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OCCURRENCE OF NON-FERMENTATIVE GRAM-NEGATIVE BACILLI AND THEIR DRUG RESISTANCE IN TERTIARY CARE RURAL HOSPITAL IN LONI, (M.S.)

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ABSTRACT **Background:** Aerobic non-fermenting Gram-negative bacilli (NF-GNB) once consider as a contaminant now associated with life-threatening infection and emerging as multi drug resistant nosocomial pathogens. Aim: Isolation and identification of NF-GNB in all the clinical samples and to determine antibiotic susceptibility pattern of the isolated NF-GNB. **Materials And Methods:** This study has been conducted in the Department of Microbiology at a tertiary care teaching hospital over a period of one year. NF-GNB were isolated and identified from clinical samples by standard procedure and antibiotic sensitivity was performed (according to CLSI guidelines). **Results:** Out of 1498 clinical specimens, 320 (21.36%) isolates were identified as NF-GNB. Maximum number of was blood, 90 (28.12%) followed by pus, 84 (26.25%), Pseudomonas Aeruginosa was the commonest isolate, 192 (60%), followed by Pseudomonas species, 58(18.12%) & Acinetobacter boumannii, 46(14.37%). All isolates most of them MDR and were sensitive to polymyxin B, colistin and tigecycline. **Conclusion:** Multi drug resistant NF-GNB was not uncommon in our hospital. All isolates were sensitive to polymyxin B, colistin and tigecycline. Indiscriminate use of antibiotics against these organisms should be avoided. It is necessary to identify NFGNB & to monitor their susceptibility pattern to guide the clinician for better care and management of patients.

KEYWORDS : NF-GNB, Multi drug resistant, Pseudomonas,

INTRODUCTION:

Aerobic non-fermenting Gram-negative bacilli (NF-GNB) are taxonomically diverse group of non -sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively.[1] Previously NF-GNB were considered to be nonpathogenic and of very little significance. Recently, rate of infection by NF-GNB is rising specially in hospitalized and immunocompromised patients.[2] In the hospital environment, they have been isolated from instrument such as ventilator machine, mattresses, and other equipment as well as from the skin of health care worker.Recent literature review shows that these organisms are now associated with life -threatening infections such as septicaemia, pneumonia, urinary tract infection, meningitis surgical site infection, ventilator associate pneumonia (VAP) ,wound infection etc.[3]

The important member of the group in non-fermenters include *Pseudomonas aeruginosa*, *Acinetobacter boumannii*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*.[4]

Currently *Pseudomonas aeruginosa*, *Acinetobacter baumannii* are the most commonly isolated non-fermenters pathogenic for humans. Infections caused by other species are relatively infrequent.[5]

Antimicrobial treatment of the infections caused by these agents is difficult due to its multi drug resistance (MDR). For this reason, accurate identification of non-fermenters is important for appropriate patient management. The main objective of this study includes to isolate and identify the Non Fermenting Gram Negative Bacilli from clinical samples. And to evaluate the antibiotic sensitivity pattern of the isolates.

MATERIALS AND METHODS:

This study was conducted for a period of one year at Rural Medical College, Loni (M.S.) India.

A total of 1498 clinical specimens were received in bacteriology laboratory, Department of Microbiology, which includes blood, Pus, Sputum, Urine, CSF, indwelling devices and body fluids etc. All the samples received were further plated on Blood agar, MacConkey agar, Nutrient agar, and incubated at 370 C for 18 - 48 hours. Growth was recorded, and non-lactose fermenting colonies were further analysed and processed as per the standard guidelines. Antibiotic sensitivity test was performed by using modified Kirby - Bauer disk diffusion method following clinical and laboratory standard institute (CLSI) guidelines.[7] The antibiotic disks used for this study were following - Ceftazidim (10mg), Amikacin (30 mg), Netilmycin (30ug), Ciprofloxacin (5ug), Ticarcillin (75ug), Cefeplme (10ug), Piperacillin/Tazobactum (100/10ug), Imipenem (10ug), Aztreonam (30ug), Colistin (10ug), Polymyxin (50ug), and Tigecycline (15 ug). MIC (minimum inhibitory concentration) of imipenem was also detected in resistant isolates. All antibiotic disks were obtained from Himedia pvt ltd, India and the E strip for MIC detection from AB BioMerieux. All the Gam-negative bacilli that grew on MacConkey agar or blood agar, whether oxidase positive or negative were inoculated on Triple sugar iron medium (TSI). Organisms that grew on Triple sugar iron agar producing an alkaline reaction were provisionally considered to be non -fermentative gram negative bacilli and were further inoculated into Huge and Leifson's medium for glucose, lactose, sucrose and maltose fermentation to find out whether a particular organism was oxidizer or non-oxidizer.

RESULTS:

A total of 1498 specimens, such as, blood, urine, pus body fluids etc were processed in the microbiology laboratory over a period of one year. Out of which, 320 (21.36%) NF-GNB were isolated and identified up to species level.

Table 1 : Distribution Of Different Non Fermenting, Gram Negative Bacterial Species From Various Clinical Specimens.

SAMPLES [total and %]	P. aeruginosa	P. Species	P.fluorescence	A. Baumannii	A.loffi	A.faecalis	S. Multophilia	Nil fermenter
Pus [84,26.25%]	45	19	8	10	1	-	-	1
Blood[90,28.12%]	71	5	-	13	1	-	-	-
Urine [58,18.12%]	33	7	-	14	2	-	-	2
Cerebrospinal fluid[12,3.75%]	2	4	1	32	1	-	1	-
Sputum[16.5%]	10	2	1	-	1	-	-	-
Vaginal swab [16,5%]	14	28	-	2	-	-	-	--
Pleural fluid[12,3.75%]	2	6	-	2	-	-	-	-
Endotracheal tube [17,5.31%]	7	6	1	-	1	-	-	-
Stool[3,30.93%]	1	-	-	-	-	2	-	-
Catheter tip [5,1.56%]	4	1	-	-	-	-	-	-
Throat swab [4,12.5%]	2	2	-	-	-	-	-	-
Ascitic fluid [3,0.93%]	1	2	-	-	-	-	-	-
Total	192[60%]	58 [18.12%]	11[3.43%]	46[14.37%]	7 [2.18%]	2 [0.62%]	1[0.31%]	3[0.93%]

Maximum no. of isolate were from blood samples, i.e. 90[28.12%]

followed by 84[26.25%] pus, 58[18.12%] urine, 17[5.31%] endotracheal tube aspiration, 16[5%] sputum and vaginal swab each, 12[3.75%] cerebrospinal fluid and pleural fluid each, 5 [1.56%] catheter tips, 4[1.25%] Throat swab and 3[0.93%] Stool and ascitic fluid each [table -1]

Table 2 :- Antibiotic Sensitivity Pattern Of Various Clinical Isolates .

Antibiotics	P. aeruginosa (n=192)	P. species (n=58)	P. fluorescence (n=11)	A. baumannii (=46)	A. loffi (n=7)	S. maltophilia (n=1)	A. faecalis (n=2)	Nil fermenter (n=3)
Ceflazidime	11 [5.72%]	8 [13.79%]	3 [27.227%]	10 [21.73%]	0	0	0	0
Amikacin	42 [21.87%]	10 [17.24%]	2 [18.128%]	12 [26.06%]	4 [57.14%]	1 [100%]	1 [50%]	2 [66.66%]
Netilmycin	25 [13.02%]	13 [27.08%]	2 [18.128%]	11 [22.91%]	3 [42.85]	1[100%]	2 [100%]	1 [33.33%]
Ciprofloxacin	21 [10.93%]	10 [17.24%]	3 [27.27%]	14 [30.43%]	3 [42.85%]	0	1 [50%]	0
Ticarcillin	9[4.685%]	3 [5.17%]	2 [18.18%]	8 [17.39%]	2 [18. 18%]	1 [100%]	0	2 [66.66%]
Piperacillin Tazobactam	47 [24.47%]	23 [39.65%]	7 [63.63%]	21 [45.65%]	4 [57.14%]	1 [100%]	1 [50%]	2 [66.66%]
Azteronam	4 [2.08%]	4 [6.89%]	2 [18.18%]	2 [4.34%]	4 [57.14%]	1 [100%]	1 [50%]	3 [100%]
Imipenem	86 [44.79%]	39 [67.24%]	8 [72.72%]	28 [60.86%]	5 [71.42%]	1 [100%]	2 {100%]	3 [100%]
Polymyxin B	192 [100%]	58 [100%]	11 [100%]	46 [100%]	7 [100%]	1 [100%]	2 [100%]	3 [100%]
Colistin	192 [100%]	58 [100%]	11 [100%]	46 [100%]	7 [100%]	1 [100%]	2 [100%]	3 [100%]
Tigecycline	192 [100%]	58 [100%]	11 [100%]	46 [100%]	7 [100%]	1 [100%]	2 [100%]	3 [100%]

All the isolate were MDR. All imipenem resistant NF-GNB [by disk diffusion method] showed high MIC values to imipenem. All the NF GNB, Isolates clinical samples was found susceptible to polymyxin B. Colistin and tigecycline [Table; 2].

DISCUSSION:

HAI is a worldwide phenomenon. In spite of awareness and hospital care, infections continue to develop in hospitalized patients and among hospital staff too. Factors promoting infections among hospitalized patients include decreased immunity; the increasing variety of medical procedure and invasive techniques creating potential routes of infection; and the transmission of drug resistant bacteria among crowded hospital populations, where poor infection control facilitate transmission.[6]

Over the last decade, NF-GNBs is rapidly emerging as a important opportunistic pathogens in the increasing population of patients who are immunocompromised by their disease or medical treatment. These organisms are aerobic, non-sporing and do not breakdown carbohydrates as a source of energy, other than fermentation.[6,7] They mainly cause hospital acquired infection. Indiscriminate use of antibiotics play major role to development of resistance to commonly used antibiotics.[8,9] Over a period of one year, we isolated 320(21.36%) NF-GNB out of 1498 clinical specimens. The most common isolate was *P. aeruginosa*, i.e., 192 (60%) and *P. species*, 58 (18.12%) followed by *A. baumannii* 46 (14.37%). Siou Cling Su et al 10 in their study ,isolated approximately 15% NF-GNB out of all Gram negative bacilli isolates. They did oligonucleotide array based test to identify NF-GNB from clinical specimens and found that *P. aeruginosa* was the commonest isolate, followed by *Acinetobacter* species. Our study was also similar to the study done by Malini et al.[2], i.e., 53.8% *P. aeruginosa* and 22.2% *A. boumannii* isolates. Upgade et al 9 isolated 43% *Pseudomonas species* and 21% *Acinetobacter* species in their study.

We isolated maximum number of NF-GNB from blood, i.e., 90 (28.12%). Some other researcher isolated majority of non- fermenters from SSI and urine samples.(11)

Multi drug resistance was considered when the organism was resistant to 3 or more classes of antibiotics.[12] Antibiotic susceptibility pattern of NF-GNB vary from country to country and from also different places within the same country.[13] All of our NF-GNB isolates were MDR. NF-GNB has got a tendency for inherent or acquired drug resistance to the commonly used antibiotics.[9] Upgade et al [9] observed 80% resistance of NF-GNB to major antibiotics. Nicasio et al[14] reported increasing resistance of NF-GNB to commonly used antibiotics, including carbapenems, cephalosporin, penicillin, fluroquinolones and aminoglycosides. They also observed that isolates were mostly sensitive to polymyxin B . Some researchers reported that to treat NF-GNB infection, some old antibiotics with more side effects were again in use.[14] All our isolates were sensitive to polymyxin B, colistin and tigecycline. Colistin and polymyxin B has got side effects like renal toxicity (27.58%) [15] Li *et al* [17] and Falaqas *et al* [18] also found usefulness of colistin and polymyxin B against MDR P. *aeruginosa*, *Acinetobacter* and *Klebsiella pneumoniae* due to low resistance rate to this drug. They also advised judicious use of the antibiotics for infection caused by these NF-GNB.

Tigecycline is a new Glycopeptides derivative of tetracycline. It has a wide range of antibacterial activity, both against Gram positive and Gram negative bacteria. However, reports regarding resistance to Colistin and Tigecycline have been observed by some workers.[18,19].

NF-GNB though regarded as contaminants are important bacteria causing wide range of nosocomial infections. Variability in sensitivity pattern emphasises the need for identification of NF-GNB and to monitor their susceptible patterns as it will help in proper management of the infections caused by them.[20]

Prevalence of pathogens often varies dramatically between communities, hospitals in the same community and among different patient populations in the same hospital.[21] Thus it is important for clinicians to remain updated with prevalence and antimicrobial susceptibility pattern of the circulating pathogens in their practice setting and their antimicrobial to be used for empiric therapy should be selected accordingly.

Most importantly these organisms have great potential to survive in hospital environment. Thus improved antibiotic stewardship and infection control measures like maintaining good housing, equipment decontamination, strict attention to hand washing and isolation procedures especially in high risk areas should be implemented to prevent the emergence and spread of multi drug resistant NF-GNB in the healthcare setting.

CONCLUSION:

Isolation of non-fermenters and their antibiotic susceptibility pattern should be regarded with all seriousness in clinical practice and clinical epidemiology because by being resistant to multiple antibiotics, their prevalence not only limits the treatment option but also act as a reservoir of drug resistance gene. They are emerging nosocomial pathogens every effort should be made for prevention and control of infections caused by them which depends on practices of effective hospital infection control measure and minimization of risk factors. Most importantly, all treating doctors should adhere to the antibiotic policy and the policy should be revised regularly depending upon the antibiotic sensitivity pattern and feed back received from clinician.

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