ESBL and MBL mediated resistance in clinical isolates of non-fermenting gram negative bacilli (NFGNB) in Nepal

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Introduction

Nonfermenter gram negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, nonsporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively [1]. They occur as saprophytes in the environment and some are also found as commensals in the human gut [2,3]. NFGNB including *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex (BCC), are emerging as important causes of blood stream infections (BSI) worldwide particularly in immune-compromised patients, patients with hematological malignancies and patients admitted in intensive care units (ICUs) [4,5]. *Pseudomonas aeruginosa* is an increasingly prevalent opportunistic human pathogen and the most common gram-negative bacterium found in nosocomial infections [6]. Despite improvements in antibiotic therapy, *Pseudomonas aeruginosa* is intrinsically resistant to a number of antimicrobial agents [7]. Members of the genus *Acinetobacter* are ubiquitous, free living, strictly aerobic, short, often capsulated, non-motile, gram-negative (or gram variable) bacilli or coccobacilli (often diplococcobacilli) with a DNA G+C content of 39 to 47 mol% that grow on simple media and prefer moist environment and can be easily obtained from soil, water, food and sewage [8]. *Acinetobacter* spp., usually considered to be opportunistic pathogen, is one of the most important notorious nosocomial health-care related pathogen especially in critically-ill hospitalized patients particularly in intensive care units and occasionally in other units too [9].

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ABSTRACT

Objective: The present study interpreted the prevalence of NFGNB from clinical specimens and assessed antibiotic resistance pattern as well as incidence of Extended Spectrun Beta lactamase(ESBL) and Metallo-Beta Lactamase(MBL) producers.

Methods: A total of 3585 clinical specimens were included and all the samples were processed for routine bacterial culture and antimicrobial susceptibility test as per standard protocol. They were further subjected to ESBL production detection by phenotypic confirmatory double disk diffusion test using cefotaxime with and without clavulanic acid. Imipenem resistant isolates were selected for the detection of MBL production by Imipenem-EDTA combined disc test. All the specimens were identified by the classical Microbiological and Biochemical tests.

Results: The result showed that 304 non fermenting gram negative bacilli were isolated from various clinical specimens. Out of 304 NFGNB,195 (64.1%) isolates were *Pseudomonas spp*. followed by *Acinetobacter spp*. i.e 109 (35.9%). Imipenem (85.1% sensitive) were the most effective drug against *P. aeruginosa* where as *Acinetobacter spp*. were most sensitive to colistin and Imipenem with 76.1% and 18.3% sensitivity respectively. Altogether, 69.1% of total NFGNB isolates were MDR and ESBL and MDR produces were determined to be 51.97% and 21.4% respectively. Forty five (14.8%) of the total isolated were found to produce both ESBL and MBL.

Conclusions: The study showed increasing trend of MDR, ESBL and MBL production in NFGNB so constant survey of antibiotic sensitivity and precautionary use of antibiotics is essential to control and manage spread of these isolates in different units of health institutions.

KEY WORDS: NFGNB
                                    ESBL
                                    MBL
                                    Acinetobacter spp
                                    Pseudomonas aeruginosa

NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory [10, 11]. In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. They have been incriminated in infections, such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections

(SSI) [12]. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases and metallo ß-lactamases. Important ESBL varieties are TEM ß-lactamases, SHV ß-lactamases, CTX-M ß-lactamases and OXA- ß-lactamases [11,12]. Acquisition of drug resistance by these pathogenic strains has posed serious challenge for the therapeutic management of clinical cases. Infections that are caused by drug resistant bacteria are associated with up to five times higher mortality rates compared with infections that are caused by susceptible bacteria [13]. These resistant organisms need to be identified and apposite action be taken before scenario gets worse. There are increasing reports of Extended Spectrum β-Lactamase (ESBL) and Metallo β-Lactamase (MBL) producing isolates expressing multidrug resistance (MDR), defined as concomitant resistance to at least two different antibiotic classes [14,15]. Patients at high risk of developing colonization or infection with ESBL producing microorganisms are often seriously ill patients with prolonged hospital stays, e.g. undergoing hemodialysis and in whom invasive medical devices are present [16]. Extended-spectrum β-Lactamase and metallo β-Lactamase producing bacteria are emerging concern for health professionals as they are associated with more severe form of disease and antibiotic resistance. ESBL and MBL have been studied well in Nepalese community [17] yet the data remains lacking for those organisms among NFGNB. As *Pseudomonas* spp and *Acinetobacter* spp are increasing threat to global health. This study was undertaken to determine the prevalence of nonfermenter gram negative bacteria with special reference to *Pseudomonas* and *Acinetobacter* and to determine their antibiotic resistance, MDR, ESBL and MBL pattern.

Materials and Methods

This cross-sectional study was conducted at B&B Hospital Kathmandu, Nepal, from April 2014 to September 2014.

Collection, isolation, and identification of bacteria

A total of 3585 samples that were sent for routine laboratory investigation were processed in this study. These samples included urine, sputum, blood, pus, throat swab, body fluids and other miscellaneous specimens such as various types of tips (Catheter tip, suction tip, CVP tip, tracheostomy tips). Processing of the specimen and identification of the isolates were done by using standard microbiological techniques which comprises of studying the colonial morphology, staining reactions and various biochemical properties [18].

Determination of antimicrobial susceptibility pattern

Susceptibility tests of the different clinical isolates towards various antibiotics were performed by modified Kirby-Bauer modified disk diffusion method for the commonly isolated pathogens using Mueller Hinton Agar (MHA) according
to Clinical Laboratory Standards Institute (CLSI) guidelines [19].

Screening and confirmation for ESBL producers

The MDR isolates were screened for possible ESBL production using ceftazidime (30µg), cefotaxime (30µg) (CLSI, 2005). According to the guidelines, isolates showing ceftazidime <22 mm, cefotaxime < 27 mm are the possible ESBL producing strains. The screen positive isolates i.e. suspected ESBL producers were subjected to Combined Disk (CD) test for confirmation of ESBL production using MASTDISCS[TM] extended spectrum β-Lactamase (ESBL) detection discs. The kit consisted of:

Set 1: Ceftazidime (30µg) and ceftazidime (30µg) plus clavulanic acid (10µg);

Set 2: Cefotaxime (30µg) and cefotaxime (30µg) plus clavulanic acid (10µg).

Screening and confirmation for MBL producers

Isolates showing resistance to Imipenem and ceftazidime were subjected for the EDTA combined disc method-by Imipenem detection of possible MBL production. Test organisms were inoculated onto plates of Mueller-Hinton agar. Two 10 µg Imipenem discs were placed on the plate, and 750µg of 0.5 M EDTA solution of pH 8 were added to one of them. The inhibition zones of the Imipenem and Imipenem -EDTA discs were compared after 16 to 18 h of incubation in aerobic condition at 35°C. All of the MBL-positive isolates were well separated from MBL-negative isolates by the criterion of a ≥7 mm increase of inhibition zone with the discs to which 750 µg of EDTA was added [20].

Ethics statement

Ethical approval was obtained from the Ethical Review Committee, B&B Hospital, Gaurko, Lalitpur, Nepal, and informed consent was obtained from the patients or their relatives. The research was fully compliant with the Helsinki Declaration.

Statistical analysis

All the results were entered in the worksheet of SPSS software version 20. Chi-square test was used to determine significant association of dependable variables like susceptibility to ciprofloxacin, sensitivity to carbapenems etc to different independent variables like ESBL and MBL production.

Results

Bacterial isolation and prevalence of pathogens

Out of 3585 clinical specimens processed, 1523 (42.24%) different bacterial isolates were obtained, of which 304 (19.97%) were NFGNB, 651 (42.74%) were other gram negative bacteria like *E. coli, K. pneumoniae* and *Proteus mirabilis* etc where as 568 (37.29%) were gram positive. Among NFGNB, 195 (64.1%) were *Pseudomonas aeruginosa,* 109 (35.9%) were *Acinetobacter* spp. The distribution pattern of NFGNB species according to the specimen is shown in Figure 1.

Figure 1. Distribution of NFGNB species according to clinical specimens.
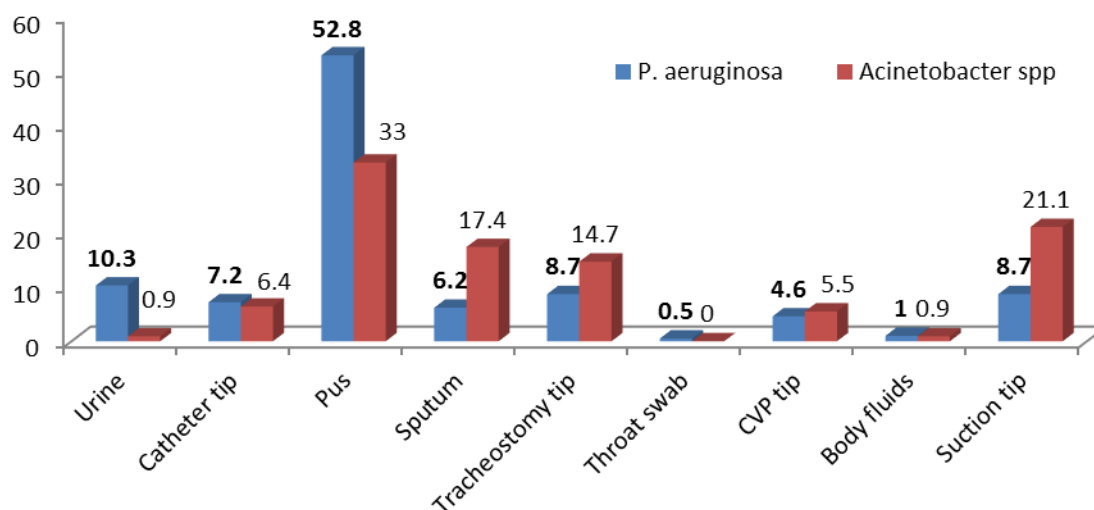
Antibiotic sensitivity pattern of NFGNB isolates

As shown in Table 1, *P. aeruginosa* strains were most sensitive towards imipenem (85.1%) followed by colistin (71.3%), amikacin (64.1%) and gentamicin (56.4%). *P. aeruginosa* were most resistant towards ceftriaxone (70.3%), followed by chloramphenicol (65.6%), ciprofloxacin (53.3%) and ofloxacin (52.8%). Similarly *Acinetobacter spp.* were more sensitive towards colistin (76.1%) followed by imipenem (18.3%) and amikacin (12.8%). Whereas the drugs most resisted was ceftriaxone (99.1%). Altogether 210 (69.1%) of MDR isolates were found in different clinical specimen. Of the total *P. aeruginosa* isolated, 53.9 % were MDR and 96.4% of Acinetobacter spp isolated were MDR followed by ciprofloxacin (97%), ofloxacin (96.3%) and gentamicin (90.8%).

Pattern of ESBL production among the NFGNB isolates

Out of 245 (80.3%) screen positive for ESBL, 158 (51.97%) were tested positive for confirmed ESBL production. Eighty-nine (45.6%) of the total *P. aeruginosa* isolates and 69(63.3%) of the total *Acinetobacter* spp. isolated were ESBL producers. On statistical analysis association between ESBL production and NFGNB isolates was significant. (p=0.003)

Pattern of MBL production among the NFGNB isolates

Out of 87 (28.6%) screen positive for MBL, 65(21.4%) were tested positive for confirmed MBL production. Seventeen (8.7%) of the total *P. aeruginosa* isolates and 48(44%) of the total *Acinetobacter* spp. isolated were MBL

producers. On statistical analysis association between MBL production and NFGNB isolates was significant. (p=0.01) Among the 158 ESBL producers 45 were also MBL co-

producers. On statistical analysis the association between MBL production and ESBL production was found to be statically significant. (p= 0.002),

Table 1. Antibiotic susceptibility pattern of NFGNB isolates.

Antibiotics	*P. aeruginosa*			*Acinetobacter* spp		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ciprofloxacin	89(45.6)	2 (1 %)	104 (53.3 %)	3(2.8%)	0(0%)	106(97%)
Ofloxacin	90(46.2)	2 (1 %)	103 (52.8 %)	3(2.8%)	1(0.9%)	105(96.3%)
Ceftriaxone	47(24.1)	11 (5.6 %)	137 (70.3 %)	0(0%)	1(0.9%)	108(99.1%)
Gentamycin	110(56.4)	17 (8.7 %)	68 (34.9 %)	9(8.3%)	1(0.9%)	99(90.8%)
Amikacin	125(64.1)	21 (10.8 %)	49 (25.1 %)	14(12.8%)	4(3.7%)	91(83.5%)
Chloramphenicol	56(28.7)	11 (5.6 %)	128 (65.6 %)	6(5.5%)	5(4.6%)	98(89.9%)
Meropenam	101(51.8)	4 (2.1 %)	90 (46.2 %)	9(8.3%)	19(17.4%)	81(74.3%)
Imipenam	166(85.1)	6 (3.1 %)	23 (11.8 %)	20(18.3%)	25(22.9%)	64(58.7%)
Piperacillin-Tazobactum	79(40.5)	19 (10 %)	97 (49.7 %)	4(3.7%)	2(1.8%)	103(94.5%)
Colistin (Methane Sulphonate)	139(71.3)	26 (13.3 %)	30 (15.4 %)	83(76.1%)	11(10.1%)	15(13.8%)

Discussion

Of the total 3585 specimens received, 1523(42.4%) specimens had shown growth, of which gram negative bacteria predominated with 955 (62.7%) isolates. In this study, the prevalence of NFGNB among total positive isolates was 304(19.97%) which was higher compared to 14.1% in a study conducted in India [21] and 9.32% in another study conducted in India [22]. Among these 304 NFGNB *P. aeruginosa* (64.1%) and Acinetobacter spp. (35.9%) were most commonly isolated. This result is in accordance to various international researches [23]. Malini et al in 2009 [24] reported 64.6% prevalence of *P. aeruginosa* and 25.3% prevalence of *Acinetobacter* spp. Among 304 isolates of NFGNB in our study, the prevalence was found to be highest from pus specimens, (45.7%) followed by suction tips (13.2%), tracheostomy tips (10.9%), sputum (10.2%) , urine (6.9%), catheter tip (6.9%) and CVP tip (4.9%), body fluids (1%). Majority of isolates were obtained from pus specimen. Lowest number of isolates was obtained from various body fluids and this result was similar to study done in India [25]. The variable prevalence of NFGNB in different studies might be due to local situation, arrangement of specimens in the study groups, type of institution, local use of antibiotics, sanitary conditions, the number of specimens processed and the rates of bacterial

isolation and the study period involved etc[23,24, 25]. The antibiotic sensitivity test of the isolates in this study, in overall showed higher sensitivity towards colistin, meropenem, imipenem, amikacin and gentamicin. Where as highest resistance was observed towards ciprofloxacin, ofloxacin, chloramphenicol. Hence antibiotics belonging to polymyxin, carbapenam and aminoglycosides are drug of choices for the treatment of the NFGNB infections. In a recent research [26] resistance to imipenem was observed in 40% of *A. baumannii* and 20% of *P. aeruginosa* isolates. Whereas 42.8 % [27] and 21 % [28] of *P. aeruginosa* and 14.2 % [27] and 23 % [28] of *Acinetobacter spp.* were resistant to imipenem as reported in different literature.

In our study 69.1% of the total isolates were found to be MDR. Among the total *P. aeruginosa* isolated, 53.9% were MDR and 96.4% of *Acinetobacter spp.* isolated was MDR. This result is consistent with various researches [26]. In our study most of the multi drug resistance isolates were isolated from pus sample. Aloush et al in 2006 [29] also found that the common site of isolation of MDR *P. aeruginosa* was pus (39%). The MDR phenotype in *P aeruginosa* could be mediated by several mechanisms including multidrug efflux systems, enzyme production, outer membrane protein loss and target mutations [30]. The number of multidrug resistant *P. aeruginosa* ranges from 20% to 85%

in various studies throughout the world [27-29]. Over 70% of strains of *Acinetobacter* spp. Isolated showed resistance to various antibiotics similar to other studies [31].

In our study among the total isolates 51.97% of isolates were conformed ESBL producers. 45.6% of the total *P. aeruginosa* and 63.3% of total *Acinetobacter* spp. were ESBL produces. This result is consistent with Thipperudraswamy et al in 2014 [21] in which total ESBL producers were 51.5% and 41.6% of *P aeruginosa* and 80% of *Acinetobacter* spp were ESBL producers. Various studies from India have reported prevalence of ESBL producers among *P. aeruginosa* ranging from 3.3% to 77.3% [32,33] and among *Acinetobacter* species ranging from 14.2% to 54.6% [32,33]. ESBL production in *Acinetobacter* has been found to be 46% in Turkey [34] and 54.6% in Korea [20].

In our study 21.4% of the total isolates were identified as MBL positive. Of the total *P. aeruginosa* isolates only 8.7% were MBL producers. Chaudhari et al in 2011 [25] found 5% of the total *P. aeruginosa* to be MBL producer and 44% of the total *Acinetobacter* spp. were MBL producer. In our study 58% of *Acinetobacter* spp. and 11% of *P. aeruginosa* were resistant to imipenem. In a recent research [26] resistance to Imipenem was observed in 40% of *A. baumannii* and 20% of *P. aeruginosa* isolates.Whereas 42.8 % [27] and 21 % [28] of *P. aeruginosa* and 14.2 % [27] and 23 % [28] of *Acinetobacter* spp. were resistant to imipenem as reported in literature. Varying rates of resistance to carbapenems 36.4% [20], 12.2% [27], 12% [35] was reported in literature. However 94% of *P. aeruginosa* and 100% *of Acinetobacter* spp. isolates were sensitive to imipenem in a study by Malini et al in 2009 [24]. In our study among imipenem resistant isolates, 73.9% of *P. aeruginosa* & 75.0% *Acinetobacter spp.* of showed MBL production whereas higher MBL production in *Acinetobacter spp.* (96.6%) and *P. aeruginosa* (100%) and lower MBL production in *Acinetobacter spp.* (56%) and *P. aeruginosa* (50%) as compared to our study was reported in literature [28,36]. A high percentage of MBL production (74.7%) was observed in our study consistent with reports where MBL production varied from 85.7% - 100% [36]. Majority of MBL positive isolates showed resistance to third generation cephalosporins, aminoglycosides and quinolones which is consistent with various studies [37]. Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option remains the potentially toxic polymyxin B and colistin [26].

This study showed that the *Pseudomonas* is the most predominant NFGNB followed by Acinetobacter spp. However, *Acinetobacter* showed more resistant to antibitotic in compare to *Pseudomonas*. The study also revealed that the increasing trend of MDR, ESBL and MBL production in NFGNB so constant survey of antibiotic susceptibility test and precautionary use of antibiotics is essential to control and manage spread of these isolates in different units of hospitals as well as distribution in different health institutions.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgments

References

1   Winn W Jr, Allen S, Janda W, Koneman E, Procop G and Schreckenberger P. Color Atlas and textbook of Diagnostic Microbiology, 6th edition USA: Lippincott Williams and Wilkins Company. 2006; 305–391 p.

2   Steinberg JP and Rio DC. Principles and Practice of Infectious diseases, 6th edition Vol. 2. Philadelphia, USA: Elsevier Publication. 2005;2751–2768 p.

3   Pinyo R, Prapassorn U, Pattarachai K and Nalinee A. Epidemiology of bacteremia caused by uncommon non-fermentative gram-negative bacteria. BMC Infec Dis. 2013;13:167.

4   Ramphal R. Infections due to Pseudomonas species and related organisms, Chapter 145. Harrison's Principles of Internal Medicine, 17 th Edition In. 2008.

5   Upgade A, Prabhu N, Gopi V and Soundararajan N. Current status of antibiotic resistant nonfermentative gram negative bacilli among nosocomial infections. Adv Appl Sci Res. 2012;3:738-742.

6   Lewis GJ, Fang X, Gooch M, Cook PP. Decreased resistance of Pseudomonas aeruginosa with restriction of ciproflxacin in a large teaching hospital's intensive care and intermediate care units. Infect Control Hosp Epidemiol. 2012;33: 368-73.

7   Samira A, Nour A, Jalil FM, Babak F and Hossein SK. Antibiotic Resistance Pattern and Evaluation of Metallo-Beta Lactamase Genes Including bla-IMP and bla-VIM Types in Pseudomonas aeruginosa Isolated

from Patients in Tehran Hospitals. ISRN Microbio. 2014;941507

8   Peleg AY, Seifert H and Paterson DL. Acinetobacter baumannii: Emergence of a successful pathogen. Clin Microbiol Rev. 2008;21:538-582.

9   Fournier PE, Vallenet D and Barbe V. Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS Genet. 2006;2:7.

10 Je M. Resistance in non-fermenting Gram-negative bacteria: multidrug resistance to the maximum. Am J Med. 2006;119 Suppl1:S29–36..

11 Rubin SJ, Granato PA and Wasilauskas BL. Manual of Clinical Microbiology, 4th edition Washington, D.C: American Society for Microbiology. 1985;330–349 p.

12 Gales AC, Jones RN, Forward KR, Linares J, Sader HS and Verhoef J. Emerging importance of multidrug-resistant Acinetobacter species and Stenotrophomonas maltophilia as pathogens in seriously ill patients: Geographic patterns, Epidemiological features, and trends in the SENTRY antimicrobial surveillance program (1997-1999). Clin Infect Dis. 2001;32:104–113.

13 Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D and Carmeli Y. Clinical and economic impact of bacteremia with extended spectrum β lactamase producing Enterobacter. Antimicrob Agents Chemother. 2006;50:1257–1262.

14 Boyd DA, Tyler S and Christianson S. Complete nucleotide sequence of a 92 kilobase plasmid harboring the CTX-M-15 extended-spectrum β-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother. 2004; 48: 3758-3764.

15 Morosini MI, Garcia CM and Coque TM. Antibiotic coresistance in extended spectrum-β-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline. Antimicrob Agents Chemother. 2006;50: 2695-99.

16 Bonomo RA and Szabo D. Mechanisms of multidrug resistance in Acinetobacter species and Pseudomonas aeruginosa. Clin. Infec.Dis. 2006;43:49-56.

17 Pokharel BM, Koirala J, Mishra SK, Dahal RK, Khadga P and Tuladhar NR. MDR and ESBL producing strains causing lower respiratory tract and urinary tract infections. J of Inst of Med. 2006;28:19-27.

18 Forbes BA, Sahm DF and Weissfeld AS. Bailey and Scott's diagnostic microbiology, 12th edition. Elesvier USA. 2007;514-618 p.

19 Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement ed. CLSI document M100-S22. CLSI: Wayne, PA; 2012.

20 Yong D, Shin S, Lim Y, Yum JH and Lee K. High prevalence of PER-1 extended spectrum beta lactamase producing Acinetobacter species in Korea. Antimicrobe Agents Chemother. 2003;47:1749-1751.

21 Thipperudraswamy T, Sudhindra KS, Shobha Nadigar and Mahesh Kumar. Prevalence of extended spectrum β lactamases (ESBL) in non-fermentating gram negative bacilli from clinical isolates. J Biomed and Pharma Res. 2014;3:72-79.

22 Juyal D, Prakash R, Shanakarnarayan S A, Sharma M, Negi V and Sharma N. Prevalence of non-fermenting gram negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Uttarakhand: A study from foothills of Himalayas. Saudi J for Health Sci. 2013;2: 2.

23 Wang H and Chen MJ. Nosocomial Pathogens Resistance Surveillance Study Group. Changes of antimicrobial resistance among nonfermenting gram-negative bacilli isolated from intensive care units from 1994 to 2001 in China. Zhonghua Yi Xue Za Zhi. 2003;83: 385-390.

24 Malini A, Deepa EK, Gokul BN and Prasad SR. Nonfermenting gram-negative bacilli infections in a tertiary care hospital in Kolar, Karnataka. J Lab Physicians. 2009;1: 62-66.

25 Chaudhari M S, Javadekar T B, Ninama G, Pandya1 N and Damor J. A study of metallo-beta-lactatamase producing Pseudomonas aeruginosa in clinical samples of s.s.g. hospital. 2011;1:60-66.

26 Gupta V, Chhina D and Kaur A. Incidence of metallo-β-lactamase (mbl) producing nonfermenters isolated from respiratory samples in icu patients. Int J Pharm Bio Sci. 2013;4: 580 – 585.

27 Gladstone P, Rajendran P and Brahmadathan KN. Incidence of carbapenem resistant nonfermenting gram negative bacilli from patients with respiratory infections in the intensive care units. Indian J Med Microbio. 2005;23: 189-191.

28 Hodiwala (Bhesania) A, Dhoke R and Urhekar AD. Incidence of metallo-beta-lactamase producing Pseudomonas, Acinetobacter & Enterobacterial isolates in hospitalized patients. Int J Pharm Bio Sci. 2013;3:79- 83.

29 Aloush V, Venezia SN, Igra YS, Cabili S and Carmeli Y. Multidrug resistant Pseudomoans aeruginosa: risk factors and clinical impact. Antimicrob Agents Chemother. 2006;50: 43-48.

30 Hirsch EB and Tam VH. Impact of multidrug-resistant Pseudomonas aeruginosa infectionon patient outcomes. Exert Rev Pharmacoecon Outcomes Res. 2010;10: 441-451.

31 Benachinmardi KK, Padmavathy M, Malini J and Naveneeth BV. Prevalence of non-fermenting Gram-negative bacilli and their in vitro susceptibility pattern at a tertiary care teaching hospital. J Sci Society. 2014;41: 162-166.

32 Aggarwal R, Chaudhary U and Bala K. Detection of extended-spectrum β-lactamase in Pseudomonas aeruginosa. Indian J PatholMicrobiol. 2008;51: 222-224.

33 Singhal S, Mathur T, Khans, Upadhyay DJ, Chugh, S, Gaind R and Rattan A. Evaluation of methods for AmpC β lactamase in gram negative clinical isolates from tertiary care hospitals. Ind J Med Microbiol. 2005;23:120-124.

34 Dora Szabó, Julia Szentandrássy, ZsuzsaJuhász, KatalinKatona, Károly Nagy and LászlóRókusz. Imported PER-1 producing Pseudomonas aeruginosa, PER-1 producing Acinetobacterbaumanii and VIM-2-producing Pseudomonas aeruginosa strains in Hungary. Annals of Cli Microbio and Antimicrob. 2008;7: 1-5.

35 Navaneeth BV, Sridaran D, Sahay D and Belwadi MR. A preliminary study on metallo-beta-lactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian J Med Res. 2002;116: 264- 267.

36 Irfan S, Zafar A, Guhar D, Ahsan T and Hasan R. Metallo-β-lactamase producing clinical isolates of Acinetobacter species and Pseudomonas aeruginosa from intensive care unit patients of a tertiary care hospital. Indian J Med Microbiol. 2008;26: 243-245.

37 Pitout JD, Gregson DB, Poirel L, McClure JA, Le P and Church DL. Detection of Pseudomonas aeruginosa producing metallo-β-lactamases in a large centralized laboratory. J Clin Microbiol. 2005;43:3129- 3135.