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Original Article

Identification and Antimicrobial Susceptibility Pattern of Clinical Isolates of Non-fermentative Gram Negative Bacilli

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ABSTRACT

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Introduction: Non-fermentative Gram-negative bacilli (NFGNB) can cause serious healthcare associated infections and are frequently resistant to multiple antibiotics. Identification of NFGNB and detecting their susceptibility pattern are important for proper management of infections caused by them. **Material & Methods:** A prospective study of 252 isolates of non-fermenters from various clinical specimens received in the Department of Microbiology was done over a period of two years (July 2004 to July 2006). Non-fermenters were identified by using a standard protocol. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method. **Results:** Among the 252 isolates, the majority of the non-fermenters were isolated from pus 124(49.20%), followed by sputum 50(19.84%), urine 32(12.69%), bronchial aspirate 23(9.12%), Pleural fluid 10(3.96%), Blood culture 3(1.19%), CSF 2(0.79%), the rest of the isolates were from other clinical specimens as indicated 8(3.17%). Out of 252 isolates, the most common isolates were from the genus *Pseudomonas* (210), among them predominant species being *Pseudomonas aeruginosa*(200), were isolated followed by *P.stutzeri*(08) and *P.putida*(02). From the genus *Acinetobacter*(41), among them *A.baumannii*(35) and *A.lwoffii*(06). Only one isolate was *Stenotrophomonas maltophilia*(01). A high level of antibiotic resistance was recorded for most of the first and second line drugs. Imipenem, Piperacillin and Amikacin were the drugs with maximum activity. **Conclusion:** Non fermenter gram negative bacilli though regarded as contaminants are important bacteria causing wide range of nosocomial infections. Irrational use of powerful antibiotics for prolonged periods added to the compromised host conditions might be responsible for multi-drug resistance (MDR). Improved antibiotic usage and infection control measures will be needed to prevent or slow the emergence and spread of multi-drug resistant NFGNB in the healthcare setting.

Key Words: NFGNB, Nosocomial, Irrational, MDR, Infection control.

1. INTRODUCTION

Non-fermenters are a heterogenous group of Gram-negative bacilli that are aerobic, non-sporing, which cannot catabolize carbohydrates and therefore are not able to ferment. This heterogeneous group includes

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organisms like *Pseudomonas.spp*, *Acinetobacter.spp*, *Alkaligenes.spp*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia complex (BCC)*. Currently *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most commonly isolated non-fermenter pathogens for humans. Being ubiquitous in nature, they were disregarded as probable contaminants, when isolated in the laboratory.^{1,2} 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. All these organisms have the potential to spread horizontally on fomites or the hands of healthcare workers.^{2,3,4} In recent years due to the indiscriminate use of antimicrobials, NFGNB have emerged as important health care associated pathogens. They have been incriminated in infections such as bacteraemia, meningitis, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis etc.⁵ Risk factors include immunosuppression (oncology patients on cytotoxic therapy/radiotherapy, organ transplant patients and even patients with AIDS), neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostic and therapeutic procedures. Emerging challenges of multi-drug resistance, both intrinsic and acquired among them is of serious concern to the treating physician.⁶ The study was conducted in the Department of Microbiology, RNT Medical College, Udaipur to isolate and identify the non-fermenters from various clinical samples and their antimicrobial susceptibility pattern was studied.

2. MATERIAL AND METHODS

In the present prospective study of 252 isolates of non-fermenters from various clinical specimens received in the Department of Microbiology, at RNT Medical College, Udaipur was done over a period of two years (July 2004 to July 2006).

2.1 Processing of Samples

All the samples were inoculated on MacConkey agar and Blood agar, incubated at 37°C for 24-48 hours and growth recorded. Morphology and motility of the organisms were determined by Gram staining and hanging drop method respectively and oxidase test was done.

All the Gram-negative bacilli or cocco-bacilli grown on MacConkey agar or blood agar, whether oxidase positive or negative were inoculated on triple sugar iron agar medium(TSI). Organisms producing acid slant/acid butt reaction were excluded and those producing no acid in TSI medium or alkaline slant in TSI medium were inoculated into Hugh and Leifson's oxidation-fermentation medium(glucose based) to find out whether a particular organism was glucose oxidizer or non-oxidizer. Cultural characteristics were studied on blood agar, nutrient agar and MacConkey agar. The nonfermentative gram negative bacilli(NFGNB) were identified up to genus or species level based on the following tests: Motility test, Indole production, Nitrate reduction, Denitrification, Citrate utilization, Urease production, H₂S production, Arginine dihydrolase production, Lysine decarboxylase production, Catalase test, Pigment production, Gelatin liquefaction, Malonate utilization and Esculin hydrolysis.

2.2 Antibiotic Sensitivity Testing

Antimicrobial sensitivity was determined by Kirby Bauer disc diffusion method on Muller Hinton agar (MHA). A suspension of each isolate was made so that the turbidity was equal to 0.5 McFarland standards and then plated as a lawn culture onto MHA. Antibiotic discs were placed and plates were incubated at 37°C for 18-24 hrs. Results were interpreted in accordance with central laboratory standards institute (CLSI) guidelines.⁷ All dehydrated media, reagents and antibiotic discs were procured from Hi-Media Laboratories Pvt. Ltd, Mumbai, India.

3. RESULTS & DISCUSSION

In the present, prospective study of 252 isolates of nonfermenters from various clinical samples was done during the period of July 2004 to July 2006 at R.N.T. Medical College, Udaipur.

In the present study, the majority of the nonfermenters were isolated from pus 124(49.20%), followed by sputum 50(19.84%), urine 32(12.69%), bronchial aspirate 23(9.12%), Pleural fluid 10(3.96%), Blood culture 3(1.19%), CSF 2(0.79%), the rest of the isolates were from other clinical specimens as indicated 8(3.17%) [Table 1]. In comparison between the present study and that of Arora et al⁸, the results were dissimilar for no. of isolates from pus, sputum and pleural fluid ($P<0.001$), urine and CSF ($P<0.05$). However isolation rates for blood and miscellaneous samples ($P>0.05$) were similar.

In the present study, the most common isolates were from the genus *Pseudomonas* (210), among them predominant species being *Pseudomonas aeruginosa*(200), were isolated followed by *P.stutzeri*(08) and *P.putida*(02). From the genus *Acinetobacter*(41), among them *A.baumannii*(35) and *A.lwoffii*(06). Only one isolate was *Stenotrophomonas maltophilia*(01). [Table 2]. These results were significantly different from the results reported by Veenu et al⁹ in the cases of the following species: *P.aeruginosa*, *Acinetobacter baumannii* ($P<0.001$) and *P.stutzeri*, *P.putida* ($P<0.05$). However the isolation rates of *Acinetobacter lwoffii* and *stenotrophomonas maltophilia* ($P>0.05$) were similar in the above study. Our results were significantly different from the results reported by Arora et al⁸ in the cases of following species *P.stutzeri* ($P<0.001$) and *Acinetobacter baumannii* ($P<0.05$). However the isolation rates of *P.aeruginosa*, *P.putida*, *Acinetobacter lwoffii* and *Stenotrophomonas maltophilia* ($P>0.05$) were similar

with our study and also correlates with vijaya et al¹⁰ study.

Table 1: Non-fermenter isolates in clinical specimens in the present study

Specimens	No.of isolates (n=252)	Percentage (%)
Pus	124	49.20
Sputum	50	19.84
Urine	32	12.69
Bronchial aspirate	23	9.12
Pleural fluid	10	3.96
Blood culture	3	1.19
CSF	2	0.79
Miscellaneous – Eye swab	1	
Ear swab	3	
Throat swab	2 = 8	3.17
Pericardial fluid	1	
Ascitic fluid	1	

Table 2: Species distribution of NFGNB in different clinical specimens

Species	Pus (124)	Sputum (50)	Urine (32)	Br. Aspirate (23)	Pleural Fluid (10)	Blood (3)	CSF (2)	Miscellaneous (8)	Total
<i>P.aeruginosa</i>	84	49	27	20	10	2	1	7	200
<i>P.stutzeri</i>	2	1	0	2	0	1	1	1	08
<i>P.putida</i>	2	0	0	0	0	0	0	0	02
<i>A.baumannii</i>	30	0	5	0	0	0	0	0	35
<i>A.lwoffii</i>	5	0	0	1	0	0	0	0	06
<i>S.maltophilia</i>	1	0	0	0	0	0	0	0	01

In the present study, majority of the isolates of *P. aeruginosa* were resistant to ceftazidime (44%), ciprofloxacin (41%) and getamincin (35.5%). The least resistance was observed in the cases of piperacillin (20%), Imipenem (17.5%) and Amikacin (14.5%). Hence it can be concluded that only Imipenem, Piperacillin and Amikacin can be recommended as empirical antibiotics against suspected *P.aeruginosa* infections. In the present study 163 (81%) isolates were found to be resistant to two or more drugs. In the comparison between the results reported by Takeyama K¹¹ and present study, it was observed that the resistance patterns were similar for Imipenem, Piperacillin and Amikacin except for

Ceftazidime (68.6% Vs 44%, $P < 0.05$) Gentamicin (64.8% Vs 35.5%, $P < 0.05\%$) and Ciprofloxacin (86.8% Vs 41%, $P < 0.001$). The antibiotic sensitivity pattern reported by Gencer et al¹² were dissimilar to the present study $P < 0.001$ in cases of Ciprofloxacin, Imipenem, Piperacillin and $P < 0.05$ for Amikacin, Ceftazidime.

The Taherikalani M¹³ study, number of *Acinetobacter* spp. are resistant to Ceftazidime, Ciprofloxacin and Gentamicin and least resistant is shown to Piperacillin, Amikacin and Imipenem. Twenty nine (70.7%) of the isolates of *Acinetobacter* spp. were resistant to two or more drugs. In the comparison between the results reported by Taneja et al¹⁴ and present study, results were similar for Ceftazidime, Ciprofloxacin and Impipenem ($P > 0.05$). Fass et al¹ reported significantly different resistance pattern of *Acinetobacter* sp. to Ceftazidime ($P < 0.001$), Ciprofloxacin and Imipenem ($P < 0.05$) whereas similar for Piperacillin ($P > 0.05$).

The variation in the antibiotic sensitivity pattern may be due to differences in the primary resistance among isolated strains due to geographical differences or in secondary resistance due to difference in the choice of empirical antibiotics.

4. CONCLUSIONS

Non fermenter gram negative bacilli though regarded as contaminants are important bacteria causing wide range of nosocomial infections. Irrational use of powerful antibiotics for prolonged periods added to the compromised host conditions might be responsible for multi-drug resistance (MDR). In the present study, it was observed that the non fermenters were isolated from almost all the clinical specimens, most common isolates being from pus. Of all the non fermenters isolated, *Pseudomonas aeruginosa* was most common followed by *Acinetobacter baumannii*, *Pseudomonas stutzari*, *Acinetobacter lwoffii*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*. More importantly these

organisms have great potential to survive in hospital environment so improved antibiotic stewardship, infection control measures that includes equipment decontamination, strict attention to hand washing and isolation procedures will be needed to prevent or slow the emergence and spread of multidrug resistant non-fermentative gram negative bacilli in the healthcare setting.

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