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RESEARCH ARTICLE

Current Trend Of Nonfermenting Gram Negative Bacilli In A Tertiary Care Hospital In Dehradun, Uttarakhand

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Abstract

Introduction: In recent years, due to the liberal and empirical use of antibiotics, Non-fermenting Gram negative bacilli (NFGNB) have emerged as important healthcare-associated pathogens. They have been incriminated in infections, such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI). (Vijaya D et al, 2000) Non-fermenters may differ in their pathogenic potential and transmissibility, and many are multidrug resistant. (Schreckenberger PC et al., 2007) **Material & methods:** 437 NFGNB isolated over a period 6 months (Nov.2012 to April 2013) were speciated and their antibiotic resistogram analysed. **Results:** NFGNB accounted for 16.18% of the total isolates. Multidrug resistance strains of *Pseudomonas aeruginosa, Acinetobacter baumannii & Burkholderia cepacia* complex were observed

Introduction

Non-fermenting Gram-negative bacilli (NFGNB) are ubiquitous in the environment and are responsible for a vast variety of infections. (Dijkshoorn L et al, 2007& LiPuma JJ et al, 2007) They are particularly associated with urinary tract infections, ventilator assocoiated pneumonia (VAP), surgical site infections and bacteraemia. *P. aeruginosa* and *Acinetobacter baumannii* account for 10% of hospital-acquired infections (Je M, 2006). *Pseudomonas aeruginosa* is the most frequently isolated micro-organism, followed by *A. baumannii* and *Stenotrophomonas maltophilia.* (LiPuma JJ et al, 2007, Blondel-Hill E et al, 2007, Schreckenberger PC et al, 2007) Non-fermenters may differ in their pathogenic potential and transmissibility, and many are multidrug resistant. (Schreckenberger PC et al, 2007) For this reason, accurate identification of NFGNB to species level is important for appropriate patient management.

Colonies on primary isolation media are presumptively identified by colonial morphology, Gram stain, oxidase activity and pigment production. The oxidase reaction is an important discriminatory test. Oxidase positive, glucose non-fermenting, Gram negative bacilli such as *Pseudomonas aeruginosa* may be termed as ‘‘pseudomonads’’. Further identification is carried out by various other phenotypic tests. All identification tests are ideally performed from non-selective agar. (Schreckenberger PC et al, 2007)

In the diagnostic clinical microbiology laboratory, identification of non-fermenters relies mainly on phenotypic characteristics. A variety of commercially available identification systems, such as API 20 NE (bioMe´rieux), VITEK 2 (bioMe´rieux) and Phoenix (Becton Dickinson) are being used for routine identification of these bacteria. Studies dealing with the performance of these commercial identification systems have shown contradictory results. (Funke G et al, 2004 & Kiska DL et al, 1996) Molecular identification techniques have emerged as alternatives for phenotypic identification methods. Among these, 16S rRNA gene sequencing is widely used. (Patel JB, 2001& Su SC et al, 2009)

Keeping this background in mind the present study was conducted with the objective of identifying and speciating NFGNB isolated from all clinical samples, assessing their clinical significance, current trend and the present antibiotic resistance pattern of the isolates and finally, to make an attempt in formulating antibiotic policy for NFGNB isolates in our hospital settings.

Material & methods:

This prospective study was conducted over a period of 6 months (Nov.2012 to April 2013) at the Department of Microbiology, SGRRIM&HS, Dehradun.

A total of 2700 clinical specimens were found culture positive in the laboratory during the study period. The organisms isolated and identified provisionally as NFGNB (n=437) were identified further by using a standard protocol for identification. (Winn W Jr et al, 2006) The characters assessed included morphology on Gram's stain, motility, pigment production, oxidase production, OF test (Hugh-Leifson's medium) for glucose, maltose and mannitol, growth in 6.5% NaCl, growth at 42°C, amino acid decarboxylation test, and gelatin liquefaction test.

Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method as per CLSI guidelines using commercially available discs.

Results:

437/2700 (16.18%) isolates of NFGNB were included in this study. Analysis of results suggest the predominance of NFGNB in respiratory (185/437, 42.33%) & pus (125/437, 28.6%) specimens. (Fig. 1)

Of the total isolates studied *Pseudomonas aeruginosa* accounted for 62.92% (275/437) & *Acinetobacter baumannii* 21.05% (92/437). *Burkholderia cepacia* complex was found in 20/437 (4.58%) of the isolates. Rarely encountered species observed were *Bordetella trematum* 15 (3.4%), *Stenotrophomonas maltophilia* 8 (1.8%) and *Massilia timonae* 6 (1.38%). *Sphingobacterium multivorum, Sphingomonas paucimobilis, Neisseria warenii* and *Achromobacter* spp. accounted for 3 (0.6%) each. (Fig. 2)

Resistance pattern of all the isolates when analysed as a group against various classes of antibiotics showed that the isolates exhibited 100% resistance to levofloxacin while, the resistance against cefixime, cefuroxime and co-trimoxazole was >60%. On the other hand, isolates showed a low level of resistance against piperacillin-tazobactam, cefoperazone-sulbactam and ceftazidime. Extremely low level of resistance was observed against imipenem and polymyxin B. (Fig. 3)

Acinetobacter baumannii, Pseudomonas aeruginosa and *Burkholderia cepacia* complex showed high level of resistance to most of the antibiotics tested (commonly used in clinical practice). When results were critically analysed for the common organism amongst these we found that *Pseudomonas aeruginosa* showed resistance to most of the drugs except polymyxin B, imipenem & ceftazidime. All the members of *Acinetobacter* species were found maximally sensitive to polymyxin B, imipenem & piperacillin-tazobactum. All the isolates of *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* showed considerable amount of sensitivity to co-trimoxazole, which is currently the drug of choice.(Winn W Jr. 2006) (Table 1)

Table 1. Antibiotic resistance pattern of NFGNBs isolated (n=437)

Isolates	Piperacillin-tazobactum	Gentamicin	Levofloxacin	Cefoperazone sulbactum	Cefixime	Ceftazidime	Cefuroxime	Imipenem	Co-trimoxazole	Polymyxin B
Achromobacter spp.(3)	0	0	3	0	3	0	0	0	3	0
Acineto. baumannii (92)	20	85	92	61	71	74	69	12	83	0
Acineto. haemolyticus (9)	1	9	9	2	2	0	2	0	9	0
Acineto. lwoffii (9)	1	9	9	7	6	8	6	2	9	0
Bordetella trematum (9)	9	9	9	0	8	6	9	3	9	0
Burkholderia cepacia	16	20	20	20	20	12	20	15	10	20

complex (20)										
Massilia timonae (3)	0	3	3	1	3	2	3	1	3	0
Neisseria warenii (3)	1	3	3	3	3	3	3	0	3	0
Pseudomonas aeruginosa (275)	91	52	275	43	157	12	154	11	152	0
Sphingobacterium multivorum (3)	0	3	3	1	0	1	2	0	3	0
Sphingomonas paucimobilis (3)	0	3	3	0	0	0	1	1	3	0
Stenotrophomonas maltophilia (8)	6	8	8	3	3	5	2	6	2	0
Total	**145**	**204**	**437**	**141**	**276**	**123**	**271**	**51**	**289**	**20**
%	**33.18**	**46.68**	**100**	**32.26**	**63.16**	**28.15**	**62.01**	**11.67**	**66.13**	**4.58**

Fig.1 SPECIMEN-WISE DISTRIBUTION OF ISOLATES

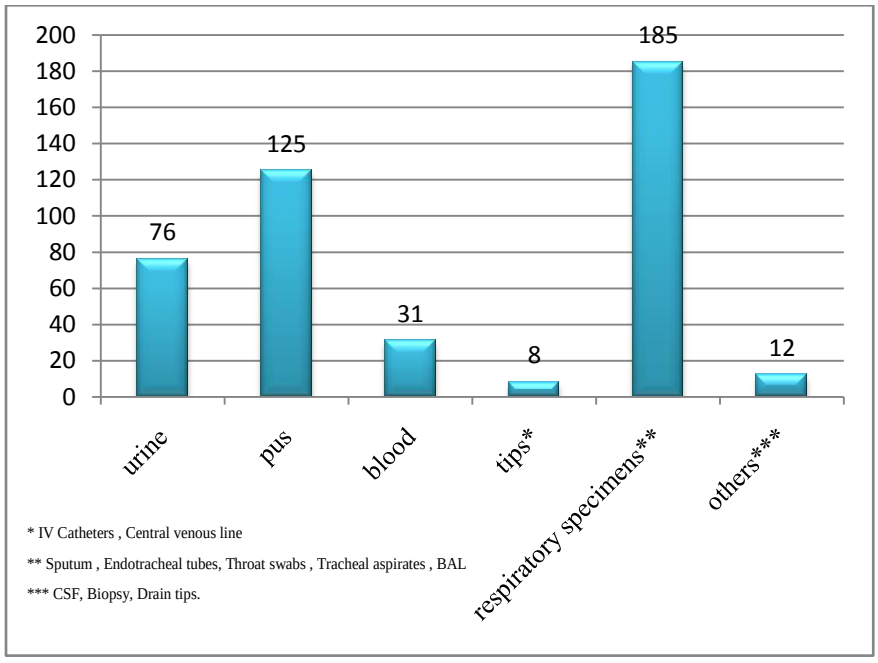


Fig. 2 SPECIES-WISE DISTRIBUTION OF THE ISOLATES

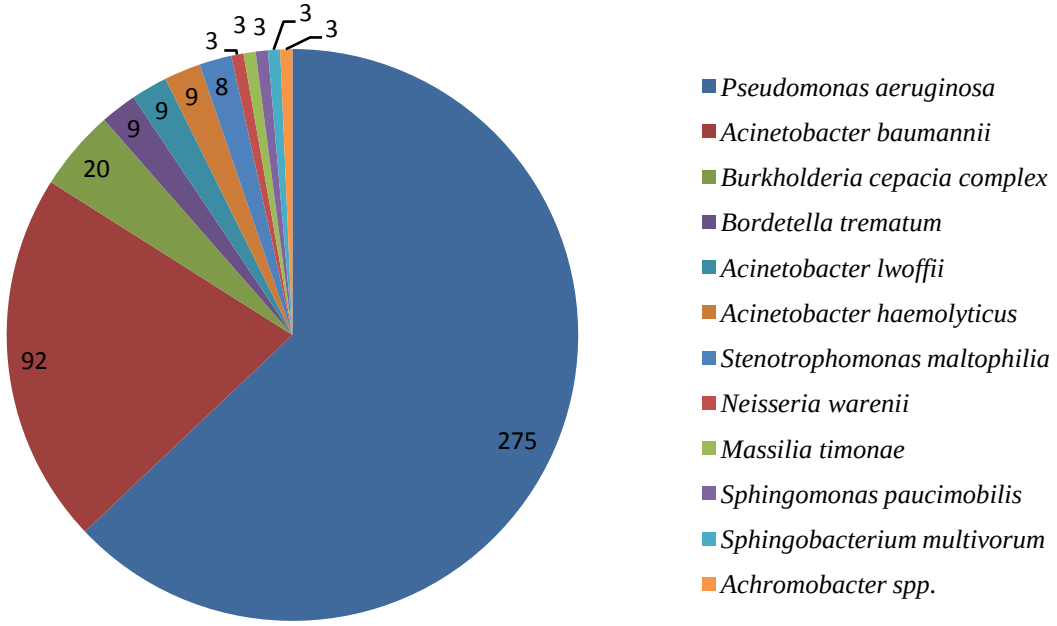
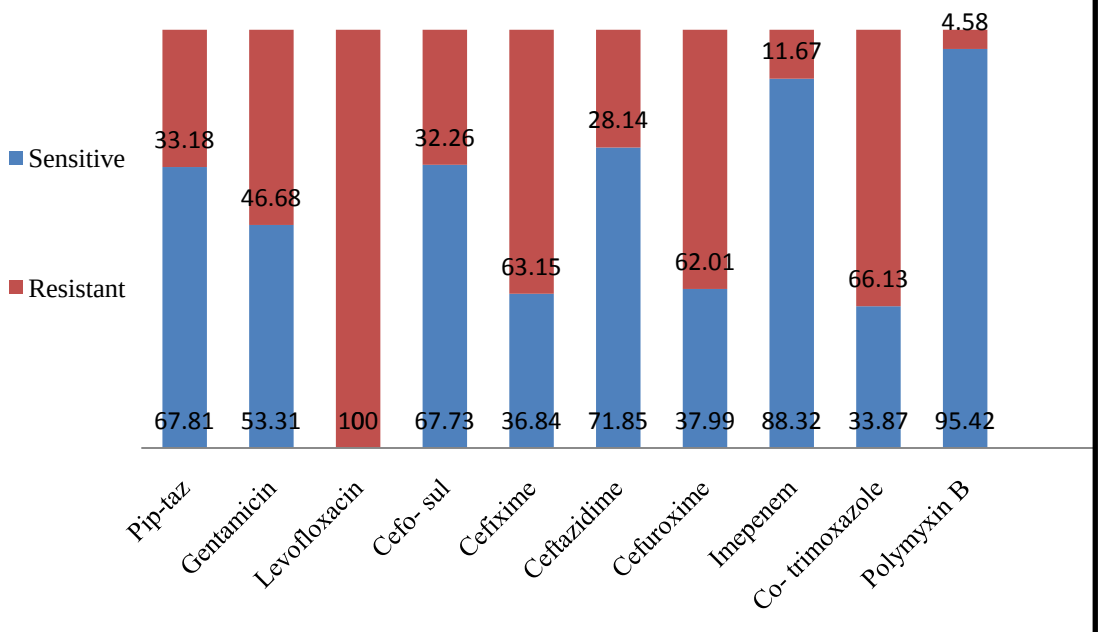


Fig 3. Distribution of resistance pattern of isolates (percentage)



Discussion:

Organisms previously classified within the genus *Pseudomonas* (rRNA homology groups I-V) are now divided among the genera *Pseudomonas, Burkholderia, Ralstonia, Comamonas, Brevundimonas* and *Stenotrophomonas.* Many identified strains have no designated species. Commercial identification systems do not provide definitive speciation of many of the clinically significant, glucose non-fermenting Gram negative bacilli. In clinical situations where precise identification is important for determining optimal therapy, patient prognosis, and appropriate infection control interventions (eg. if querying the first isolation of a member of the *Burkholderia cepacia* complex in a respiratory sample from a patient with cystic fibrosis), referral of such an isolate to a Reference Laboratory is usually appropriate. (Schreckenberger PC et al, 2007)

Because of these variations a surveillance of the nosocomial pathogens for resistograms in a given set up is needed in order to guide appropriate selection of empiric therapy. Various international authorities emphasize that every hospital should have its individual antibiotic policy since the standard antibiotic policy may not hold true for every area. (Vijaya D et al, 2000)

Because of high intrinsic resistance of different NFGNB to different antimicrobial agents, the value of proper identification comes to the forefront.

The available data suggests that NFGNB are microorganisms worth mentioning because of their epidemiological complexity, propensity to cause outbreaks of infection and antimicrobial resistance. (Rahbar M et al, 2006, Japoni A et al, 2006 & Taherikalani M et al, 2008) They have emerged as important nosocomial pathogens especially in immunocompromised hosts and are responsible for causing variety of infections. (Je M, 2006) Studies carried out by various workers have reported a wide range of isolation rate of NFGNBs, from 9.32% to 45.9%.(Juyal D et al, 2013 & Sidhu S et al, 2010) The commonest isolated strains in our study were *P. aeruginosa* (62.92%), *Acinetobacter* species (25.17%) and *Burkholderia cepacia* complex (4.57%). *Pseudomonas aeruginosa* and *Acinetobacter* species as commonest NFGNB isolated, correlates with the data published by various workers. (Vijaya D et al, 2000, Malini et al, 2009 & Upgade A et al, 2012)

We report a high prevalence of resistance to different classes of antibiotics, including β-lactams, cephalosporins and aminoglycosides.

Imipenem is a carbapenem antibiotic, which is highly active against *P. aeruginosa* and *Acinetobacter spp.*(Taneja N et al, 2003) Unfortunately paralleling its increasing use, resistance to this agent has also increased.(Ouinn PJ, 1998) We found 11.67% of the NFGNB to be collectively resistant to imipenem.

Resistance to antimicrobials is common and has increased over the years among NFGNB and a number of strains are now resistant to nearly all commonly used antibiotics. Multi drug resistance among these organisms makes the treatment of infections caused by them difficult and expensive.(Kharangate NV et al, 2001) Polymyxins are the remaining antibiotic drug class with fairly consistent activity against multidrug-resistant strains of *P. aeruginosa, Acinetobacter spp,* and *S. maltophilia*. Resistance may compromise treatment, leading to increased mortality, extended hospital stay and greater healthcare costs. (Je M., 2006) In our study only the *Burkholderia cepacia* complex isolates were resistant to polymyxin B.

Conclusion:

As combinations of antimicrobial agents are often prescribed as empiric therapy for suspected infections, appropriate choice of antimicrobials is very important as it improves outcome and cost to the patients in terms of the expenses of costly antibiotics as well as duration of hospital stay. Thus, it is important for the clinicians to remain updated with current antimicrobial susceptibility pattern of the circulating pathogens, and the antimicrobials to be used for empiric therapy should be selected accordingly.

We recommend periodic surveillance of the isolates (especially from critical care units) up to species level as well their antibiograms at regular intervals, critical analysis of such data and formulating a rotational antibiotic policy at hospital level to tackle the problems related to nosocomial infections due to drug resistant strains.

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