



ISOLATION AND IDENTIFICATION OF NON-FERMENTING GRAM NEGATIVE BACILLI IN ALL CLINICAL SPECIMENS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN TERTIARY CARE HOSPITAL OF CENTRAL INDIA

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ABSTRACT

Objective: This prospective observational study was done to identify the non-fermenting Gram negative bacilli (NFGNB) and performing their antimicrobial susceptibility pattern for commonly used antibiotics. Also to know the prevalence of different species of non-fermenting Gram negative bacilli in various clinical specimens. **Materials and Methods:** During period of one year (April 2017 to March 2018) all clinical specimens received in Microbiology Department of M.G.M. Medical College, Indore (M.P.) were processed according to the standard microbiological techniques. **Result:** 100 isolates of NFGNB were obtained by the processing of 1095 samples. The incidence of nonfermenting gram negative bacilli was 9.13%. Nonfermenters isolated predominantly were *P.aeruginosa* (78%) followed by *Acinetobacter baumannii* (19%) and *Burkholderia cepacia complex* (3%). NFGNB isolation was higher in male patients (54%) and most common in the age group of 60-70 years (18%). Majority of NFGNB were isolated from pus samples, (30%) followed by blood (20%). NFGNB had showed maximum susceptibility to meropenem (70%) followed by imipenem (67%) and amikacin (55%). **Conclusion:** Observations from the our study showed that the Different sensitivity pattern and multidrug resistance exhibited by nonfermenters pose a great problem in treating these infections and leads to high morbidity and mortality. These organisms have great potential to survive in hospital environment, so effective methods of sterilization and infection control measures should be implemented. Care in detection, evaluation of effective antibiotic options, judicious use of antibiotics by instituting antibiotic policy of combination therapy and rigorous infection control measures will help to fight against nonfermenters for effective management of patients. The wide spread variability of sensitivity profile of common hospital isolates, indicate that every hospital should monitor their antibiogram profile of these isolates from time to time to serve as a basis for empirical therapy in emergency situation.

KEYWORDS: *Acinetobacter baumannii*, *P.aeruginosa* and *Burkholderia cepacia complex*.

INTRODUCTION

Aerobic non-fermenting Gram-negative bacilli (NFGNB) are taxonomically diverse group of non sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively.^[7] NFGNB are ubiquitous in nature and emerged as important health care associated pathogens, which can cause opportunistic infections in immunocompromised hosts.^[2] The term nonfermenting gram negative bacilli is used to all aerobic gram negative bacilli that show abundant growth within 24 hours on the surface of Kliglers iron agar (KIA) or Triple sugar iron agar (TSI) medium, but neither grow in nor acidify the butt of these media.^[4] They have been incriminated in infections such as, septicemia, meningitis, pneumonia, urinary tract infection and surgical site infections.^[4] In recent years, the problem is further compounded by the emergence of resistance to antimicrobial agents which

may be due to liberal and empirical use of antibiotics.^[8] *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas*, *Burkholderia*, *Alcaligenes*, *Weeksella* etc. are common nonfermenter pathogen responsible for health care associated infection¹⁰. NFGNB show resistance to a wide range of antibiotics, leading to serious infections. Multi-drug resistance (aminoglycosides, fluoroquinolones, ureidopenicillins and third generation cephalosporins) exhibited by *Acinetobacter* species and *Pseudomonas aeruginosa* poses a major clinical problem in treatment.^[5]

MATERIALS AND METHODS

The present study was done in the Department of Microbiology MGM medical College, Indore (M.P.) from April 2017 to March 2018. During this period all clinical specimens received were processed for detection

of aerobic NFGNB bacteria without delay in the following manner.^[7]

1. Direct smear examination by Gram staining.
2. Culture on Blood agar, MacConkey agar and Nutrient agar.
3. Motility by hanging drop method.
4. Identification with the help of biochemical tests.
5. Antimicrobial susceptibility testing on Mueller Hinton agar by Kirby Bauer's disc diffusion method.^[9]
6. Interpretation of result.

Antibiotics used in our study were piperacillin/tazobactam (100/10 µg) amikacin (30 µg), ceftazidime (30 µg), imipenem (10 µg), meropenem (10µg), gentamicin (10µg), cefepime (30µg) and levofloxacin (5µg), ceftriaxone (30µg), doxycycline (10 µg), polymyxin (300 µg), norfloxacin (10 µg), nitrofurantoin (300 µg).

Table 1: Sex wise distribution of NFGNB.

Sex	Number of cases	Percentage
Males	54	54
Females	46	46
Total	100	100

Table 2: Age wise distribution of NFGNB.

Age in years	Number of cases	Percentage
<1	11	11
1 -10	07	7
11-20	03	3
21-30	16	16
31-40	14	14
41-50	17	17
51-60	14	14
61-70	18	18
Total	100	100

Table 3: Distribution of various specimens and no of isolates.

S. No	Specimen	No of isolates	Percentage
1	Pus	30	30
2	Blood	20	20
3	Aural swab	18	18
4	Sputum	10	10
5	Urine	08	8
6	Pleural fluid	05	5
7	Tracheal swab	03	3
8	CSF	03	3
9	Throat swab	03	3
	Total	100	100

Table 4: Antibiotic susceptibility pattern of NFGNB.

Antibiotics	Sensitive		Intermediate Sensitive		Resistant	
	No of cases	%	No of Cases	%	No of Cases	%
Piperacillin +Tazobactam	52	52	13	13	35	35
Ceftazidime	41	41	15	15	44	44
Gentamycin	49	49	11	11	40	40
Levofloxacin	51	51	13	13	36	36
Ceftriaxone	13	13	20	20	67	67
Amikacin	55	55	8	8	37	37
Imipenem	67	67	7	7	26	26
Meropenim	70	70	11	11	19	19
Doxicyclin	23	23	12	12	65	65
Cefepime	22	22	11	11	67	67
Polymyxin B	50	50	12	12	38	38
Cotrimoxazole	20	20	11	11	69	69
Norfloxacin*	03	37.5	01	12.5	04	50
Nitrofurantoin*	01	12.5	01	12.5	06	75

*For urinary isolates only.

RESULTS AND DISCUSSION

100 isolates of NFGNB were obtained by the processing of 1095 samples. The incidence of nonfermenting gram negative bacilli was 9.13%. Nonfermenters isolated predominately were *P.aeruginosa* (78%) followed by

Acinetobacter baumannii (19%) and *Burkholderia cepacia* complex (3%). NFGNB isolation was higher in male patients (54%) and most common in the age group of 60-70 years (18%). (Table1&2) Majority of NFGNB were isolated from pus samples, (30%) followed by blood (20%). (Table 3) NFGNB had showed maximum

susceptibility to meropenem (70%) followed by imipenem (67%) and amikacin (55%). (Table 4). In our study the incidence of nonfermenting gram negative bacilli was 9.13%, which resembles with the studies of Deepak Juyal *et al.*^[10] (9.62%) and Grewal *et al.*^[3] (11.6%). In our study nonfermenters isolated predominately were *P.aeruginosa* followed by *Acinetobacter baumannii* followed by *Burkholderia cepacia* complex resembling to studies done by Grewal *et al.*^[3] Jitendranath *et al.*⁵ and Kaur A *et al.*¹ NFGNB are known to cause infection in extremes of age which was seen in our study (18% in the age group of 61 to 70 years and 11% in <1 year of age) similar to study by Sorabh Singh Sambyal *et al.*^[11] (30% in <1 year) and Sachdev *et al.*^[12] (20% in 71 to 80 years of age group), which could be due to physiologically deficient immune system. Out of total 100 patients, 54% were males and 46% were females which was about similar to study of Sorabh Singh Sambyal *et al.*^[11] (58.58% were males while 41.42% were females). In our study majority of NFGNB were isolated from pus samples, (30%) which was similar to study done by Malini *et al.*^[6] (62.2%), Jitendarnath *et al.*^[5] (53.3%). NFGNB displays a wide and variable spectrum of antibiotic sensitivity pattern. There is no antibiotic for which all isolated NFGNB were susceptible in our study. Various studies depict that NFGNB are uniformly resistant to penicillin group of drugs.^[10,12] According to our study sensitivity of Meropenem was 70% and the sensitivity ranged from 40% to 85% in other studies.^[1,2,5,10] NFGNB showed resistance of 67% to Ceftriaxone, 44% to Ceftazidime, 40% to Gentamicin, 35% to Piperacillin-tazobactam which are commonly used by the clinicians in our hospital. For urinary isolates, NFGNB showed 62.5% resistance to Norfloxacin while 87.5% to Nitrofurantoin in our study. NFGNB showed a good sensitivity to Amikacin 55% in our study. NFGNB showed an overall 26% resistance to Imipenem in our study, compared to study by Taneja *et al.*^[13] which showed 36%. The differences in the percentage may be due to the variation in the sample size.

CONCLUSION

Observations from the our study showed that the Different sensitivity pattern and multidrug resistance exhibited by nonfermenters pose a great problem in treating these infections and leads to high morbidity and mortality. These organisms have great potential to survive in hospital environment, so effective methods of sterilization and infection control measures should be implemented. Care in detection, evaluation of effective antibiotic options, judicious use of antibiotics by instituting antibiotic policy of combination therapy and rigorous infection control measures will help to fight against nonfermenters for effective management of patients. The wide spread variability of sensitivity profile of common hospital isolates, indicate that every hospital should monitor their antibiogram profile of these isolates from time to time to serve as a basis for empirical therapy in emergency situation.

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