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RESEARCH ARTICLE

PREVALENCE OF NON-FERMENTING GRAM NEGATIVE BACILLI FROM CLINICAL ISOLATES AND THEIR ANTIBIOGRAM PROFILE

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ABSTRACT

Background and Objectives: Non fermenting gram negative bacilli (NFGNB) are an increasing cause of concern in the hospitals as they produce a therapeutic dilemma for the treating physician.The present study was undertaken to know the prevalence and the resistance pattern of non-fermenting gram negative bacilli from clinical isolates.
Methods: 2758 bacterial isolates 389 (14.1%) were Non-fermenting gram negative bacilli recovered from various clinical specimens**.** All the samples were processed for routine bacterial culture and antimicrobial susceptibility test as per standard protocol (CLSI guidelines). **Results:** Among NFGNBs, 274(70.43%) were *Pseudomonas aeruginosa,* 99 (25.44%) *Acinetobactercalcoaciticus-baumaniicomplex,* 10 (2.57%) *Acinetobacterlwoffii*and6 (1.54%) *Acinetobacterhemolyticus*. All the *Pseudomonas aeruginosa* isolates were sensitive to Polymyxin B and least resistance was observed towards Amikacin 9.85%.All the Acinetobacterisolates were sensitive to Polymyxin B. Only 20.20 % of *Acinetobactercalcoaciticusbaumannii complex* were resistant to Imipenemwhereas rest all strains were sensitive. **Conclusion:** The prevalence of NFGNB among clinical isolates was 14.1%. Significantly higher resistance rate was observed by these isolates to almost all the drugs routinely used.

Keywords: NFGNB, Pseudomonas, Acinetobacter, Amikacin, Polymyxin B

INTRODUCTION:

Non fermenting gram negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, nonsporingbacilli that do not utilize glucose as a source of energy or utilize it oxidatively.They occur as saprophytes in the environment and some are also found as commensals in the human gut[1]. NFGNB are known to account for about 15% of all bacterial isolates from a clinical Microbiology laboratory. In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogen. They have been incriminated in infection such as, septicemia, meningitis, pneumonia, urinary tract infection and surgical site infection.[1]*Acinetobacter*and *Pseudomonas* are important nosocomial pathogens with high mortality rates. Both have intrinsic resistance to the extended spectrum cephalosporins and have the outer membrane with selective permeability to beta-lactams. By modification of porins diminish permeability to other antibiotics. Also they have chromosomal beta-lactamases.[2]

MATERIALS AND METHODS:

The present study was undertaken at the Department of Microbiology, Karnataka Institute of Medical Sciences (KIMS), Hubli from Dec 2010 to Nov 2011.

Source of data:

Clinical samples such as pus, urine, blood, body fluidsetc. obtained from patients admitted in Karnataka Institute of Medical Sciences hospital and received at the department of Microbiology.

Inclusion criteria:

Non repetitive, consecutive non-fermenting gram negative bacilli isolated from clinical samples obtained from hospitalised patients (IPD) received during study period.

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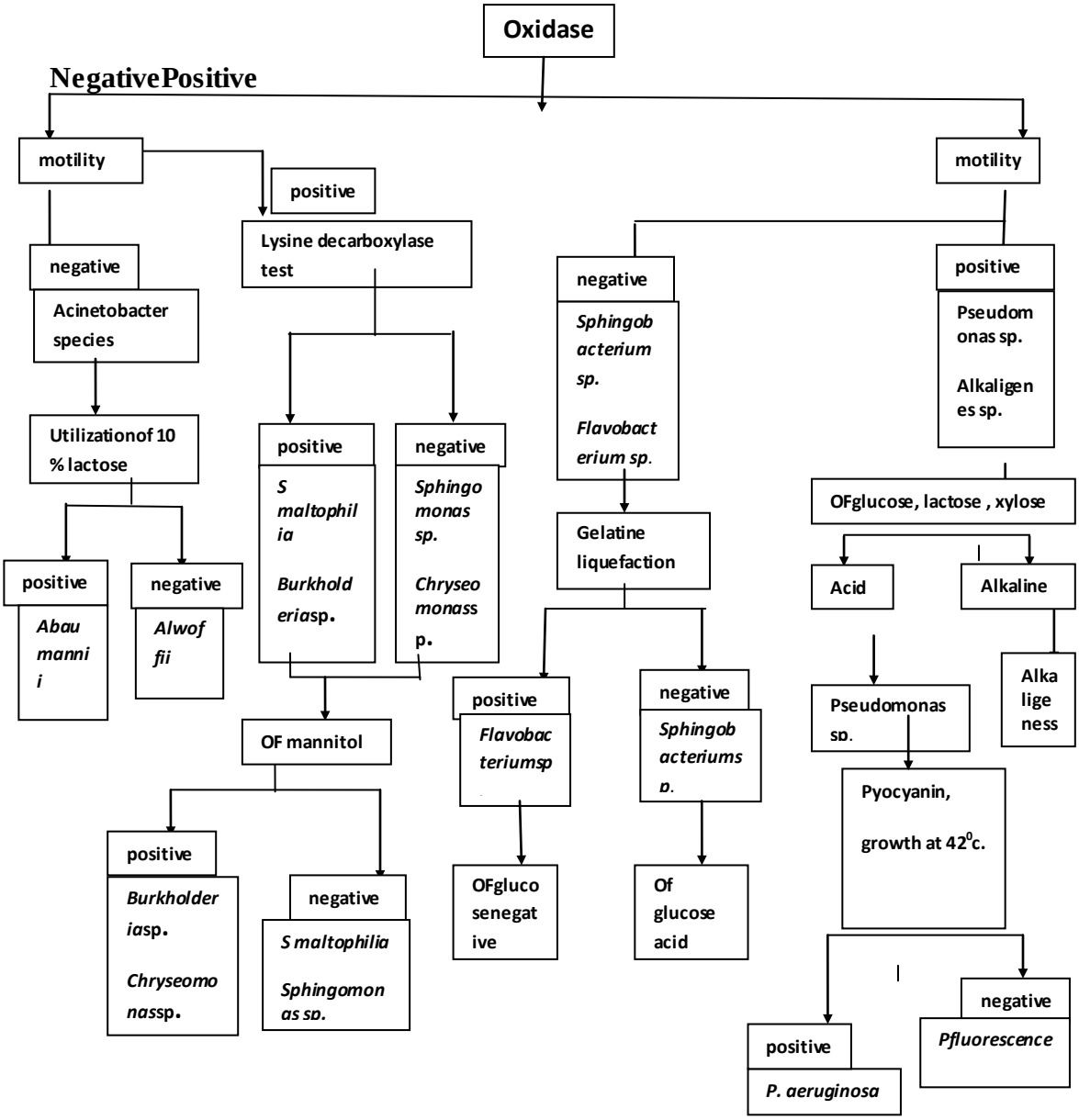
Sample processing:

All the samples were processed for routine bacterial culture as per standard protocol.[3] Smears were prepared on clean glass slides. Gram stain performed and observed for the presence of any gram negative bacilli or gram variable cocco-bacilli. Samples were inoculated into Thio-glycollate broth, chocolate agar, MacConkey's agar and Blood agar. They were incubated at 37^0 C in ambient air for 24 to 48hours. Isolates were identified based on colony morphology, motility and relevant biochemical reactions. All 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 [2,3].

Scheme for identification of NFGNBs:[2,3]



Antimicrobial susceptibility test:[4,5]

Antimicrobial susceptibility test was carried out with modified Kirby-Bauer disk diffusion method using current CLSI[9] recommendations. Commercially available antibiotic disks (Himedia, Mumbai) were used. The antibiotic susceptibility profile against Gentamicin, Amikacin, Gatifloxacin, Levofloxacin, Cephalosporins (Cefoxitin, Ceftazidime, Cefotaxime, Ceftriaxone, Cefepime), Piperacillin-Tazobactam, Imipenem and Polymyxin B were studied. *Pseudomonasaeruginosa* ATCC 27853 was used as control strain[4].

RESULTS:

2758 bacterial isolates 389 (14.1%) were Non-fermenting gram negative bacilli recovered from various clinical specimens like pus (207), sputum(61), urine(55), ear discharge (31), blood (8), cerebrospinal fluid (8), pleural fluid (6), ascitic fluid (6), post operative drain (3), aspiration from liver abcess (2), corneal scraping (1) and tracheal secretion (1).

Table 1: Organisms isolated from different clinical samples.

Organisms	Pus no (%)	Sputum no (%)	Urine no (%)	Ear discharge no (%)	Others no (%)	Total no (%)
Pseudomonas aeruginosa	153(53.83)	35(12.77)	36(13.13)	24(8.75)	26(9.48)	274(70.43)
Acinetobactercalcoaciticus-baumaniicomplex	45(45.45)	25(25.25)	18(18.18)	6(6.06)	5(5.05)	99(25.44)
Acinetobacter lwoffii	6(60)	1(10)	1(10)	0	2(20)	10(2.57)
Acinetobacterhemolyticus	3(50)	0	0	1(16.66)	2(33.33)	6(1.54)
TOTAL	207(53.21)	61(15.68)	55(14.13)	31(7.96)	35(8.99)	389

- Majority 207(53.21%) were isolated from pus followed by sputum 61(15.68%).
- *Pseudomonas aeruginosa* was the most common isolate 274 (70.43%) followed by *Acinetobactercalcoaciticus-baumaniicomplex* 99 (25.44%) and *Acinetobacterlwoffii*10(2.57).
- Of 389 NFGNB isolates 214 were resistant to ceftazidime (zone of inhibition less than 18 mm) taken as possible ESBL producers and subjected to phenotypic confirmatory disc diffusion method using ceftazidime with and without clavulanic acid.

Table 2: Age and Sex distribution of the patients in the study group

n=214

Age (yrs)	Males		Females	
	Number	%	Number	%
0—10	8	5.88	4	5.12
11-20	20	14.7	12	15.38
21-30	20	14.7	21	26.92
31-40	27	19.85	18	23.07
41-50	16	11.76	12	15.38
51-60	28	20.58	4	5.12
61-70	12	8.82	4	5.12
>70 yrs	5	3.67	3	3.84
Total(214)	136	63.55	78	36.44

Male to female ratio was 1.74: 1.

Mean age in the study group was 38.1± 18.48 years.

Table 3: Antibiotic resistance pattern of *Pseudomonas aeruginosa*:

Antibiotics tested	*Pseudomonas aeruginosa* Resistance (n=274)	
	No	(%)
Amikacin	27	9.85
Cefipime	35	12.77
Cefoxitin	42	15.32
Ceftazidime	120	43.79
Ciprofloxacin	53	19.34
Gatifloxacin	48	17.51
Gentamicin	49	17.88
Imepenem	31	11.31
Levofloxacin	47	17.15
Piperacillin-tazobactam	33	12.04
Polymyxin B (300ug)	0	0
Ticarcillin	43	15.69
Tobramycin	46	16.78

All the isolates were sensitive to Polymyxin B and least resistance was observed towards Amikacin 9.85%.

Table 4: Antibiotic resistance pattern of Acinetobacter species

Organism	*Acinetobactercalcoaciticus-baumaniicomplex* (99)		*Acinetobacter Lwoffii* (10)		*Acinetobacterhemolyticus*(6)		TOTAL (115)	
	No	%	No	%	No	%	No	%
Amikacin	18	18.18	0	0	1	16.66	19	16.52
Cefipime	23	23.23	0	0	1	16.66	24	20.86
Cefotaxime	81	81.81	3	30	2	33.33	86	74.78
Cefoxitin	48	48.48	3	30	2	33.33	53	46.08
Ceftazidime	88	88.88	3	30	2	33.33	93	80.86
Ceftriaxone	88	88.88	3	30	2	33.33	93	80.86
Cotrimoxazole	39	39.39	2	20	1	16.66	42	36.52
Gatifloxacin	25	25.25	3	30	2	33.33	30	26.08
Gentamicin	47	47.47	3	30	2	33.33	52	45.21
Imepenem	20	20.20	0	0	0	0	20	17.39
Levofloxacin	45	45.45	3	30	2	33.33	50	43.47
Piperacillin-tazobactam	22	22.22	0	0	1	16.66	23	20
Polymyxin B (300ug)	0	0	0	0	0	0	0	0
Tetracycline	36	36.36	1	10	1	16.66	38	33.04
Ticarcillin	41	41.41	2	20	2	33.33	45	39.13
Tobramycin	45	45.45	3	30	2	33.33	50	43.47

All the isolates were sensitive to Polymyxin B. All the strains of *Acinetobacterlwoffii* and *Acinetobacterhemolyticus*were also sensitive to imipenem, where as 20.20 % of *Acinetobactercalcoaciticusbaumannii complex* were resistant to imipenem.

Table 5: Analysis of the risk factors for non-fermenting gram negative bacilli infectionin the Hospital

Risk factors	NFGNB infection in the Hospital No (n=389) (%)
Burns	30(7.71)
Carcinomas	18(4.62)
Catheterization	136(34.96)
Chronic ilment	116(29.82)
Diabetis ellitus.	18(4.62)
HIV Positive	9(2.31)
Hospitalization of 5 days or more	200(51.41)
ICUs (Intensive care units)	6(1.54)
Neurological Disorders	6(1.54)
Sepsis	9(2.31)
Surgical Intervention	173 (44.47)

The major risk factors for infection with non-fermenting gram negative bacilliwerehospitalization of 5 days or more, surgical intervention and catheterization.

DISCUSSION

Nonfermentative gram-negative bacilli (non-fermenters) cause a significant number of infections, particularly in the hospitalised patients and immunocompromised hosts. *Pseudomonas aeruginosa* and *Acinetobacterbaumanii*are the most common nonfermenters pathogenic for humans. Infections caused by other species are relatively infrequent.[6] In the present study, 389 (14.1%) isolates were non-fermenting gram negative bacilli recovered from various clinical specimens at the department of Microbiology, Karnataka Institute of Medical Sciences, Hubli from Dec 2010 to Nov 2011. Out of which 274(70.43%) were *Pseudomonas aeruginosa*, 99 (25.44%) were *Acinetobactercalcoaciticus-baumanii complex*, 10(2.57%) were *Acinetobacterlwoffiiand* 6(1.54%) were *Acinetobacterhemolyticus.* Study conducted by Malini A, Deepa E K, et al. reported nonfermenting gram negative bacilli isolation rate as 4.5%. *Pseudomonasaeruginosa* as the most common isolate (53.8%).[2]

Maximum number of non fermenting gram negative bacilli were isolated from pus (53.21%) followed by sputum (15.68%) and urine (14.13%).NoyalMariya Joseph, SujathaSistla et al.[7] reported, non-fermenters (77.8%) were the most predominant pathogens causing Ventilator-Associated Pneumonia in the Critical Care Units and the Medicine Intensive Care Unit (48.3%). Baheraet al.[8] isolated 37.36 % *P.aeruginosa* from bronchoalveolar lavage, 23.07 %from blood, 15.38%from tracheal aspirate. K PrabhatRanjan, NeelimaRanjan, et al.[9] reported that *P. aeruginosa* was the most prevalent (29.6%) among all the pathogens isolated from the surgical wound. Anupurba and colleagues[10] quoted 32%, where asHani and colleagues[11] found a prevalence rate of 27.78%.Iraida E. Robledoet al.[12] reported 60% of resistant strains Acinetobacter species were from ICU. Male to female ratio was 1.74: 1. Mean age in the study group is 38.1 ± 18.48 years.

There was no statistically significant difference observed between male and female gender regarding NFGNB infection.

ANTIBIOTIC SUSCEPTIBILITY:

Antibiotic resistance pattern of *Pseudomonas aeruginosa*: A total of 122 (45.18%) isolates were multidrug resistant being resistant to three or more antibiotics tested in the present study. *Pseudomonas aeruginosa* exhibited maximum resistance to ceftazidime120(43.79%) and least to Amikacin 27 (9.85%). All 100% sensitive to Polymyxin B. Vandana A Agarwal, Shruthi A D, et al.[13] reported, 4.4% *P. aeruginosa* isolates as multidrug resistant. Taneja N et al.[6] observed 22.58 % of *P. aeruginosa*were multi drug resistant. S. Nagaveni, H. Rajeshwari*et al.*[14]*stated, P. aeruginosa* exhibiting high degree of resistance against three groups of antibiotics i.e. β-lactams (74%) followed by aminoglycosides (70%) and flouroquinolones (100%).

Several studies have reported [8,13,6,14,15,16,17], occurrence of range of resistance in *P. aeruginosa* to amikacin 3-74%, ciprofloxacin 12-79%, ceftazidime 9-70%, piperacillin 2.6 75%, and imipenem 32.9-69%[15,16,18,19,20].

Antibiotic resistance pattern of Acinetobacter species:

Acinetobacter species exhibited higher drug resistance to ceftazidime 93(80.96%), ceftriaxone 93(80.86%) and Cefotaxime 86 (74.78%). All were 100% sensitive to Polymyxin B.

Many studies have reportedrangeof occurrence of resistance in *Acinetobacter* spp. to gentamicin was 0 – 81%, amikacin 10 – 51%, ciprofloxacin 19 – 81%, ceftazidime 0 – 81%, piperacillin-tazobactam 36 – 75%, and imipenem 5 – 19%[21,22,23,24].

Therapeutic options:

Imipenem is a carbapenem antibiotic, which is active against *P. aeruginosa*and Acinetobactersp. This drug is highly β-lactamase stable and has an unusual property of causing a post antibiotic effect on gram negative bacteria. It is a small molecule, which can over come the poor outer membrane permeability of β-lactams for *Pseudomonas* by penetrating through the porinomp D[17,21].

Piperacillin and imipenem either alone or in combination with amikacin were used for treating the patients not responding to treatment with fluoroquinolones, aminoglycosides and ceftazidime[6].

Risk factors for different β-lactamase producing non-fermenting gram negative bacilli infection.

In our study the major risk factors for infection with β-lactamase producing non-fermenting gram negative bacilli were Hospitalization of 5 days or more, Surgical intervention and Catheterization.

CONCLUSION:

- A prospective study conducted to know the prevalence of different β-lactamases among 389(14.1%) non-fermenting gram negative bacilli isolated from various clinical specimens.
- Of2758 bacterial isolates 389 (14.1%) were Non-fermenting gram negative bacilli recovered from various clinical specimens like pus (207), sputum(61), urine(55), ear discharge (31), blood (8), cerebrospinal fluid (8), pleural fluid (6), ascitic fluid (6), post operative drain (3), aspiration from liver abcess (2), corneal scraping (1) and tracheal secretion (1).
- Of these 274(70.43%) were *Pseudomonas aeruginosa*, 99(25.44%) were*Acinetobactercalcoaciticus-baumanii complex*, 10(2.57%) were *Acinetobacterlwoffiiand* 6(1.54%) were *Acinetobacterhemolyticus.*
- All the isolates of *Pseudomonas aeruginosa* were sensitive to Polymyxin B and least resistance was observed towards Amikacin 9.85%.
- All the isolates ofAcinetobacter were sensitive to Polymyxin B. All the strains of *Acinetobacterlwoffii* and *Acinetobacterhemolyticus*were also sensitive to imipenem, where as 20.20 % of *Acinetobactercalcoaciticusbaumannii complex* were resistant to imipenem.
- The major risk factors for infection with non-fermenting gram negative bacilli infectionwerehospitalization of 5 days or more, surgical intervention and catheterization.

REFERENCES:

1. Hauk Vahaboglo, FigenCoskunkan, OzlemTansel et al. Clinical importance of Extended spectrum beta lactamases (PER-1-Type) produced in Acinetobacter species and *Pseudomonas aeruginosa*strains. *J Med Microbiol*2001; 50 (7) :642-5.
2. Malini A, Deepa E K, Gokul B N, S R Prasad. Non-fermenting gram negative bacilli infections in a tertiary care hospital in Kolar. *J lab physicians* 2009; 1 (2): 62-66.
3. Washington Winn, Jr., Stephen Allen, WilliumJanda, Elmer Koneman, Gary Procop, Paul Schrenkenberger, Gail Woods. The non-fermenting gram negative bacilli. Koneman'sColour atlas and text book of diagnostic microbiology. Sixth edition. Philadelphia Lippincott Willium and Wilkins.2006: 309-375.
4. Clinical and Laboratory Standards Institute M100-S21 (2011). Performance standards for antimicrobial susceptibility testing. Twenty-first informational supplement. Wayne, PA: CLSI 2011; vol 31: no 1.
5. Sinha M, Srinivasa H. Antibiotic resistance profile and extended spectrum beta-lactamase (ESBL) production in Acinetobacter species *Indian J Med Res* 2007; 25 :21-5.
6. Taneja N, Maharwal S, Sharma M. Imipenem resistance in nonfermenters causing nosocomial urinary tract infections. *Int J Biol Med Res*. 2011; 2(3): 766-770.
7. NoyalMariya Joseph, SujathaSistla, Tarun Kumar Dutta, Ashok Shankar Badhe, Desdemona Rasitha, Subhash Chandra Parija. Ventilator-associated pneumonia in a tertiary care hospital in India: role of multi-drug resistant pathogens. *J Infect DevCtries*2010; 4 (4): 218-225.
8. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-β-lactamase producing *Pseudomonas aeruginosa.Indian J Med Microbiol*2008; 26: 233-7.
9. K PrabhatRanjan, NeelimaRanjan, Satish K Bansal and D R Arora. Prevalence of *Pseudomonas aeruginosa* in Post-operative Wound Infection in a

Referral Hospital in Haryana. *IndJLabPhysicians* 2011; 3(2): 129.

10. Anupurba S, Sen MR. Antimicrobial resistance profile of bacterial isolates from Intensive Care Unit: Changing trends. *J Commun Dis* 2005; 37:58-65.

11. Masaadeh Hani A, Jaran AS. Incident of Pseudomonas aeruginosa in post-operative wound infection..Am J Infect Dis. 2009; 5: 1–6.

12. Iraida E. Robledo, Edna E. Aquino, Marı´a I. Sante´, Jorge L. Santana, Diana M. Otero, Carlos F. Leo´n and Guillermo J. Va´zquezo. Detection of KPC in Acinetobacter spp. in Puerto Rico. *J Anti Chemother* 2010; 54 (3): 1354–1357.

13. Agarwal VA, Dongre SA, Powar RM. Antimicrobial resistance profile of metallo-beta-lactamase *Pseudomonas aeruginosa* producing metallo-beta-lactamases. *Indian J Med Res* 2006; 124: 588-90.

14. Nagaveni S, S. Nagaveni, H. Rajeshwari, Ajay Kumar Oli, S. A. Patil and R. KelmaniChandrakanth. Widespread Emergence of Multidrug Resistant *Pseudomonas aeruginosa*Isolated from CSF Samples. *Indian J Microbiol* 2011; 51 (1): 2–7.

15. Y. Khosravi, S. Tee Tay, and J. Vadivelu, "Metallo- β-lactamase-producing imipenem-resistant *Pseudomonas aeruginosa* clinical isolates in a university teaching hospital in Malaysia:detection of IMP-7 and first identification of IMP-4, VIM-2 and VIM-11,". *DiagMicrobiolInf Dis* 2010; 67 (3): 294–296.

16. Japoni A, Alborzi A, Kalani M. Susceptibility patterns and cross-resistance of antibiotics against *Pseudomonas aeruginosa* isolated from burn patients. *Ind J MedMicrobiol* 2006: 32: 343–347.

17. Gibb, A. P., C. Tribuddharat, R. A. Moore, T. J. Louie, W. Krulicki, D. M. Livermore, M.-F. Palepou and N. Woodford. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla IMP allele, bla IMP-7. *JAntimicrob. Agents Chemother* 2002; 46: 255–258.

18. HoriehSaderi, HoseinLotfalipour, ParvizOwlia and Hassan Salimi.Detection of Metallo-β-Lactamase Producing *Pseudomonas aeruginosa* Isolated From Burn Patients. *Lab Medicine* 2010;41: 609-612.

19. Gales AC, Jones RN, Turnidge J, Rennie T, Ramphal R. Characterization of *Pseudomonas aeruginosa* isolates: occurrence rates, antimicrobial susceptibility patterns and molecular typing in the global SENTRY antimicrobial surveillance program. *Clin Infect Dis* 2001; 32: S146-55.

20. Manoharan A, Chatterjee S, Madhup S K, Gautam V, Das A. Detection and characterization of metallo-beta-lactamases producing *Pseudomonas aeruginosa.Indian J Med Microbiol*2010; 28 (3): 241-4.

21. Gales AC, Jones RN, Forward KR, Linares J, Sader HS, Verhoef J. Emerging importance of multi drug-resistant Acinetobactersp and *Stenotrophomonasmaltophilia* as pathogens in seriously ill patients: Geographic patterns, epidemiological features and trends in the SENTRY antimicrobial surveillance program 1997-1999. Clin Infect Dis 2001; 32: S104-12.

22. Chaudhary U, Aggarwal R. Extended spectrum β-lactamases (ESBL) - An emerging threat to clinical therapeutics. *Ind J Med Microbiol* 2004; 22: 75-80.

23. Prashanth K, Badrinath S. In vitro susceptibility pattern of Acinetobacter species to commonly used cephalosporins, quinolones and aminoglycosides. *Indian J Med Microbiol*2004; 22: 2: 97-103.

24. Gladstone P, Rajendra P et al. Incidence of carbepenem resistant non-fermenting gram negative bacilli from patients with respiratory infection in the intensive care unit. *Indian J Med Microbiol*2005; 23 (3): 189-191.

