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Non-fermenters other than *Pseudomonas* species: Characterization and susceptibility pattern

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Abstract

Background: Importance of non-fermenting gram-negative bacilli (NFGNB) in the aetiopathogenesis of human infections has increased considerably in recent years. Their higher rate of isolation from a wide variety of clinical specimens necessitates their identification and characterization.

Material and Method: A total of 5,369 bacterial isolates were obtained from 12,591 various clinical samples. All the samples were processed on Blood agar and MacConkey agar medium and identified according to standard protocol.

Result: Out of 5,369 isolates, 478 were identified as non-fermentative bacteria. Out of NFGNB strains isolated, the predominant numbers were that of *P. aeruginosa* 337 (70.5%) followed by other Pseudomonad's 63 (13.1%) and other Non-fermenters 78 (16.4%). Out of strains of other non-fermenters (78) predominant number was that of *Acinetobacter anitratus* (34), followed by *Acinetobacter lwoffii* (17), *Alcaligenes xylosoxidans* (10), *Alcaligenes denitrificans* (10) and *Alcaligenes faecalis* (7). The most sensitive drugs against non-fermenter isolates were found imipenem followed by amikacin.

Conclusion: There is enough reason to believe that they are associated with human disease process. Therefore, careful attempts must be made for their isolation and identification from various clinical specimens.

Keywords: NFGNB, *Acinetobacter anitratus*, *Alcaligenes denitrificans*, Imipenem

Introduction

The term non-fermenters (NF) refers to a group of aerobic non-spore forming gram-negative bacilli that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative rather than fermentative metabolic pathways. They comprise about 20% of all the gram-negative bacilli isolated from clinical specimens received in routine in a microbiology laboratory.[1,2] Previously, NF were considered as non-pathogenic and commensals of little clinical significance. However, nowadays their frequent isolation from clinical specimens strengthens their role in human disease process. Various host factors like immune status, nutritional status, environmental factors, respiratory viral infections like influenza and measles causing transient immunosuppression contribute further for enhancing their role as pathogens to humans.[3] *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus* and *Alcaligenes* species are important species among the heterogeneous group of NF bacteria.[4] These organisms are not identified as a routine because some NF are slow growing and require the use of special culture media and biochemical tests for their isolation and identification.

This study was aimed to find out the incidence of NF other than *Pseudomonas* species with emphasizes the importance of confirmatory tests for their isolations and identification scheme, in different clinical specimens and subjected them to antibiotic sensitivity pattern that can throw more light on their prevalence and pathogenic role.

Material and Method

This prospective study was conducted in the Department of Microbiology, in a tertiary care hospital from January 2013 to December 2013. A total of 12,591 clinical specimens received in the department for culture and sensitivity testing for aerobic bacteria. All the samples were processed on Blood agar and MacConkey agar medium according to standard microbiological procedure.[5]

Identification of non-fermenters: All the non-lactose fermenting colonies grown on MacConkey agar were picked up and subjected to oxidase test, Gram's staining and carbohydrate utilization (triple sugar iron) test. The organisms which were gram negative, glucose oxidizers, grown on MacConkey agar and had positive oxidase reaction, were processed further using various tests such as indole test, gas from nitrate, growth at 42°C, maltose fermentation, arginine dehydrolase, motility, pigment production, mannitol fermentation and gelatin liquefaction.[4] The organisms that were gram negative, glucose oxidizers, grown on MacConkey agar and had negative oxidase reaction, were processed further using various tests such as motility, mannitol fermentation, lactose fermentation and arginine dehydrolase as described in Table 1.[6]

The organisms, which were gram negative, glucose non-oxidizers, grown on MacConkey agar, had positive oxidase reaction and motile, were processed further using various tests such as nitrate reduction, gas from nitrite, H_2S in TSI and xylose fermentation as stated in Table 2.[6] The organisms that were gram negative,

glucose non-oxidizers, grown on MacConkey agar, had positive oxidase reaction and non-motile, were processed further using various tests as stated in Table 3.[6] The organisms, which were gram negative, glucose non-oxidizers, grown on MacConkey agar and had negative oxidase reaction, were processed further using various tests such as maltose fermentation, motility, beta hemolysis on Blood agar and citrate test as stated in Table 4.[6]

Antimicrobial Sensitivity Testing: Sensitivity to relevant antibiotics was determined by the Kirby-Bauer disk diffusion method as per the Clinical and Laboratory Standards Institute (CLSI, 2003) guidelines using the commercially available antibiotic disks from Hi-Media (Mumbai, India). NCCLS reference strains, *Pseudomonas aeruginsa* ATCC 27853 and *Escherichia coli* ATCC 25922 were included as control strains.[7,8]

Table 1: Different tests for identification of Gram negative, glucose oxidizers, growth on MacConkey and oxidase negative organisms

Organisms	Motility Test	OF Mannitol	OF Lactose	Arginine Dehyrolase
Burkholderia cepacia complex	+	A	A	_
Stenotrophomonas maltophilia	+	ALK	ALK	_
Acinetobacter anitratus	_	ALK	A	_

Note: + (Positive), - (Negative), A (Acid), ALK (Alkaline)

Table 2: Different tests for identification of Gram negative, glucose non-oxidizers, growth on MacConkey, oxidase positive and motile organisms

Organisms	Nitrate Reduction Test	Gas from Nitrate	Gas from Nitrite, Nitrate not reduced	H_2S in TSI	OF Xylose
Alcaligenes denitrificans	+	+	_	_	_
Alcaligenes putrifaciens	+	_	_	+	ALK
Alcaligenes faecalis	V	_	_	_	_
Bordetella bronchiseptica	+	_	_	_	_

Note: + (Positive), - (Negative), ALK (Alkaline), V (Variable)

Table 3: Different tests for identification of Gram negative, glucose non-oxidizers, growth on MacConkey, oxidase positive and non-motile organisms

Organisms	OF Glucose	Citrate Test	PPA	Pigment	Growth at 42°C
Acinetobacter calcoaceticus var.lwoffii.	ALK	±	-	-	V
Moraxella Species	ALK	_	+	Tan	+

Note: + (Positive), - (Negative), ALK (Alkaline), V (Variable)

Table 4: Different tests for identification of Gram negative, glucose non-oxidizers, growth on MacConkey, oxidase negative organisms

Organisms	OF Maltose	Motility	Beta Hemolysis	Citrate Test
Acinetobacter calcoaceticus var. lwoffii	_	_	+/-	+
Stenotrophomonas maltophilia	A	+	_	+/-
Bordetella parapertusis	_	_	+	_

Note: + (Positive), - (Negative), A (Acid)

Result

A total of 5,369 bacterial isolates were obtained from 12,591 various clinical samples. Out of 5,369 isolates, 478 were identified as non-fermentative bacteria. The maximum numbers of isolates were from pus (52.0%) followed by urine (15.7%), aural swab (11.3%), blood culture (8.6%), sputum (5.6%), endocervical swab (1.3%),

burn swab (1.3%) and other specimens. The category of others (4.2%) included various specimens like cerebrospinal fluid, endotracheal tube secretions, pleural fluid, CVC (central venous catheter) tips, conjunctival swab, bronchoalveolar lavage, throat swab, subcutaneous tissue and liver abscess.

Majority of the isolates were gram-negative bacilli (89%) and rests were gram-negative coccobacilli (11%). They were also differentiated into oxidase positive (87.6%) and oxidase negative (12.4%) organisms. Out of non-fermenting gram-negative bacilli (NFGNB) strains isolated from various clinical specimens, predominant numbers were that of *P. aeruginosa* 337 (70.5%) followed by other Pseudomonad's 63 (13.1%) and other Non-fermenters 78 (16.4%). Other Pseudomonad's included the isolates of *P. stutzeri* (44.6%) followed by *P. fluorescence* (26.9%), *S. maltophilia* (11.1%), *P. putida* (07.9%), *P. pickettii* (7.9%) and *B. cepacia complex* (1.6%). Out of strains of other non-fermenters (78) predominant number was that of *Acinetobacter anitratus* (34), followed by *Acinetobacter lwoffii* (17), *Alcaligenes xylosoxidans* (10), *Alcaligenes denitrificans* (10) and *Alcaligenes faecalis* (7). All the isolates were equally found in male and female patients. Majority of the isolates were isolated from pus samples (Table 5). The most sensitive drugs against non-fermenter isolates were found imipenem followed by amikacin. Some strains of *Acinetobacter anitratus* (3), *Alcaligenes denitrificans* (2), *Alcaligenes xylosoxidans* (1) and *Alcaligenes* faecalis (1), were resistance to all drugs. None of the isolates were sensitive to all drugs. Majority of isolates were resistant to two or more drugs (Table 6).

Table 5: Incidence of NFGNB in relation to specimens

Organisms (n=78)	Pus	Urine	Aural Swab	Blood Culture	Sputum	EC Swab	Burn Swab	Others
Acinetobacter anitratus (34)	11	6	2	6	5	3	0	1
Acinetobacter lwoffii (17)	7	0	0	3	2	0	0	5
Alcaligenes xylosoxidans (10)	5	5	0	0	0	0	0	0
Alcaligenes denitrificans (10)	6	3	0	0	0	1	0	0
Alcaligenes faecalis (07)	4	0	0	3	0	0	0	0

Table 6: Antibiotic susceptibility pattern of other non-fermenters (n=78)

Drugs	*Acinetobacter anitratus* (34)	*Acinetobacter lwoffii* (17)	*Alcaligenes xylosoxidans* (10)	*Alcaligenes denitrificans* (10)	*Alcaligenes faecalis* (7)
Ampicillin	R*	R	R	R	R
Amoxy-Clav	7 (20.5%)	4 (23.5%)	R	R	R
Gentamicin	R	2 (11.7%)	3 (30.0%)	1 (10.0%)	R
Carbenicillin	2 (5.8%)	R	1 (10.0%)	R	R
Ciprofloxacin	3 (8.8%)	1 (5.8%)	1 (10.0%)	2 (20.0%)	1 (14.2%)
Co-trimoxazole	2 (5.8%)	1 (5.8%)	R	R	R
Tetracycline	4 (11.7%)	2 (11.7%)	R	R	R
Netilmycin	4 (11.7%)	7 (41.1%)	1 (10.0%)	R	R
Piperacillin	5 (14.7%)	3 (17.6%)	1 (10.0%)	2 (20.0%)	1 (14.2%)
Nitrofurantoin	R	R	R	R	R
Amikacin	17 (50.0%)	10 (58.8%)	4 (40.0%)	6 (60.0%)	4 (57.1%)
Imipenem	33 (97.0%)	15 (88.2%)	9 (90.0%)	8 (80.0%)	6 (85.7%)

* Resistant

Discussion

Non-fermenters comprise about one-fifth of all gram-negative bacilli recovered on aerobic cultures from extra intestinal lesions. Until recently, a number of them were considered to be nonpathogenic commensals of little clinical importance. However, their repeated association with the disease has brought these organisms in limelight.[2]

In this study, NFGNB were most commonly isolated from pus samples (52.0%) followed by urine sample (15.7%), aural swab (11.3%), blood culture (8.6%), sputum (5.6%), endocervical swab and Burn swab 6 each (1.3%) others (4.2%). NFGNB were also

isolated in majority of cases from pus sample (33%) followed by respiratory tract specimen (21.2%), urine sample (13.8%), blood culture (4.6%), CSF (4.6%), gastrointestinal specimen (8.3%) and other (12.9%) in a study done by Paramsivan et al.[3] Veenu et al.[10] in their study isolated NFGNB in most of cases from pus sample (52.6%), followed by urine and catheter tip (15.3%), sputum (13.3%), blood culture (9%), tracheal secretion (5.3%), high vaginal swab (2%) and others (2.3%).

However, the commonest species in the present study was *P. aeruginosa* (337/478) but we have concentrated on other NFGNB. In this context, the common NFGNB were *Acinetobacter calcoaceticus var. anitratus* (7.1%) followed by *Acinetobacter lwoffii* (3.6%), *Alcaligenes xylosoxidans* (2.1%), *Alcaligenes denitrificans* (2.1%), *Alcaligenes faecalis* (1.5%). Pickett and Pedersen, in their study on 486 cases, reported the incidence of *Acinetobacter anitratus* (7%) and *Acinetobacter lwoffii* (2%) was also similar to the present study i.e. 7.1% and 3.6% respectively.[11] The incidence of *Acinetobacter anitratus* was reported 20% at Swedish Medical Centre that was much higher than the present study.[6] A study done by Yashodhara and Shyamala[12] showed the incidence of *Acinetobacter anitratus* (13%) that was much higher than the present study (7.1%). On the other hand lower incidence was found in *Alcaligenes species* (4%) and *Acinetobacter lwoffii* (2%) as compared to the present study i.e. 5.7% and 3.6% respectively. In this study, the incidence of *Alcaligenes denitrificans* was 2.1% but in another study on 300 cases conducted by Veenu et al.[10] the incidence of *Alcaligenes denitrificans* was 1.3% that was much lower than the present study.

In the present study, out of 34 isolates of *Acinetobacter anitratus*, most of the strains were isolated from pus sample (11), followed by urine sample (6), blood culture (6), sputum sample (5), endocervical swab (3), aural swab (2) and one from conjunctival swab.(Table 5) From the 17 isolates of *Acinetobacter lwoffii*, 7 strains were from pus sample, 3 from blood culture, 2 from sputum and 1 each from pleural fluid, liver abscess, CVC line, conjunctival swab and cerebrospinal fluid respectively.(Table 5) Yasodhara and Shyamala[12] in their study reported 13 strains of *Acinetobacter anitratus* that were isolated from urine sample (7), blood culture (2), pus sample (1), tracheal washing (1), CSF (1). *Acinetobacter lwoffii* was reported in 2 cases, one each from sputum and urine sample. Veenu et al.[10] isolated only 2 strains of *Acinetobacter anitratus* one each from pus and high vaginal swab and only 1 strain of *Acinetobacter lwoffii* was from pus specimen.

In the present study, out of 10 isolates of *Alcaligenes xylosoxidans*, five each were isolated from pus and urine specimens. *Alcaligenes denitrificans* (10) were mainly isolated from pus sample (6), followed by urine sample (3) and endocervical swab (1). Veenu et al.[10] in their work reported 4 strains of *A. denitrificans* that were isolated from pus sample (3) and urine sample (1).

In this study, *Alcaligenes faecalis* was isolated in 7 cases out of which 4 were from pus and 3 were from blood culture. Veenu et al.[10] reported 11 strains of *A. faecalis* in their study that were isolated from pus sample (6), urine sample and catheter tip (1), sputum sample (1), tracheal secretion (1), blood culture (2). However, Paramasivan et al.[3] did not report any strain of *Alcaligenes species*.

Antibiotic susceptibility pattern on other NF showed that majority of isolates of *Acinetobacter species* were sensitive to imipenem followed by amikacin. The strains of *Alcaligenes* species also showed marked sensitivity to imipenem followed by amikacin.(Table 6) Yasodhara and Shyamla[12] reported amikacin to be the best drug against *Acinetobacter* species and *Alcaligenes* species. Veenu et al.[10] also found amikacin to be the best drug against *Acinetobacter* species. The differences observed in the antibiotic resistance pattern of the isolates might be due to geographical differences and varying prescribing habits of clinicians. The antibiogram pattern of the NF isolated in the present study shows multidrug resistance pattern and marked resistance was observed to commonly used drugs like ampicillin, gentamicin, amoxy-clav, co-trimoxazole and nitrofurantoin. None of the isolates were sensitive to all the drugs. Majority of the isolates were resistant to two or more drugs. On the whole the drugs that have shown good *in-vitro* efficacy were imipenem and amikacin respectively.

Conclusion

Thus this study draws attention to the fact that a large number of non-fermenters other than *Pseudomonas* species are isolated from different clinical specimens. With the available evidence from the current literature and considering the changing concept of pathogenic potentials of these microorganisms, there are enough reasons to believe that they are also associated with human disease process. Therefore, all the attempts must be made for repeated isolation of NFGNB from various clinical specimens. Such attempts will definitely highlight the poorly understood group, the non-fermenting bacteria and their role in human infections.

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