ–Ceftazidime (30 μg); CEF–Cefepime (30 μg); PIP–Piperacillin (100 μg); CES–Cefoperazone-sulbactam (75/30 μg); PTZ–Piperacillin-tazobactam (110/10 μg); TCL–Ticarcillin-clavulanic acid (75/10 μg); CIP–Ciprofloxacin (5 μg); STX–Trimethoprim-sulfamethoxazole (1.25/23.75 μg); GEN–Gentamicin (10 μg); AMK–Amikacin (30 μg); MER–Meropenem (10 μg) ; NT–Not tested

BCC was isolated in 12.1% of cases, though its association with CF was not studied. Rahbar *et al.,*[25] have shown the isolation of BCC as 4.66% of all the nonfermenters isolated from different type of specimens (respiratory, blood, urine, wound, etc.). BCC is known for its intrinsic resistance to many beta-lactam drugs, aminoglycosides, colistin and polymixin B, the first-line therapeutics of choice against serious pseudomonal infections.[24] Our strains have demonstrated 100% sensitivity to ceftazidime and meropenem.

Achromobacter xylosoxidans is an aerobic, gram-negative bacillus found in a variety of aquatic environments and has proved to survive on inanimate surfaces in hospital settings, connected to its role as a nosocomial colonizer. It is generally considered an opportunistic pathogen and has attracted attention as an emerging pathogen in CF.[26] Reported prevalence rates of *A. xylosoxidans* have increased in recent years, although this may in part result from growing attention or improved microbiologic techniques. Our study has observed the isolation of *A. xylosoxidans* in 9.1% cases. These isolates have shown 100% sensitivity to beta-lactam-beta-lactamase inhibitor combination antimicrobials.

B. pseudomallei the causative agent of melioidosis, is an emerging NFGNB that causes lung infections. It is responsible for forming abscesses in multiple organs besides lungs like kidney, skin, musculoskeletal tissue, heart, etc. One of the nasty things about this bacterium is that the incubation period can be anywhere from a couple of days to couple of years. The other interesting fact about this pathogen is that it demands prolonged treatment first in intensive phase, followed by prolonged continuation phase.[27] The current study observed its isolation in 6.1% cases. Both the patients presented with cough and expectoration of 1-2 weeks duration. History of alcohol intake was present in both cases for last 15 years. One of them was diabetic for last 5 years and his blood culture also grew same pathogen. The patient was on piperacillin-tazobactam for 7 days. No improvement was observed in this patient and he expired. The other patient was empirically started on amoxicillin-clavulanic acid and amikacin but after culture report was changed to ceftazidime and trimethoprim-sulphamethoxazole and he showed improvement.

Differentiation between colonization and infection by these pathogens is of utmost importance. Otherwise, unnecessary institution of antibiotics will contribute to further increase in resistance. The present study observed that 69.7% of these NFGNB were pathogens, whereas 30.3% were colonizers. Most of the patients were having hospital stay for more than 9-10 days. It supports the fact that these patients may have acquired these pathogens in hospital settings from one source of other. But tracing of source giving rise to these infections was not observed in the present study.

Most of these NFGNB were sensitive to cefoperazone-sulbactam (75.9%), meropenem (73.3%), and piperacillin-tazobactam (71.4%) [Table 2]. The aminoglycosides that are considered as good option for life-threatening lower respiratory infections have shown high resistance in the present study for these non-fermenters wherever tested. It may be due to poor penetration of aminoglycosides from blood into infected respiratory tissues so as to reach the local drug concentration above the minimum inhibitory concentration necessary for the infecting organisms. This observation has also been discussed by earlier studies.[28] All these non-fermenters are known for their inherent resistance to multiple groups of antibiotics. Hence, correct identification of these non-fermenters is very important for choosing correct antibiotic so as to reduce the morbidity and mortality. In the present study, 29 (87.9%) cases showed improvement after institution of correct treatment, whereas 3 (9.1%) expired. The mortality may be due to underlying illness contributing to their immuno-compromised state or late institution of treatment.

Any NFGNB culture isolate from respiratory tract infection should not be ignored as just contaminant but correlated clinically for its pathogenic potential and identified using standard methods, so as to institute appropriate and timely antibiotic coverage.

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