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Research Paper

A Study of Non-Fermenting Gram Negative Bacilli With Special Reference To Mbl Production In A Rural Tertiary Care Hospital.

Medical Science

KEYWORDS : NFGNB, meropenem, metallo-β-lactamases, MDR

Dr. Santosh Tathe — Department of Microbiology, Dean Office buildings, ground floor S.R.T.R. Govt. Medical College & Hospital, Ambajogai- 431517

Dr. Rajesh Ovhal — Department of Microbiology, Dean Office buildings, ground floor S.R.T.R. Govt. Medical College & Hospital, Ambajogai- 431517

Dr. Sandeep Nilekar — Department of Microbiology, Dean Office buildings, ground floor S.R.T.R. Govt. Medical College & Hospital, Ambajogai- 431517

ABSTRACT

Background: The most active β-lactams, namely carbapenems are mainstay of treatment for serious infections. Acquired metallo-β-lactamases (MBL) in Non-fermenters have recently emerged as one of the most worrisome resistance mechanisms with capacity to hydrolyze all β-lactams, including carbapenems & also other group of antibiotics & ability to transfer it to other bacteria via plasmids or tansposones. We conducted this study to find the prevalence of MBL-producing non- fermenting bacilli & their comparative antibiogram.

Materials and Methods: The study was conducted over a period of 24 months in a tertiary rural hospital. Isolates were screened for meropenem resistance by conventional methods & were confirmation of MBL by 'Double Disk Synergy Test' (DDST), 'Disc Potentiation Test' (DPT) and 'E-Test'.

Results: Our findings show that isolates having MBL production also have high degree of multidrug resistance compaired to MBL non producers. Active surveillance to detect MBL producers is the need of time.

INTRODUCTION:

Aerobic non fermenting gram negative bacilli (NFGNB) are heterogeneous group of organisms that are either incapable of utilizing carbohydrates as source of energy or degrade them via oxidatively rather than fermentative pathway. [1]

These organisms are common inhabitants of soil and water or parasites in normal microbial flora of humans and animals.[2] Though primarily regarded as contaminants, over past decade they have emerged as important opportunistic pathogens in patients who are critically ill or immunocompromised, causing broad spectrum infections like ventilator associated pneumonia (VAP), urinary tract infection (UTI), wound infection, septicaemia, osteomyelitis and rarely meningitis.5a

Among the species that are opportunistic pathogens in immunologically compromised host either by disease or treatment Pseudomonas aeruginosa (P. aeruginosa) is eminent followed by Acinetobacter baumannii (A baumannii), P. fluorescence, P. stutzeri ,Stenotrophomonas maltophilia (S. maltophilia), P.putida, P. cepacia.[7,8]

Non Fermenting Gram Negative Bacilli (NFGNB) are innately resistant to many antibiotics and are known to produce extended spectrum Beta-lactamases and metallo Beta-lactamases.9a total of 1078 Acinetobacter species and 842 Stenotrophomonas maltophilia isolates were collected between January 1997 and December1999 from5geographic regions (Canada, the United States, Latin America, Europe, and the Asia-Pacific,10,11we evaluated the existence of classes A, B and D \u03b2-lactamases among Pseudomonas aeruginosa (P.aerug- inosa

Antimicrobial treatment of the infection caused by these agents is difficult due to its multidrug resistance (MDR)[6] and rapid selection of high level MDR to various groups of antibiotics like beta-lactam, aminoglycosides and fluroquinolones posing problem for both treatment and infection control.9a total of 1078 Acinetobacter species and 842 Stenotrophomonas maltophilia isolates were collected between January 1997 and December1999 from5geographic regions (Canada, the United States, Latin America, Europe, and the Asia-Pacific,12 In recent years due to the liberal and empirical use of antibiotics, NFGNB have emerged as important health associated pathogens[13] and the isolation rate of NFGNB was increasing in our laboratory, hence this study was undertaken to identify, speciate and study the sensitivity pattern of NFGNB.

Material and Methods

A total of 4727 clinical specimens were received in the laboratory during November July 2012 to October 2014. These samples were plated on blood agar, chocolate agar, and Mac Conkey's agar, and incubated at 37°C for 18-24 hours. The organisms isolated were identified using the appropriate biochemical tests.[1]

All the organisms that grew on Triple Sugar Iron agar and produced an alkaline reaction were provisionally considered to be NFGNB and identified further by using a standard protocol for identification.[1] The characters assessed included morphology on Gram's stain, motility, pigment production, oxidase production, OF test (Hugh-Leifson's medium) for glucose, lactose, sucrose, maltose, mannitol, and xylose,[14] growth on 10% lactose agar, lysine decarboxylase test, and gelatin liquefaction test.

The clinical significance of the isolated NFGNB was assessed retrospectively by analyzing the case sheets for a combination of relevant laboratory and clinical criteria.

The sensitivity test was performed with the help of the Kirby-Bauer disc diffusion method using commercially available discs (Hi-media). The different antimicrobials tested were imipenem, piperacillin, ticarcillin, amikacin, ciprofloxacin, ceftazidime, cefepime, cefoperazone, ceftriaxone and cotrimoxazole. The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[15]

Strains of NFGNB resistant to meropenem (MRP) by Kirby Bauer disc diffusion method were considered as screening test positive for MBL production and selected for confirmation of MBL by 'Double Disk Synergy Test' (DDST), 'Disc Potentiation Test' (DPT) and 'E-Test'. Isolates which were positive by E-test considered to be MBL producers.[16Vitek 2 and E test,17Solna, Sweden]

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E. coli ATCC 25922 and P. aeruginosa ATCC 27853 were used as the control strains.

Results:

A total of 260 (5.5%.) isolates of NFGNB were obtained from 4727 various clinical samples like pus, swab of wound/ discharge, sputum, blood, urine, CSF, ET tube, central line tip culture & other body fluids etc. during the study period.

"Table 1 about here".

All the standard biochemical tests were performed as per the guidelines to identify NFGNB to the species level. Pseudomonas aeruginosa was the commonest species isolated (55 %) followed by Acinetobacter baumannii (33 %) & Pseudomonas fluorescens (8 %) Burkholderia cepacia (3 %) & Stenotrophomonas maltophilia (1 %) were among the least isolated.

"Table 2 about here"

Antibiotic susceptibility testing of all these isolates was done as per CLSI 2012 guidelines. Among isolated NFGNB, Acinetobacter baumannii followed by Pseudomonas aeruginosa showed significant resistance to higher antibiotics like carbapenems & lipopeptides.

"Table 3 about here"

All these meropenem resistant strains (n=42) were considered as screen test positive and further confirmed for MBL production by 'Double Disk Synergy Test' (DDST), 'Disc Potentiation Test' (DPT) and 'E-Test'.

"Table 4 about here"

By the E-test and DPT 41 isolates were positive for MBL production (97.61%) & by the DDST 39 isolates were positive (92.85%). So the 41 isolates which were positive by E-test were considered to be MBL producers.

"Table 5 about here"

We then compared the antibiogram of the confirmed MBL producers & MBL non producers & we noted that there is statistically significant (p-value <0.05) resistance to other antibiotics by MBL producers than MBL non producers.

"Table 6 about here"

Discussion:

In our study, the MBL producing organism showed significant resistance to other class or group of antibiotics & is a unique problem with MBLs that shows a broad-spectrum resistance profile. The genes encoding MBLs are often procured by class 1 (sometimes class 3) integrons. Other gene cassettes within the integrons confer resistance to other antibiotics such as fluoroquinolones, aminoglycosides and co-trimoxazole. Integrons are, in turn, embedded in transposons, resulting in a highly transmissible genetic apparatus that can be transferred between bacteria.[18] This highly transmissible nature of MBL makes them a problem statement. Therefore regular screening, prompt notification and strict isolation of the infected patient along with other barrier methods to prevent its transmission in hospital environment are necessary.

Although our MBL producers showed resistance towards many antibiotics, all MBL isolates were susceptible to Colistin. Similar findings were noted by Deshmukh & Damle et al (2012)[19], Seema Bose et al (2012)[20], Shikha Rajan et al (2014)[21].

Conclusion:

So Nonfermenter's ubiquitous nature, ability to survive & transmit via environment, innate resistance to many antibiotics & add on ability to acquire rapid resistance to antibiotics by producing MBL & ESBL & transmit it to other strains makes them a very notorious organisms in hospital environment causing havoc in many places worldwide.

Similar studies to map out NFGNB incidence, prevalence & antibiogram profile are the need of time to effectively if not eliminate but control the emerging epidemic.

Table 1: Percentage of NFGNB in clinical samples

Total no. of non-fermenters isolated	260	5.5 %
Total no. of samples processed	4727	100 %

Table 2: Percentage of different NFGNB isolated

Organism	No.	% percentage
Pseudomonas aeruginosa	143	55%
Acinetobacter baumannii	86	33.08%
Pseudomonas fluorescens	21	8.07%
Burkholderia cepacia	7	2.69%
Stenotrophomonas maltophilia	3	1.16%
Total	260	100%

Table 3: Comparison of antibiotic sensitivity of all the isolates

Organisms Antibiotics	P. aeruginosa	A. baumanii	P. fluorescens	B. cepacia	S. maltophilia
Pipracillin	69 (48.25%)	32 (37.20%)	11 (52.38%)	NA	NA
Ticarcillin	81 (56.64%)	42 (48.83%)	13 (61.90%)	NA	NA
Carbencillin	NA	NA	17 (80.95%)	NA	NA
Pipracillin-tazobactum	110 (76.92%)	55 (63.95%)	21 (100%)	NA	NA
Ticarcillin-clavulanic acid	99 (69.23%)	51 (59.30%)	19 (90.47%)	7 (100%)	3 (100%)
Ceftazidime	49 (34.26%)	16 (18.60%)	13 (61.90%)	6 (85.71%)	2 (66.66%)
Cefepime	56 (39.16%)	22 (25.58%)	9 (42.85%)	NA	NA
Cefotaxime	NA	43 (50%)	10 (47.62%)	NA	NA
Aztreonam	74 (51.74%)	NA	18 (85.71%)	NA	NA
Imipenem	126 (88.11%)	75 (88.37%)	21 (100%)	8 (100%)	
Meropenem	119 (83.21%)	69 (80.23%)	20 (95.23%)	NA	NA
Colistin	143 (100%)	86 (100%)	21 (100%)	NA	NA
Polymyxin B	143 (100%)	78 (90.69%)	21 (100%)	NA	NA
Gentamicin	54 (37.76%)	41 (47.67%)	13 (61.90%)	NA	NA
Amikacin	102 (71.32%)	52 (60.46%)	15 (71.42%)	NA	NA
Ciprofloxacin	54 (37.76%)	28 (32.55%)	9 (42.85%)	NA	NA
Levofloxacin	106 (74.12%)	58 (67.44%)	21 (100%)	6 (85.71%)	3 (100%)
Lomefloxacin	94 (65.73%)	NA	14 (66.67%)	NA	NA
Tetracycline	NA	32 (37.21)	9 (42.85%)	NA	NA
Doxycycline	NA	50 (58.13)	18 (85.71%)	NA	NA
Trimethoprim-sulfamethoxazole	NA	29 (33.72%)	10 (47.61%)	4 (57.14%)	1 (33.34%)

Table 4: Antibiotic susceptibility pattern of NFGNB for carbapenems & lipopeptides

Antibiotics	Sensitive		Resistant	
	No. of cases	%	No. of cases	%
Imipenem	223	89.2	27	10.8
Meropenem	208	83.2	42	16.8
Colistin	250	100	0	0
Polymyxin B	242	96.8	8	3.2

Table 5: Number of MBL producing isolates confirmed by various phenotypic tests:

Screen test positive isolates (Meropenem resistant)	42
MBL confirmed by E- Test	41
MBL confirmed by DDST	39
MBL confirmed by DPT	41

Table 6: Comparison of antimicrobial susceptibility pattern in MBL and non-MBL producing NFGNB isolates (n=250)*

Antimicrobial Agents	**Sensitivity of MBL +ve *NFGNB.* n= 41**	**Sensitivity of MBL -ve *NFGNB.* n=209**	Total n=250	p-Value
Amikacin	21 (51.21%)	148 (70.81%)	169 (67.6%)	<0.05
Gentamicin	10 (24.39%)	98 (46.88%)	108 (46.8%)	<0.05
Ciprofloxacin	7 (17.07%)	84 (40.19%)	91 (36.4%)	<0.05
Cefepime	19 (46.34%)	149 (71.29%)	168 (67.2%)	<0.05
Ceftazidime	6 (14.63%)	72 (34.44%)	78 (31.2%)	<0.05
Piperacillin+ Tazobactam	23 (56.09%)	163 (77.99%)	186 (74.4%)	<0.05
Aztreonam	9 (21.95%)	83 (39.71%)	92 (36.8%)	<0.05
Polymyxin B	33 (80.48%)	209 (100%)	242 (96.8%)	-
Meropenem	0 (0%)	209 (100%)	209 (83.6%)	-

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