ORIGINAL RESEARCH PAPER

Volume-8 | Issue-2 | February-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

bla_{NDM-1} GENE CARRIAGE IN THE ICU OF TERTIARY CARE HOSPITAL IN NORTH INDIA

Microbiology

Dr. Anuj Sud* MD, Senior Resident, Department of Microbiology, Lok Nayak Hospital and Maulana Azad Medical College, New Delhi-110002, India *Corresponding Author

Dr. Swati Sharma MD, Senior Resident, Department of Microbiology, Janakpuri Superspeciality Hospital, Janakpuri, Delhi, India

Dr. Beena Uppal MD, Previous Director Professor and HOD, Department of Microbiology, Maulana Azad Medical College, New Delhi-110002, India

ABSTRACT

BACKGROUND:- New Delhi metallo-β-lactamase 1 (NDM-1) enzyme leads to multidrug resistance and has been detected from bacteria in many countries. The study was done To detect bacteria carrying blaNDM-1 gene from intensive care unit (ICU) patients and correlate the change with increasing duration of ICU stay.

METHODS:- Blood and urine samples were collected from 140 patients thrice- 0-2 days, 3-7 days and 7 days of ICU admission. All bacterial isolates resistant to meropenem were evaluated for Metallo-β-lactamase (MBL) production. blaNDM-1 gene was detected using Real Time PCR from the MBL producers.

RESULT:- Overall, 458 samples (229 each) of blood and urine were collected and 75 gram negative bacteria were isolated. From these, 46.7% (35/75) strains were found to be carbapenem resistant and blaNDM-1 gene was detected in 17.3% (13/75).

CONCLUSION:- High prevalence of bacteria carrying blaNDM-1 gene was seen in ICU patients increasing the burden on healthcare.

KEYWORDS

Carbapenem resistance, Metallo-β-lactamase producers, NDM-1 producers, blaNDM-1 gene, ICU infections

1. INTRODUCTION

Nosocomial infections are common in the Intensive care unit (ICU) due to various factors like high antibiotic use, invasive procedures, prolonged hospital stay, critically ill patients, central venous catheterization, mechanical ventilation, tracheostomy [1-3]. Nosocomial infections like urinary tract infections, ventilator associated pneumonia, catheter site infection, bacteremia are commonly seen in the ICU and the causative agents frequently encountered include *Acinetobacter spp, Pseudomonas aeruginosa, Klebsiella spp, Staphylococcus aureus, Escherichia coli* etc. [1,4]. Infections with carbapenem resistant organisms like *Acinetobacter* can lead to increased mortality [5].

Different mechanisms by which bacteria become resistant to antimicrobials include reduced uptake, efflux pumps, drug inactivating enzymes like β-lactamases etc. New Delhi Metallo-β-lactamase (NDM-1) is one such enzyme, which was first identified in *E. coli* in 2008 from the urine of a 59-year old Swedish patient with type II diabetes who had a history of stroke and was admitted to a hospital in India in 2007 [6]. It is placed in class B of Ambler classification as it carries zinc at the active site, and the gene bla_{NDM-1} is located on the plasmid enabling it to spread through horizontal transfer. Bacteria carrying this gene have been reported from most parts of the world including United States of America, Canada, Africa, several European countries, New Zealand etc [7-13].

Overpopulation, lack of basic sanitation and access to clean water, tropical climate, rampant over-the-counter antibiotic usage can lead to rapid dissemination of multidrug resistant organisms. Further, the spread of these resistant microorganisms is going to harm the health care system by increasing the days of hospitalisation, thus increasing the cost of treatment and eventually leading to an increase in mortality rate. The success of treatments like organ transplantation, cancer chemotherapy and other major surgeries is compromised [14].

The study was designed to document the magnitude of carbapenem resistance, production of MBL and NDM-1, in routine clinical isolates of ICU patients. Follow up of the patients was also done to correlate the change with increasing duration of ICU stay.

2. MATERIALS AND METHODS

The study was conducted at a tertiary care centre in New Delhi, India through co-ordination between the Department of Microbiology and Department of Anaesthesia. The study was approved by Institutional Ethical Committee. One hundred and forty adult patients (above 18 years of age) admitted to the ICU were included in the study, after taking informed consent from the guardian.

Blood and urine samples were collected using strict aseptic technique within the first two days of ICU admission and further samplings were done on 3-7 days and 7 days of admission. The blood samples were inoculated in brain heart infusion broth such that the ratio of blood:broth was 1:5 and subcultures on 5% sheep blood agar and MacConkey's agar were done after 24 hours and 5 days. The urine samples were inoculated onto 5% sheep blood agar and MacConkey's agar within one hour of collection. The plates were incubated at 37°C for 18-24 hrs and bacterial growths were subjected to preliminary tests like Gram staining, hanging drop for motility, catalase and oxidase tests. Their identity was established using biochemical tests such as fermentation of sugars, indole test, citrate utilization test, urease production test, production of H_2S on TSI, etc.

2.1 SCREENING OF ISOLATES FOR CARBAPENEM RESISTANCE

The isolates were screened for carbapenem resistance using the initial screening test recommended by the CLSI guidelines. A 0.5 McFarland bacterial suspension was prepared and Lawn culture was done on Mueller Hinton agar petri plate; meropenem disc (10µg) was then placed using sterile method in the centre of inoculum. A strain was tentatively labelled as a carbapenem resistant when the zone diameter was ≤19 mm [15].

2.2 PHENOTYPIC CONFIRMATORY TEST FOR PRODUCTION OF METALLO-β-LACTAMASES (MBL):-

All isolates with positive screening test were evaluated for MBL production using combination of Imipenem and Imipenem-EDTA discs by Double disc synergy test. Another lawn culture of the suspected strain was prepared on Mueller Hinton agar plate using a sterile cotton tipped swab and allowed to dry for 3-5 min. Two discs of Imipenem (10µg each) were placed 25 mm apart. 10 µl of 0.1M anhydrous EDTA (292 µg) was added to one of the imipenem disc using a sterile micropipette. Incubation was done at 37°C for 18-24 hours in ambient air. A strain showing difference of ≥7 mm between the zone around imipenem and imipenem plus EDTA discs was considered positive for MBL production [16].

2.3 DETECTION OF bla_{NDM-1} GENE USING REAL TIME PCR TECHNIQUE

Strains showing positive result in the double disc diffusion technique were further tested by real-time PCR to detect the presence of bla_{NDM-1} gene. DNA was extracted from the isolates using Magnapure Compact and preserved at -70°C till further testing. Real Time PCR using SYBR green dye was carried out to amplify and detect bla_{NDM-1} gene, if present. Following primer sequences were used:

Primer NDM-1S: 5'-GCTGGCGGTGGTGACTC
Primer NDM-1A: 5'-GGCAAGCTGGTTCGACAAC

The reporter is the double-strand DNA-specific dye SYBR Green dye which binds double-stranded DNA at the minor groove. The resulting DNA-dye-complex absorbs blue light (λ_{max} = 488 nm) and emits green light (λ_{max}=522 nm). Thus, as a PCR product accumulates, fluorescence increases. Fluorescence measurement at the end of the elongation step of every PCR cycle is performed to monitor the increasing amount of amplified DNA. Melting curve analysis was also done to detect any non specific amplifications.

3. RESULTS

Out of the 140 patients recruited for the study, 60.7% (85/140) were males and 39.3% (55/140) were females. Maximum patients were in the younger age group with 34.3% (48/140) patients between 21 and 30 years, followed by 17.9% (25/140) in the 31 to 40 years age group. Twenty six patients (18.6%) had fever during ICU stay and total leukocyte count was found to be raised in 61 patients (43.6%). Half of the patients (70/140) expired while the remaining half were transferred to respective wards. (Table 1)

One hundred and forty (140) blood and urine samples each were collected during the first two days of ICU stay, while 63 samples each were collected during the next sampling (3-7 days). In the last sampling (>7 days), 26 samples each of blood and urine were collected. The no. of patients on follow up was less as some patients were transferred to the respective wards while some expired in the ICU. Thus a total of 458 specimens were collected and significant growth was obtained in 26.9% (123/458) samples which included gram negative bacteria, gram positive bacteria and budding yeast cells.

From the 229 blood samples, 43 gram negative bacteria were isolated, out of which *Klebsiella pneumoniae* was the most common bacteria followed by *Escherichia coli.* Other gram negative bacteria recovered were *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Proteus mirabilis.* From the 43 strains isolated, 18 were found to be resistant to meropenem. Majority of the carbapenem resistant strains belonged to *Klebsiella pneumoniae* (08/18) while all the *Acinetobacter baumannii* strains obtained were found to be resistant to meropenem. (Table 2)

TABLE 1:- DEMOGRAPHIC PROFILE AND CLINICAL CHARACTERISTICS OF THE PATIENTS

		Fever (n=26)	TLC Raised (n=61)	Culture Positive (n=59)	Pts. Expired (n=70)
18-20 yr (n=15)	M (n=7)	0	1 (14)	0	2 (29)
	F (n=8)	2 (25)	6 (75)	6 (75)	3 (37)
21-30 yr (n=48)	M (n=28)	3 (11)	10 (36)	8 (29)	11 (39)
	F(n=20)	3 (15)	7 (35)	8 (40)	8 (40)
31-40 yrs (n=25)	M (n=17)	5 (29)	9 (53)	11 (65)	8 (47)
	F (n=8)	0	4 (50)	6 (75)	6 (75)
41-50 yrs (n=14)	M (n=6)	1 (17)	5 (83)	2 (33)	3 (50)
	F (n=8)	2 (25)	2 (25)	3 (37)	5 (62)
51-60 yrs (n=18)	M (n=15)	3 (20)	6 (40)	6 (40)	10 (67)
	F (n=3)	2 (67)	2 (67)	2 (67)	1 (33)
>60 yrs (n=20)	M (n=12)	4 (33)	7 (58)	6 (50)	9(75)
	F (n=8)	1 (12)	2 (25)	1 (12)	4 (50)

*Figures in paranthesis represent percentage; M=Male; F=Female

The carbapenem resistant bacteria were further tested for MBL production, and 44.4% (08/18) of these turned out to be positive. *Acinetobacter baumannii* strains comprised 50% of the MBL producers. Real time PCR showed that 75% (06/08) MBL producing bacteria were carrying blaNDM-1 gene; among which *Acinetobacter* and *Klebsiella* accounted for 50% (03/06) and 33.3% (02/06) respectively.

TABLE 2:- CARBAPENEM RESISTANCE, METALLO-β-LACTAMASE AND NDM-1 PRODUCERS IN BLOOD SAMPLES

		E.coli	Klebsiella pneumoniae	Acinetobacter spp	Ps. aeruginosa	Proteus mirabilis	Total
0-2 days	No.	12	08	02	05	01	28
	CR	03 (25)	03 (37)	02 (100)	02 (40)	00	10 (36)
	MBL	01 (33)	00	02 (100)	01 (50)	00	04 (40)
	NDM-1	01 (100)	00	01 (50)	00	00	02 (50)
3-7 days	No.	01	05	01	02	01	10
	CR	01 (100)	03 (60)	01 (100)	00	00	05 (50)
	MBL	00	01 (33)	01 (100)	00	00	02 (40)
	NDM-1	00	01 (100)	01 (100)	00	00	02 (100)
>7 days	No.	01	03	01	00	00	05
	CR	00	02 (67)	01 (100)	00	00	03 (60)
	MBL	00	01 (50)	01 (100)	00	00	02 (67)
	NDM-1	00	01 (100)	01 (100)	00	00	02 (100)
Total	No.	14	16	04	07	02	43
	CR	04 (29)	08 (50)	04 (100)	02 (29)	00	18 (42)
	MBL	01 (25)	02 (25)	04 (100)	01 (50)	00	08 (44)
	NDM-1	01 (100)	02 (100)	03 (75)	00	00	06 (75)

From the 229 urine samples, 32 gram negative bacteria were obtained with followed by *Pseudomonas aeruginosa* (25% or 08/32). From them, 17 strains were carbapenem resistant and 10 of these were producing MBL. 80% (4/5) of the *Acinetobacter* strains recovered were found to be carbapenem resistant while 75% (3/4) of them were found to be producing MBL. Production of NDM-1 was seen in 70% (7/10) of the MBL producers with *Escherichia coli* and *Acinetobacter spp* accounting for the majority. A single strain of *Enterobacter cloacae* was recovered during the second urine sampling (3-7 days) and it was found to be producing NDM-1 (Table 3).

Thus, a total of 75 gram negative bacteria were obtained in the study with *Escherichia coli* and *Klebsiella spp* accounting for the majority. Carbapenem resistance was seen in 35 isolates with 18 of them turning out to be MBL producers. The bla_{NDM-1} gene was detected in 72% of the MBL producers. The proportion of bacteria which were carbapenem resistant, producing MBL and carrying the bla_{NDM-1} gene was predominantly seen to increase with increase in duration of ICU stay. (Table 4)

TABLE 3:- CARBAPENEM RESISTANT, METALLO-β-LACTAMASE AND NDM-1 PRODUCERS IN URINE SAMPLE

		E.coli	Klebsiella pneumoniae	Acinetobacter spp	Ps. aeruginosa	En. cloacae	Total
0-2 days	No.	05	05	02	05	00	17
	CR	01 (20)	02 (40)	01 (50)	03 (60)	00	07 (41)
	MBL	01 (100)	01 (50)	00	01 (33)	00	03 (43)
	NDM-1	01 (100)	00	00	00	00	01 (33)
3-7 days	No.	03	00	01	02	01	07
	CR	01 (33)	00	01 (100)	01 (50)	01 (100)	04 (57)
	MBL	00	00	01 (100)	01 (100)	01 (100)	03 (75)
	NDM-1	00	00	01 (100)	00	01 (100)	02 (67)

>7 days	No.	04	01	02	01	00	08
	CR	03 (75)	01 (100)	02 (100)	00	00	06 (75)
	MBL	02 (67)	00	02 (100)	00	00	04 (67)
	NDM-1	02 (100)	00	02 (100)	00	00	04 (100)
Total	No.	12	06	05	08	01	32
	CR	05 (42)	03 (50)	04 (80)	04 (50)	01 (100)	17 (53)
	MBL	03 (60)	01 (33)	03 (75)	02 (50)	01 (100)	10 (59)
	NDM-1	03 (100)	00	03 (100)	00	01 (100)	07 (70)

TABLE 4:- CARBAPENEM RESISTANT, METALLO-β-LACTAMASE AND NDM-1 PRODUCING BACTERIA RECOVERED IN THE STUDY

		E. coli	*Klebsiella pneumoniae*	*Acinetobacter spp*	*Ps. aeruginosa*	*Proteus mirabilis*	*E. cloacae*	Total
0-2 days	No.	17	13	04	10	01	00	45
	CR	04 (23)	05 (38)	03 (75)	05 (50)	00	00	17 (38)
	MBL	02 (50)	01 (20)	02 (67)	02 (40)	00	00	07 (41)
	NDM-1	02 (100)	00	01 (50)	00	00	00	03 (43)
3-7 days	No.	04	05	02	04	01	01	17
	CR	02 (50)	03 (60)	02 (100)	01 (25)	00	01 (100)	09 (53)
	MBL	00	01 (33)	02 (100)	01 (100)	00	01 (100)	05 (56)
	NDM-1	00	01 (100)	02 (100)	00	00	01 (100)	04 (80)
>7 days	No.	05	04	03	01	00	00	13
	CR	03 (60)	03 (75)	03 (100)	00	00	00	09 (69)
	MBL	02 (67)	01 (33)	03 (100)	00	00	00	06 (67)
	NDM-1	02 (100)	01 (100)	03 (100)	00	00	00	06 (100)
Total	No.	26	22	09	15	02	01	75
	CR	09 (35)	11 (50)	08 (89)	06 (40)	00	01 (100)	35 (47)
	MBL	04 (44)	03 (27)	07 (87)	03 (50)	00	01 (100)	18 (51)
	NDM-1	04 (100)	02 (67)	06 (86)	00	00	01 (100)	13 (72)

*Figures in paranthesis represent percentage; No.- Number isolated; CR- Carbapenem resistance; MBL- Metallo-β-lactamase producers; NDM-1- Bacteria carrying bla_{NDM-1} gene

4. DISCUSSION

Infection with multidrug resistant organisms can lead to adverse outcomes especially in critically ill patients admitted in the ICU. Walsh et al found the presence of bacteria carrying bla_{NDM-1} gene in the drinking water and sewage samples in Delhi suggesting that the problem of these multidrug resistant bacteria was not just limited to the hospitals [17]. The present study was done to find out the prevalence of infection with bacteria carrying bla_{NDM-1} gene in patients who were recently admitted to the ICU.

A total of 140 patients were included in the study and 18.6% (26/140) of them had fever while 43.6% (61/140) had raised TLC count. From them, 458 blood and urine samples were collected and 26.9% (123/458) of them showed significant growth with gram negative bacteria being isolated in most cases (75/123 or 61%). Most common gram negative bacteria recovered from blood samples was *Klebsiella spp* which was also reported in the study by Wattal C et al [18]. Other predominant gram negative bacteria isolated were *E. coli* and *Pseudomonas* similar to other studies [1, 4].

Infections in the ICU become even more alarming due to the presence of multidrug resistant organisms in the hospital environment especially in the ICU leading to increased hospital stay [3,4]. Further, the higher occurence rates for most antimicrobial resistant pathogens in ICU compared to non ICU patients has been confirmed in the studies conducted at The National Healthcare Safety Network [19]. In our study, 46.7% (35/75) of the strains were found to be carbapenem resistant. Among all the bacteria obtained in our study, maximum carbapenem resistance was seen in *Klebsiella* (11/35 or 31.4%) followed by *Escherichia coli* and *Acinetobacter spp* comprising 25.7% (09/35) and 22.9% (08/35) of the carbapenem resistant bacteria respectively. However, Wattal C et al found much higher resistance to meropenem in *Klebsiella*, *Acinetobacter* and *Pseudomonas* isolates [18].

Metallo-β-lactamases are zinc dependent β-lactamases including VIM, IMP, NDM-1 etc. and their presence in various bacterial strains constitutes a serious threat to β-lactam therapy leading to treatment failure. In our study, 51.4% (18/35) of the carbapenem resistant strains were found to be producing MBL, the major contributor being *Acinetobacter* accounting for 38.9% (07/18) of the MBL producing strains. Studies have shown high incidence of MBL producers from the carbapenem resistant strains of *Acinetobacter* with studies reporting it to be >70% and in our study it was 87.5% i.e. 7/8 [20,21]. We found that 24% (18/75) of the gram negative bacteria isolated were producing MBL somewhat similar to that shown in a study in Mysore, India where 18% MBL producers were obtained from the 100 Enterobacteriaceae strains isolated from urine, blood, sputum and exudates [22]. Multiple risk factors like IV line, previous antibiotic use, prolonged hospital stay, catheterisation, mechanical ventilation can lead to infection with bacteria producing MBL as observed by Kumar SH et al. Thus, the chances of multidrug resistant infections in the patients in ICU are even more [23].

The MBL producing strains were evaluated for carriage of bla_{NDM-1} geneand 13 isolates were found positive for NDM-1 production. Out of the 7 *Acinetobacter* isolates which were found to be producing MBL, 6 showed the production of the enzyme NDM-1 by real time PCR. Studies have shown wide variation in the prevalence of bla_{NDM-1} gene in bacteria producing MBL with a study in Pune, India reporting it 32% (20/62) to another study in Varanasi, India reporting it as 84.4% (54/64) whereas it was 72.2% (13/18) in our study [24,25]. The proportion of carbapenem resistant bacteria, producers of MBL and NDM-1 were predominantly seen to increase with ICU stay. However, a study on a larger scale needs to be done to confirm this finding as the number of bacteria recovered on subsequent samplings were less due to loss of patients on follow up.

Since NDM-1 producing organisms frequently carry other resistance enzymes including ESBL, AmpC β-lactamases, the treatment options are quite limited with only tigecycline and polymyxins (colistin and polymyxin B) showing considerable efficacy [26-28]. However, colistin can cause renal toxicity and tigecycline is not much useful in systemic and urinary tract infections thus the treatment options are very much limited and the best option that remains is preventing the spread of these multidrug resistant bacteria by antimicrobial stewardship and infection control practices. [26,27]

2. CONCLUSION

High prevalence of carbapenem resistance and bacteria carrying bla_{NDM-1} gene was found which is quite significant since the bla_{NDM-1} gene makes the bacteria resistant to most antibiotics including carbapenems leaving few treatment options while increasing the duration of hospitalisation, the cost of treatment and may ultimately lead to an increase in the mortality rate. Providing clean water and basic sanitation, adherence to standard precautions including hand hygiene, strict infection control practices, antimicrobial stewardship are some of the measures which can help reduce the burden on healthcare due to these multi drug resistant pathogens.

REFERENCES

1) Ak O, Batirel A, Ozer S, Çolakoğlu S. Nosocomial infections and risk factors in the

intensive care unit of a teaching and research hospital: a prospective cohort study. Med Sci Monit. 2011 May;17(5):PH29-34.
2) Fournier PE, Richet H. The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin Infect Dis. 2006 Mar 1;42(5):692-9.
3) Playford EG, Craig JC, Iredell JR. Carbapenem-resistant Acinetobacter baumannii in intensive care unit patients: risk factors for acquisition, infection and their consequences. J Hosp Infect. 2007 Mar;65(3):204-11.
4) Pradhan NP, Bhat SM, Ghadage DP. Nosocomial infections in the medical ICU: a retrospective study highlighting their prevalence, microbiological profile and impact on ICU stay and mortality. J Assoc Physicians India. 2014 Oct;62(10):18-21.
5) Wareham DW, Bean DC, Khanna P, Hennessy EM, Krahe D, Ely A, et al. Bloodstream infection due to Acinetobacter spp: epidemiology, risk factors and impact of multi-drug resistance. Eur J Clin Microbiol Infect Dis. 2008 Jul;27(7):607-12.
6) Yong D, Toleman MA, Giske CG , Cho HS , Sundman K , Lee K et al. Characterization of a new metallo-beta-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 2009 Dec;53(12):5046-54.
7) Pasteran F, Albornoz E, Faccone D, Gomez S, Valenzuela C, Morales M et al. Emergence of NDM-1-producing Klebsiella pneumoniae in Guatemala. J Antimicrob Chemother. 2012 Jul;67(7):1795-7.
8) Pannaraj PS, Bard JD, Cerini C, Weissman SJ. Pediatric carbapenem-resistant Enterobacteriaceae in Los Angeles, California, a high-prevalence region in the United States. Pediatr Infect Dis J. 2015 Jan;34(1):11-6.
9) Kus JV, Tadros M, Simor A, Low DE, McGeer AJ, Willey BM et al. New Delhi metallo-β-lactamase-1: local acquisition in Ontario, Canada, and challenges in detection. CMAJ 2011 Aug 9;183(11):1257-61.
10) Gaibani P, Ambretti S, Berlingeri A, Cordovana M, Farruggia P, Panico M et al. Outbreak of NDM-1-producing Enterobacteriaceae in northern Italy, July to August 2011. Euro Surveill. 2011 Nov 24;16(47):20027.
11) Zarfel G, Hoenigl M, Leitner E, Salzer HJ, Feierl G, Masoud L et al. Emergence of New Delhi metallo-β-lactamase, Austria. Emerg Infect Dis. 2011 Jan;17(1):129-30.
12) Revathi G, Siu LK, Lu PL, Huang LY. First report of NDM-1-producing Acinetobacter baumannii in East Africa. Int J Infect Dis. 2013 Dec;17(12): e1255-8.
13) Williamson DA, Sidjabat HE, Freeman JT, Roberts SA, Silvey A, Woodhouse R et al. Identification and Molecular characterization of New Delhi Metallo beta lactamase-1 (NDM-1) and NDM-6 producing Enterobacteriaceae from New Zealand Hospitals. Int. J. Antimicrob. Agents 2012 Jun; 39(6):529-33.
14) Karczewski M, Tomczak H, Piechocka-Idasiak I, Cichanska L, Adamska Z, Stronka M. Is multiresistant Klebsiella pneumoniae New Delhi metallo-beta-lactamase (NDM-1) a new threat for kidney transplant recipients? Transplant Proc. 2014 Sep;46(7):2409-10.
15) Clinical and Laboratory Standards Institute (CLSI) document M100-S22-Performance Standard for Antimicrobial Susceptibility Testing; Approved Standards: Jan 2012;32(3).
16) Shahcheraghi F, Nikbin VS, Feizabadi M. Identification and genetic characterization of metallo-beta-lactamase producing strains of Pseudomonas aeruginosa in Tehran, Iran. New Mirobiologica 2010; 33: 243–8.
17) Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health; an environmental point prevalence study. Lancet Infect Dis. 2011 May;11(5);355-62.
18) Wattal C, Raveendran R, Goel N, Oberoi JK, Rao BK. Ecology of bloodstream infection and antibiotic resistance in intensive care unit at a tertiary care hospital in north India. Braz J Infect Dis. 2014 May-Jun;18(3):245-51.
19) Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA et al: NHSN annual update: Antimicrobial resistant pathogens associated with healthcare associated infections: Annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol 2008; 29:996–1011.
20) Kabbaj H, Seffar M, Belefquih B, Akka D, Handor N, Amor M et al. Prevalence of Metallo-β-Lactamases Producing Acinetobacter baumannii in a Moroccan Hospital. ISRN Infectious Diseases, vol. 2013, Article ID 154921, 3 pages, 2013. doi:10.5402/2013/154921.
21) Eser OK, Ergin A, Hasçelik G. Antimicrobial resistance and existence of metallo-beta-lactamase in Acinetobacter species isolated from adult patient. Mikrobiyol Bul. 2009 Jul;43(3):383-90.
22) Wadekar MD, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int Jour Curr Res Aca Rev: 2013 1(3):p89-95.
23) Kumar SH, De AS, Baveja SM, Gore MA. Prevalence and Risk Factors of Metallo β-lactamase Producing Pseudomonas aeruginosa and Acinetobacter species in Burns and Surgical Wards in a Tertiary Care Hospital. J Lab Physicians 2012 Jan-Jun;4(1):39-42.
24) Bharadwaj R, Joshi S, Dohe V, Gaikwad V, Kulkarni G, Shouche Y. Prevalence of New Delhi metallo-β-lactamase (NDM-1)-positive bacteria in a tertiary care centre in Pune, India. Int J Antimicrob Agents. 2012 Mar;39(3):265-6.
25) Seema K, Sen MR, Upadhyay S, Bhattacharjee A. Dissemination of the New Delhi metallo-β-lactamase-1 (NDM-1) among Enterobacteriaceae in a tertiary referral hospital in north India. J Antimicrob Chemother. 2011 Jul;66(7):1646-7.
26) Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. Int J Antimicrob Agents. 2011;37:415–9.
27) Albur M, Noel A, Bowker K, MacGowan A. Bactericidal activity of multiple combinations of tigecycline and colistin against NDM-1-Producing Enterobacteriaceae. Antimicrob Agents Chemother.2012;56:3441-3.
28) Chien JMF, Koh TH, Chan KS, Chuah THC, Tan TT. Successful treatment of NDM-1 Klebsiella pneumoniae bacteraemia in a neutropenic patient. Scand J Infect Dis. 2012;44:312–4.