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OCCURRENCE OF INFECTIONS CAUSED BY NON FERMENTING GRAM NEGATIVE BACILLI IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Background: Non fermenting Gram Negative Bacilli (NFGNB) have been incriminated as important nosocomial pathogens, often exhibiting multidrug resistance and creating treatment problems and failures.
Objectives: To determine the frequency of isolation, identification and antibiogram pattern of NFGNB from various clinical specimens and to correlate with age, gender, sample type, ward/unit where admitted, clinical diagnosis and risk factors.
Methods: Various samples received for cultural analysis for aerobic bacteria were subjected to primary gram staining and culture on solid and liquid media. Bacterial species isolated were identified and tested for antibiogram pattern using standard laboratory procedures.
Results: A total of 1980 NFGNB were isolated from 27774 samples (7.1%). The commonest non fermenters were Acinetobacter species (55.5%) and Pseudomonas aeruginosa (42.9%). Acinetobacter species showed 67.9% sensitivity to Imipenem / Meropenem and 65.9% sensitivity to Colistin. Pseudomonas aeruginosa was found to be sensitive to Piperacillin – Tazobactam (70.6%) and Colistin (64.9%). Infections with these organisms were common in older individuals, above 60 years of age (26.7%) and the male:female ratio was 1.2:1. Samples yielding NFGNB were predominantly pus (40.6%), Endotracheal (ET) tubes (23.9%) and urine (18.6%). Patients were from ICU (32.1%) and Surgery wards (24.3%). Clinically, wound infections predominated (42.3%). Important risk factors were use of ventilator and prolonged antibiotic therapy (24.7% and 14.3% respectively).
Conclusion: Occurrence of NFGNB and their antibiogram pattern varies across geographic boundaries and within hospital units, necessitating periodic surveillance to achieve appropriate selection of antimicrobial therapy.

KEYWORDS

Non fermenters, nosocomial pathogens, antibiogram pattern

INTRODUCTION

Non fermenting Gram Negative Bacilli (NFGNB) are a group of aerobic bacteria that do not utilize glucose as an energy source.[1] They exist predominantly as saprophytes in nature, although they may also be found as human gut commensals.[2]

The pathogenic potential of these NFGNB is now accepted, due to their repeated isolation from clinical samples and their association with various clinical conditions.[3] These organisms vary in their transmissibility and degree of pathogenic potential.

NFGNB have been associated with infections in hospitalized patients and immunocompromised hosts, making them notorious nosocomial pathogens. They have been incriminated as causative agents of various clinical conditions such as bacteremia, meningitis, pneumonia, wound and bone infections and urinary tract infections.[2]

An emerging problem with this group of bacteria is their multidrug resistance. Production of ESBLs and metallobeta lactamases (MBLS) are important means of resistance, in addition to them being inherently resistant to a large number of antibiotics.[2] This has limited treatment options available in management of infections by NFGNB.

As these organisms have been associated with nosocomial infections, evaluation of their occurrence in various clinical samples is gaining importance. Periodic monitoring of the spectrum of NFGNB and their antibiogram pattern will serve as a guide in selecting appropriate antibiotics, especially when required to be given empirically, in life threatening situations.

This study was therefore undertaken to determine the occurrence of NFGNB from various clinical samples, to determine their antibiogram pattern, to evaluate a clinico – bacteriological correlation and to assess associated risk factors.

MATERIALS AND METHODS

A total of 27774 clinical samples were received in the Department of Microbiology, Goa Medical College and Hospital, from admitted patients, over a period of one year, for aerobic culture.

All samples were subjected to primary gram staining, for pus cells and bacteria, following which, culture was done on Blood agar, MacConkey agar and Glucose Broth. Urine culture was done semiquantitatively, using an inoculating loop, having a diameter of 1.3mm. All inoculated media were incubated at 37°C for 24 hours. Growth on culture media was identified by standard microbiological techniques.[1] These included gram staining, motility, colony morphology and biochemical reactions.[1]

Every oxidase positive and negative Gram Negative Bacilli / Coccobacilli was inoculated on Triple Sugar Iron Agar media. Organisms giving alkaline reactions were considered as non fermenters and identified upto the species level, using catalase and oxidase test, nitrate reduction, oxidation – fermentation reaction using Hugh Leifson Media containing 1% glucose, IMViC Reactions, urea hydrolysis, lysine decarboxylation, arginine hydrolase test, growth at 40°C and 42°C, growth on 6.5% NaCl and gelatin liquefaction test.[1] Some strains were confirmed by automated Identification System, VITEC, Biomerieux).

Antimicrobial sensitivity testing was done on all isolates by Kirby Bauer Disc diffusion technique as per CLSI Guidelines.[4]

RESULTS

A total of 27774 samples were processed over a period of one year. The occurrence of gram negative non fermenting bacilli was 7.1% i.e. 1980 NFGNB were isolated

The species of NFGNB isolated in the present study is depicted in the Table No 1. Acinetobacter species predominated (55.5%), followed by Pseudomonas aeruginosa (42.9%). Other non fermenting gram

negative bacilli were isolated less frequently (Burkholderia cepacia - 0.55%, Chryseobacterium indologenes - 0.5%; amongst others).

The antimicrobial sensitivity pattern of the isolates is seen in Table No 2. Acinetobacter species were sensitive to Imipenem / Meropenem (67.9%) and Colistin (65.9%). Pseudomonas aeruginosa showed 70.6% sensitivity to Piperacillin-Tazobactam and 64.9% sensitivity to Colistin. Burkholderia species were sensitive to Quinolones (91.7% overall) and Trimethoprim-Sulphamethoxazole (90.9% overall).

These infections were more common in individuals belonging to the older age group i.e. above 60 years (26.7%), 51-60 years (16.2%) and 41-50 years (17.9%). The male female ratio was 1.2: 1 (Table Nos 3 and 4).

Table No 5 depicts the isolation rate of NFGNB from various samples. Pus samples yielded 40.6% NFGNB, followed by ET tubes (23.9%) and urine (18.6%). A similar sample wise predominance was observed with individual bacteria.

Patients whose samples yielded NFGNB were predominantly admitted in the ICU (32.1%), surgery wards (24.3%) and Burn ward (15.6 %) (Table No 6).

Table No 7 depicts the clinical diagnosis of patients yielding NFGNB. Wound infections predominated (42.3%), followed by UTI (18.6%), Septicemia (15.6%) and Pneumonia (15.3%).

Various risk factors associated with NFGNB can be seen in Table no 8. Association with ventilator use was present in 24.7% patients, prolonged antibiotic therapy in 14.3% individuals and presence of urinary catheter and immunocompromised state in 11..6 % each.

DISCUSSION

Non fermenting gram negative bacilli, once believed to be contaminants, have established themselves as important nosocomial etiological agents.

The percentage isolation of NFGNB was 7.1% in the present study. This finding is in concurrence with that obtained in the studies of Juyal et al (9.3%)[5] and Samanta et al (10%).[6] However a high isolation rate of 45.9% was seen in the study of Sidhu et al in seriously ill patients with bacteremia.[7] It is possible that the isolation rate of NFGNB is determined by selection of patients. Critically ill patients, when selected are more likely to yield a high rate of isolation, as compared to all groups of admitted patients.

In the present study, the commonly isolated NFGNB were Acinetobacter species (55.5%) and Pseudomonas aeruginosa (42.9%). Similar predominance can be seen in the study of Juyal et al.[5] These two organisms were also frequently isolated in the study of Bhargawa et al,[3] although Pseudomonas predominated (56.9%), as compared to Acinetobacter baumanii (20.8%). These organisms are capable of surviving in hospital environment, due to their inherent resistance to antiseptics and their ability to colonise inanimate surfaces, causing serious infections in already sick hospitalized patients. Other NFGNB were also isolated, but less frequently in the present study. These species also have the propensity to survive in the environment and cause infections in immunocompromised subjects.

Acinetobacter species and Pseudomonas aeruginosa have shown low sensitivity to most antimicrobials, in the present study. The problem of drug resistance is escalating at a rapid rate, in most hospitals, causing a dilemma to Clinicians, limiting treatment options. Apart from self administered, injudicious and overuse of antimicrobials, the organisms' ability to form biofilms adds to their survival and virulence.[3]

In the present study most of the patients with these infections were in the older age bar i.e. 16.2% in the age group 51-60 years and 26.7% in the group of individuals above 60 years. As age advances, the immune status gets compromised and comorbid conditions set in, predisposing these individuals to infection with nosocomial pathogens.

The NFGNB were predominantly isolated from pus (40.6%), ET tubes (23.9%) and urine (18.6%), in the present study. Bhargawa et al, similarly found higher isolation of NFGNB from pus (41.6%), urine (33.05%) and blood (15.8%).[3]
Patients yielding the NFGNB were admitted predominantly in the ICU (32.1%) and surgical wards (24.3%), in the present study. This correlates well with the samples yielding the isolates and the clinical conditions that these organisms caused.

Clinical correlation revealed that these organisms were predominantly isolated from wound infection (42.3%), UTI (18.6), Septicemia (15.6%) and Pneumonia (15.3%). It stands to reason that type of sample involved will correlate with the clinical condition of the study patients and the ward/unit where he/she is admitted.

The NFGNB have been associated with various clinical conditions. They have been incriminated as notorious agents of chronic non healing ulcers and ventilator associated pneumonias.

Very often, risk factors serve as predictors of outcome and patient survival. Ventilator use (24.7%) and prolonged antibiotic therapy (14.3%) were important risk factors in patients in the present study. These factors in conjunction with underlying co morbidities contribute to susceptibility of admitted patients to acquire these nosocomial pathogens.

CONCLUSION

Prevalence of NFGNB probably varies between patient groups and geographic areas. Constant surveillance and knowledge of these circulating pathogens along with their antibiogram pattern is essential to select antimicrobials, if required, to be given empirically. Survival of these agents in the hospital environment can be curtailed by stringent infection control measures.

Table 1: Frequency Of Bacterial Species Isolated During The Study Period

Organisms	Total No. isolated	Percentage
Acinetobacter species	1098	55.5
Pseudomonas aeruginosa	850	42.9
Stenotrophomonas maltophilia	1	0.05
Burkholderia cepacia	11	0.55
Burkholderia pseudomallei	3	0.15
Elizabethkingia meningoseptica	4	0.2
Sphingomonas paucimobilis	3	0.15
Chryseobacterium indologenes	10	0.5
Total	1980	100

Table 2: Antimicrobial Sensitivity Pattern Of Gram Negative Bacilli (percentage)

Antibiotic	Acinetobacter species	P. aeruginosa	S. maltophilia	B. cepacia	B. pseudomallei	E. meningoseptica	S. paucimobilis	C. indologenes
Gentamicin	21.9	59.4	100	45.4	66.6	50	0	10
Amikacin	64.9	63.5	100	63.6	66.6	50	66.6	50
Tobramycin	21.9	59.4	100	-	-	-	66.6	-
Amoxycillin + Clavulanate	-	-	0	27.3	33.3	0	-	-
Ampicillin + Sulbactam	23.2	-	0	-	-	0	-	0
Piperacillin + Tazobactam	64.9	70.6	100	63.6	66.6	100	66.6	70
Cefuroxime	-	-	-	-	-	0	-	0
Cefepime	18.9	20.5	0	27.3	33.3	0	33.3	10
Ceftriaxone	18.9	-	0	27.3	33.3	-	33.3	10
Ceftazidime	18.9	20.5	0	27.3	33.3	0	33.3	30
Ciprofloxacin	40.9	59.4	100	83.3	100	0	100	90
Levofloxacin	-	59.4	100	83.3	100	50	100	90

Ofloxacin	-	-	100	83.3	100	50	100	-
Imipenem	67.9	62.0	100	81.8	100	0	66.6	40
Meropenem	67.9	62.0	100	81.8	66.6	0	66.6	30
Trimethoprim + Sulphamethoxazole	40.9	-	0	81.8	100	75	100	80
Aztreonam	-	59.4	-	27.3	33.3	25	33.3	20
Colistin	65.9	64.9	0	0	-	0	-	0
Chloramphenicol	-	-	0	-	33.3	-	33.3	50
Tigecycline	-	-	0	45.4	0	0	0	50
Netilmycin	-	59.4	100	-	-	50	-	10

Table 3: Gender Wise Distribution Of Patients

Gender	Number	Percentage
Male	1072	54.1
Female	908	45.9
Total	1980	100

Table 4: Age Wise Distribution Of Subjects

Age in years	Number	Percentage
0-10	216	10.9
11-20	128	6.4
21-30	189	9.5
31-40	245	12.4
41-50	354	17.9
51-60	320	16.2
> 61	528	26.7
Total	1980	100

Table 5: NFGNB Isolated From Various Clinical Samples

Organism	Pus	Sputum	ET tube	Urine	Blood	Body fluid	Suction tip
Acinetobacter species (n=1098)	452 (41.2)	30 (2.7)	262 (23.9)	200 (18.2)	62 (5.7)	86 (7.8)	6 (0.5)
Pseudomonas aeruginosa (n=850)	342 (40.2)	20 (2.4)	208 24.5)	169 (19.9)	42 (4.9)	64 (7.5)	5 (0.6)
Stenotrophomonas multophilia (n=1)	-	-	1 (100)	-	-	-	-
Burkholderia cepacia (n=11)	-	-	-	-	6 (54.5)	5 (45.5)	-
Burkholderia pseudomallei (n=3)	3 (100)	-	-	-	-	-	-
Elizabethkingia meningoseptica (n=4)	-	-	-	-	1 (25)	3 (75)	-
Sphingomonas paucimobilis (n=3)	3 (100)	-	-	-	-	-	-
Chryseobacterium indologenes (n=10)	4 (40)	1 (10)	2 (20)	-	1 (10)	2 (20)	-
Total (n=1098)	804 (40.6)	51 (2.6)	473 (23.9)	369 (18.6)	112 (5.7)	160 (8.1)	11 (0.5)

Figures in parenthesis indicate Percentages

Table 6: Ward / Unit Wise Distribution Of Patients With Nfgnb Infections

Ward / Unit	Acinetobacter species	P. aeruginosa	S. maltophilia	B. cepacia	B. pseudomallei	E. meningoseptica	S. paucimobilis	C. indologenes	Total
ICU	356(32.4)	270(31.8)	1(100)	1(9.1)	-	1(25)	3(100)	3(30)	635(32.1)
Medicine wards	119(10.8)	97(11.4)	-	4(36.35)	-	-	-	2(20)	222(11.2)
Paediatric wards	33(3.0)	26(3.1)	-	4(36.35)	-	3(75)	-	1(10)	67(3.4)
Pulmonary Medicine wards	37(3.4)	32(3.8)	-	1(9.1)	-	-	-	1(10)	71(3.6)
Skin ward	21(1.9)	16(1.8)	-	-	-	-	-	-	39(1.9)
Surgical wards	273(24.9)	202(23.8)	-	1	3(100)	-	-	3(30)	482(24.3)
Burns Ward	168(15.3)	140(16.5)	-	(9.1)	-	-	-	-	308(15.6)
Orthopaedic wards	21(1.9)	13(1.5)	-	-	-	-	-	-	34(1.7)
Gyaenecology wards	62(5.6)	45(5.3)	-	-	-	-	-	-	107(5.4)
ENT ward	8(0.8)	9(1.0)	-	-	-	-	-	-	17
Total	1098	850	1	11	3	4	3	10	1980

Figures in parenthesis indicate Percentages

Table 7: Clinical Diagnosis Of Patients With Nfgnb Infections

Clinical diagnosis	Acinetobacter species	P. aeruginosa	S. maltophilia	B. cepacia	B. pseudomallei	E. meningoseptica	S. paucimobilis	C. indologenes	Total
Pneumonia	170(15.5)	128(15.1)	1(100)	3(27.2)	-	-	-	1(10)	303(15.3)
Septicemia	160(14.6)	135(15.9)	-	4(36.4)	-	2(50)	-	7(70)	308(15.6)
Wound infections	472(42.9)	360(42.3)	-	-	3(100)	-	3(100)	-	838(42.3)
UTI	200(18.2)	169(19.9)	-	-	-	-	-	-	369(18.6)
Meningitis	96(8.8)	58(6.8)	-	4(36.4)	-	2(50)	-	2(20)	162(8.2)
Total	1098	850	1	11	3	4	3	10	1980

Figures in parenthesis indicate Percentages

Table 8: Risk Factors Associated With Isolation Of NFGNB (n=1980)

Risk factor	Acinetobacter species	P. aeruginosa	S. maltophilia	B. cepacia	B. pseudomallei	E. meningoseptica	S. paucimobilis	C. indologenes	Total
Ventilator	268(54.7)	213(43.5)	1(0.2)	1(0.2)	-	1(0.2)	3(0.6)	3(0.6)	490(24.7)
Urinary catheter	120(52.2)	110(47.8)	-	-	-	-	-	-	230(11.6)
Dialysis	48(62.3)	29(37.7)	-	-	-	-	-	-	77(3.9)
Prolonged Antibiotic therapy	135(47.5)	143(50.4)	-	2(0.7)	2(0.7)	-	-	2(0.7)	284(14.3)
Prolonged Hospital stay	90(57.7)	63(40.4)	-	-	-	-	-	3(1.9)	156(7.9)

Corticosteroid use	102(52)	94(48)	-	-	-	-	-	-	196(9.9)
Immunocompromised state	115(50.2)	100(43.7)	-	8(3.5)	1(0.4)	3 (1.3)	-	2(0.9)	230(11.6)

Figures in parenthesis indicate Percentages

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