# Detection of Metallo-Beta-Lactamase among *Pseudomonas aeruginosa* Isolated from Wound Infections in Two Tertiary Care Hospitals of Bangladesh

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**Abstract**

**Background:** Metallo-Beta-Lactamase (MBL) enzyme is one of the causes of resistance among *Pseudomonas* aeruginosa against Carbapenem drug which is a broad spectrum antibiotic and is the drug of choice to treat severe infections. The rapid spread of MBL producing *Pseudomonas aeruginosa* represent a matter of great concern worldwide. The study was undertaken to determine MBL producing *Pseudomonas aeruginosa* by phenotypic method.

**Mmethods:** A cross sectional analytical study was conducted from January, 2016 to December, 2016 among the patients with wound infections cases attending the in-patient department of Surgery and Burn unit of Sir Salimullah Medical College and Mitford Hospital and also Burn and Plastic Surgery unit of Dhaka Medical College Hospital. Total 288 wound infection cases were taken purposively. *Pseudomonas aeruginosa* were isolated and subjected to antimicrobial susceptibility test by Kirby-Bauer Disc Diffusion method. The isolates showing resistance to imipenem were subjected to Imipenem-EDTA combined disc diffusion test for phenotypic detection of MBL.

**Result:** Culture of 288 wound swab yielded 229 (79.51%) organisms. Out of 229 isolated bacteria, Gram negative bacteria were 216 (94.32%) and Gram positive bacteria were 13 (5.68%). Out of 216 Gram negative isolates, *Pseudomonas aeruginosa* 92 (42.59%). In the present study the isolates of *Pseudomonas aeruginosa* showed a pattern of resistance to Ciprofloxacin and Levofloxacin 75.00%, Gentamicin 72.83%, Amikacin 71.74%, Ceftazidime 70.65%, Cefepime 65.22%, Aztreonam 64.13%, Imipenem and Meropenem 38.04%, Piperacillin-tazobactam 28.26%, Colistin 4.35%. None of the isolates were resistant to Polymyxin B. Among 92 *Pseudomonas aeruginosa*, 35 (38.04%) were Imipenem resistant strains and 27 (29.35%) were MBL producing stains detected by Imipenem-EDTA combined disc diffusion test. MBL producing *Pseudomonas aeruginosa* were showed sensitivity to colistin 23 (85.18%) and to polymyxin 27 (100%).

**Conclusion:** The study indicates that continuous supervision of MBL producing *Pseudomonas aeruginosa* is necessary in our country for effective treatment purpose as well as for control of infection.

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**Keywords**: *Pseudomonas aeruginosa*, MBL, Imipenem-EDTA combined disc diffusion test.

## Introduction

*Pseudomonas aeruginosa* is a Gram negative bacillus of clinical significance and the reason of severe infections in patients with diseases including major surgery, burn, diabetes.[1] Carbapenems are drug of choice for severe infections with *Pseudomonas aeruginosa* because of their broad spectrum activity.[2] However, the scenario has changed with the entry of carbapenem resistant *Pseudomonas aeruginosa*.[3] Decreased outer membrane permeability, increased efflux system, alteration of penicillin binding proteins and production of carbapenem hydrolyzing enzymes such as metallo-beta-lactamase, serine carbapenemase are the causes of resistance of *Pseudomonas aeruginosa* against carbapenem antibiotic.[4] Production of Metallo-Beta-Lactamase enzyme is one of the causes of resistance against Carbapenem antibiotic in *Pseudomonas aeruginosa*.

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MBLs are carbapenemases belong to the molecular class B of Ambler's scheme that correspond group 3 under Bush-Jacoby-Medeiros classification scheme.[5,6] MBLs are able to hydrolyze all Beta-Lactam drugs as well as carbapenems except monobactams.[7,8] They require zinc ($Zn^{2+}$) as cofactor to degrade Beta-Lactam drugs and are inhibited by metal chelators such as ethylene-diamine-tetra-acetic acid (EDTA); 0-1, 10-phenanthroline; dipicolinic acid and mercaptopropionic acid (MPA).[9] MBL producing *Pseudomonas aeruginosa* was first reported from Japan in 1991[10] and since then it has been detected from different parts of the world such as Asia,[11] Europe,[12] Australia,[13] South America,[14] North America.[15] Several phenotypic methods are available for the detection of MBL production. All these methods are based on the ability of metal chelators such as EDTA and thiol based compounds to inhibit the MBL activity.

The present study is designed to identify *Pseudomonas aeruginosa* from wound swabs and to detect the presence of MBL producing *Pseudomonas aeruginosa* phenotypically by Imipenem-EDTA combined disc diffusion test.

## Methods

*Sample size*

A cross sectional analytic study was carried out in the department of Microbiology in Sir Salimullah Medical College and Mitford Hospital during the period of January, 2016 to December, 2016. The research protocol was approved by Ethical Review Committee of Sir Salimullah Medical College and Mitford Hospital. Total 288 wound infection cases attending the in-patient department of Surgery and Burn unit of Sir Salimullah Medical College and Mitford Hospital and also Burn and Plastic Surgery unit of Dhaka Medical College and Hospital were enrolled for this study irrespective of age, sex and antibiotic use. Among 288 samples, 174 were burn wound swabs, 78 samples were surgical wound swabs and 36 were diabetic wound swabs. Clean surgical wounds without sign of infection were excluded for this study.

*Sample collection*

Wound swab samples were collected by sterile cotton tipped swab moistened with sterile saline. During collection, the swab was taken by zigzag pattern by rotating the swab stick across the wound without touching the surrounding wound edge or skin. The specimens were immediately kept in a sterile test tube, capped properly and labelled. Then swabs were transferred to microbiology lab without any delay.

*Isolation and identification of Pseudomonas aeruginosa*

All wound swabs were inoculated onto Blood agar media and MacConkey agar media and were incubated aerobically at 37°C for 18-24 hours. Pale colonies on MacConkey agar media, Gram-negative bacilli on Gram staining, pink-red slope and butt without production of gas and $H_2S$ on KIA media, motile, negative urease and indole on MIU media, positive citrate and oxidase test were identified as *Pseudomonas* spp.[16] Suspected *Pseudomonas* spp. were subcultured on Cetrimide agar media and incubated at 37°C for 18-24 hours for confirmation of *Pseudomonas aeruginosa*. Cetrimide agar media is a selective media for *Pseudomonas aeruginosa*.[17]

*Antimicrobial susceptibility test*

Antimicrobial susceptibility test was done by Kirby-Bauer modified disc diffusion technique according to the CLSI guidelines.[18] *Pseudomonas aeruginosa* ATCC 27853 was used as negative control. The antibiotics tested were Gentamicin (10µg/disc), Amikacin (30µg/disc), Ceftazidime (30µg/disc), Cefepime (30µg/disc),

Piperacillin-tazobactam (100/10µg/disc), Aztreonam (30µg/disc), Ciprofloxacin (5µg/disc), Levofloxacin (5µg/disc), Imipenem (10µg/disc), Meropenem (10µg/disc), Colistin (10µg/disc) and Polymyxin B (300 units/disc).

*Detection of MBL by Imipenem-EDTA combined disc diffusion test*

Imipenem resistant *Pseudomonas aeruginosa* were subjected to Imipenem-EDTA combined disc diffusion test for detection of MBL production phenotypically.[19] 3-5 isolated colonies of *Pseudomonas aeruginosa* were taken from MacConkey agar media by the help of sterile wire loop and then emulsified with 3-4 ml of sterile normal saline to achieve a turbidity equivalent to a 0.5 McFarland standard. A Mueller-Hinton agar plate was inoculated as per as standard procedure. Two imipenem (10 µg/disc) discs were placed on the plate at the distance of 25 mm from center to center. 10 µl of 0.5 M EDTA solution was added to one of them by the help of micropipette tips. The inhibition zones of the imipenem and imipenem-EDTA discs were observed after overnight incubation at 37°C. A ≥ 7 mm increase in zone diameter of imipenem-EDTA versus the zone diameter of the imipenem alone indicate the presence of MBL production. (Figure 4).

**Results**

Culture of 288 wound swab yielded 229 (79.51%) organisms. Among 229 isolates, 149 were from burn wound, 46 were from surgical wound and 34 were isolated from diabetic wound (Table I). Out of 229 isolated bacteria, Gram negative bacteria were 216 (94.32%) and Gram positive bacteria were 13 (5.68%) (Figure1). Among 13 Gram-positive bacteria, 09 (69.23%) were Coagulase negative *Staphylococcus* and 4 (30.77%) were *Staphylococcus aureus.* Out of 216 Gram negative isolates, *Pseudomonas aeruginosa* 92 (42.59%). In the present study, among 142 Gram negative bacteria isolated from burn wound patients, *Pseudomonas aeruginosa* were 75 (52.82%); out of 43 Gram negative bacteria isolated from surgical wound, *Pseudomonas aeruginosa* were 05 (11.63%) and among 31 Gram negative bacteria isolated from diabetic wound, *Pseudomonas aeruginosa* were 12 (38.71%). Among 92 *Pseudomonas aeruginosa*, 75 (81.52%) were from burn wound, 05 (5.44%) were from surgical wound and 12 (13.04%) were from diabetic wound (Figure 2). In the present study the isolates of *Pseudomonas aeruginosa* showed a pattern of resistance to Ciprofloxacin and Levofloxacin 75.00%, Gentamicin 72.83%, Amikacin 71.74%, Ceftazidime 70.65%, Cefepime 65.22%, Aztreonam 64.13%, Imipenem and Meropenem 38.04%, Piperacillin-tazobactam 28.26%, Colistin 4.35% (Figure 3). None of the isolates were resistant to Polymyxin B. Among 92 *Pseudomonas aeruginosa*, Imipenem resistant strains were 35 (38.04%) and MBL producing stains detected by Imipenem-EDTA combined disc diffusion test were 27 (29.35%) (Table II). MBL producing *Pseudomonas aeruginosa* were showed sensitivity to colistin 23 (85.18%) and to polymyxin 27 (100%).

Table I: Distribution of bacteria isolated from different types of wound swab (n=288)

| Type of samples | Number of samples | Number of isolates | | Total (%) |
|---|---|---|---|---|
| | | Gram positive | Gram negative | |
| Burn wound | 174 | 07 | 142 | 149 (85.63%) |
| Surgical wound | 78 | 03 | 43 | 46 (58.97%) |
| Diabetic wound | 36 | 03 | 31 | 34 (94.44%) |
| Total | 288 | 13 | 216 | 229 (79.51%) |

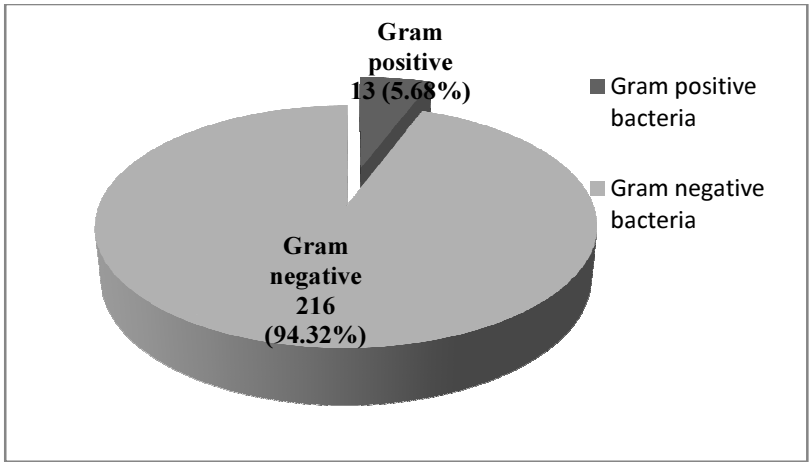


Figure 1. Distribution of Gram negative and Gram positive bacteria out of 229 isolates (n=229)

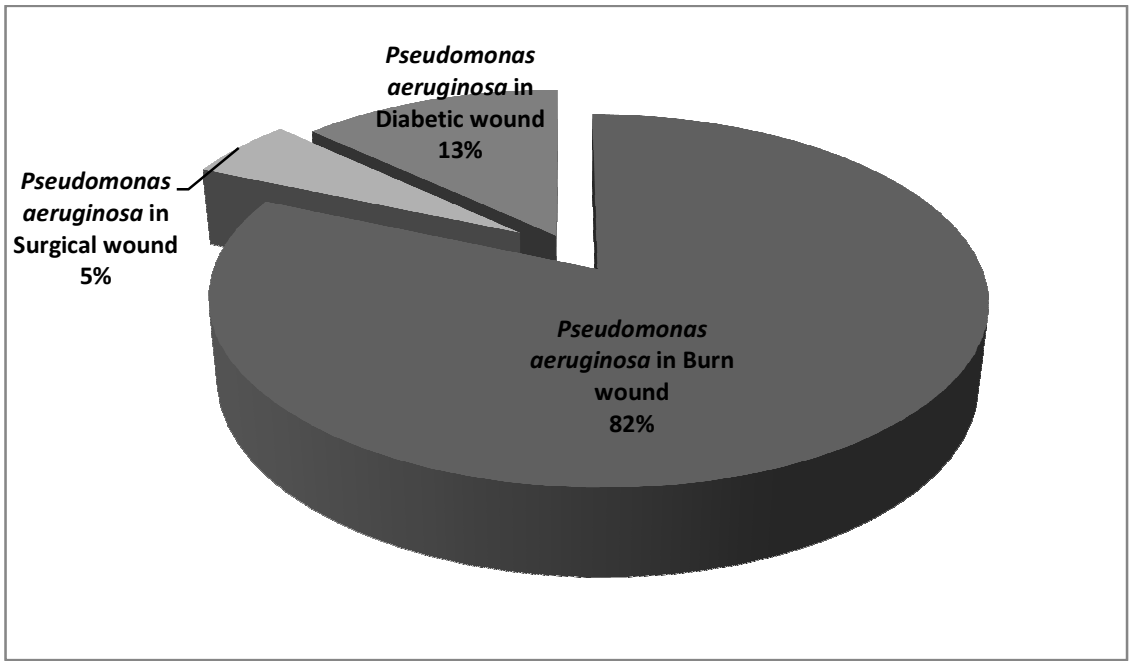


Figure 2. Distribution of *Pseudomonas aeruginosa* isolated from different type of wound infections (n=92)

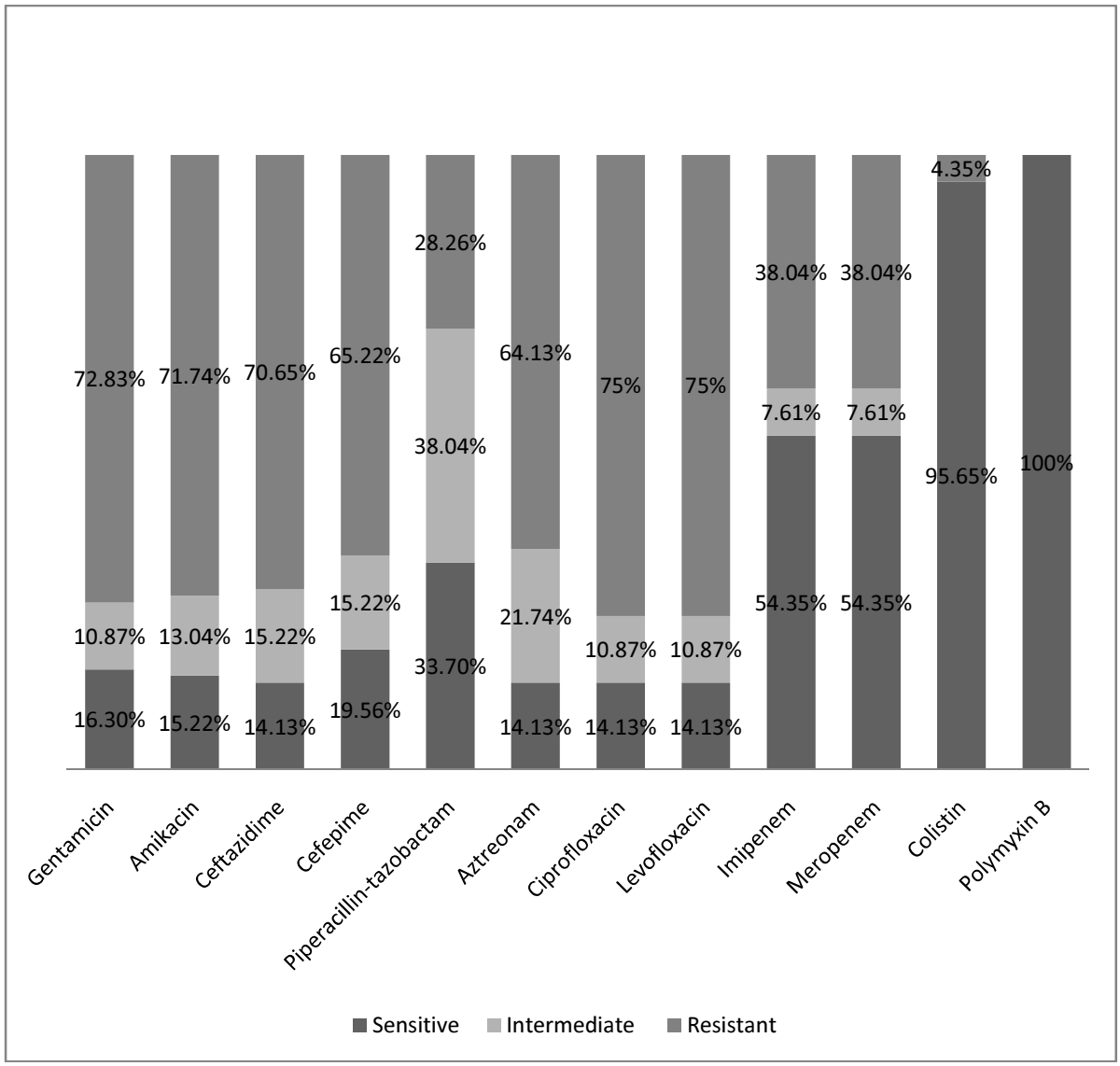


Figure 3. Antimicrobial susceptibility pattern of isolated *Pseudomonas aeruginosa*

Table II: Isolation rate of imipenem sensitive, imipenem resistant and MBL producing *Pseudomonas aeruginosa*

| No of isolates | Imipenem sensitive strains | Imipenem resistant strains | MBL producers |
|---|---|---|---|
| | No (%) | No (%) | No (%) |
| 92 | 50 (54.35%) | 35 (38.04%) | 27 (29.35%) |

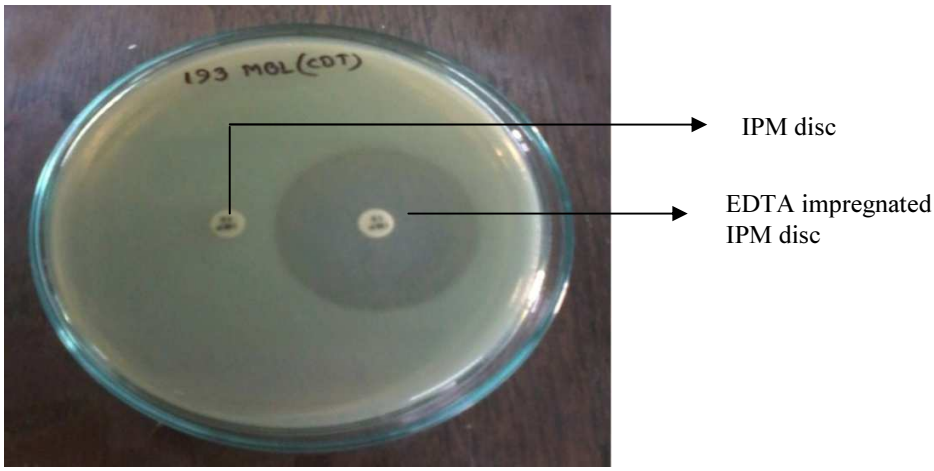


Figure 4. Imipenem-EDTA combined disc diffusion test shows MBL detection evidenced by ≥7 mm inhibition zone of IPM with EDTA as compared with IPM disc alone.

**Discussion**

Carbapenems are used as last resort antibiotics for the treatment of infection with multi-drug resistant Gram negative bacilli. It is a broad spectrum beta lactam antibiotic that shows stability to extended spectrum beta lactamase and Amp C- beta lactamase.[20] However, there has been increasing reports of resistance to this life saving antibiotics in *Pseudomonas aeruginosa*.[21] Indiscriminate use of carbapenems could have resulted in the increase of carbapenem resistant *Pseudomonas aeruginosa*. Production of Metallo-Beta-Lactamase is one of the important cause of resistance to carbapenem drugs which is plasmid mediated, so the resistance can be spread among hospital pathogens. So early detection of MBL producing *Pseudomonas aeruginosa* in clinical laboratories is important, the benefit of which include implementation of appropriate antimicrobial treatment and infection control policy.

In the present study, isolated *Pseudomonas aeruginosa* in burn wound patients were 52.82%, in surgical wound 11.63% and in diabetic wound 38.71%. In our study, *Pseudomonas aeruginosa* was the most frequently isolated pathogen in burn wound and diabetic foot wound which correlates with the findings of Saxena *et al*. (2013)[22] in India and Parsa *et al*. (2015)[23] in Iran respectively. However the finding of *Pseudomonas aeruginosa* in burn wound is near to the findings of Farzana (2013)[24] in Bangladesh. In this study, *Pseudomonas aeruginosa* in surgical wound swab was 11.63% which correlates with the findings of Raza *et al*. (2013)[25] in Nepal.

In the present study the isolates of *Pseudomonas aeruginosa* showed a pattern of resistance to Ciprofloxacin and Levofloxacin 75.00%, Gentamicin 72.83%, Amikacin 71.74%, Ceftazidime 70.65%, Cefepime

65.22%, Aztreonam 64.13%, Imipenem and Meropenem 38.04%, Piperacillin-tazobactam 28.26%, Colistin 4.35%.and 0.00% to Polymyxin B. Abedin (2016)[26] in Bangladesh reported similar findings like the present study. This study reported decreased susceptibility of the *Pseudomonas aeruginosa* to several antimicrobials. This can be interpreted in part by the increase in consumption of antimicrobial agents leading to selective pressure of antibiotics on *Pseudomonas aeruginosa* and consequently the bacteria modify the resistance mechanisms.

Among 92 *Pseudomonas aeruginosa*, 38.04% isolates were found to be imipenem resistant and 29.35% were found to be MBL producers detected by Imipenem-EDTA combined disc diffusion test. A study carried by Farzana in Bangladesh (2013)[24] who reported 44% MBL producing *Pseudomonas aeruginosa*. Another study by Najim *et al*. (2014)[27] in Iraq were found 12.7% imipenem resistant and MBL producing *Pseudomonas aeruginosa*. However, all the imipenem resistant *Pseudomonas aeruginosa* were not MBL producers. This is might be due to presence of impermeability via loss of Opr D porin or up regulation of active efflux pump system which are also responsible for carbapenem resistance in *Pseudomonas* aeruginosa. MBL producing *Pseudomonas aeruginosa* were showed sensitivity to colistin 85.18% and to polymyxin 100%. Our study revealed Polymyxin B and Colistin showed more sensitivity against *Pseudomonas aeruginosa* which may be drug of choice for the treatment of life threatening infection caused by MBL producing *Pseudomonas aeruginosa.*

*Conclusion*

The present study showed 29.35% were MBL producers. In this study, Polymyxin B and Colistin were found to be most effective drug for the treatment of infection caused by MBL producing *Pseudomonas aeruginosa*.

**References**


1. Osman KM, Alabady MS, Ata NSSM, Ezzeldin NA and Aly MAK. Genotypic characterization of *Pseudomonas aeruginosa* isolated from human and animal sources in Egypt. *Zoonoses and Public Health* 2010; 57(5):329-338.
2. Cornaglia G, Akova M, *et al*. Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. *International journal of antimicrobial agents* 2007;29(4):380-8.
3. Chaudhary U, Bhaskar H, Sharma M. Imipenem-EDTA disk method for rapid identification of Metallo- β-lactamase producing gram-negative bacteria. Ind J Med Res 2008; 127:406-7.
4. Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant nonfermenting gram-negative bacilli from patients with respiratory infections in intensive care unit. *Indian J Microbial* 2005; 23:189-91.
5. Bush K, Jacoby GA and Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995.; 39(6): 1211-33.
6. Livermore DM and Woodford N. Carbapenemases: a problem in waiting? *Curr Opin Microbiol* 2000; 3(5):489-495.
7. Walsh TR, Toleman MA, Poirel L and Nordmann P. Metallo-β-lactamases: the Quiet before the Storm? *Clin Microbiol Rev* 2005; 18(2):306-325.
8. Toney JH and Moloughney JG. Metallo-beta-lactamase inhibitors: promise for the future? *Curr Opin Investig Drug* 2004; 5(8):823-6.
9. Watanabe M, Iyobe S, Inoue M and Mitsuhashi S. Transferable Imipenem Resistance in *Pseudomonas aeruginosa.*

*Antimicrob Agents Chemother* 1991; 35(1):147-151.
10. Yatsuyanagi J, Saito S, Harata S, Suzuki N, Ito Y, Amano K and Enomoto K . Class 1 Integron Containing Metallo-β-Lactamase Gene $bla_{VIM-2}$ in *Pseudomonas aeruginosa* Clinical Strains Isolated in Japan. *Antimicrob Agents* Chemother 2004; 48(2):626-628.
11. Lagatolla C, Tonin EA, Monti-Bragadin C, Dolzani L, Gombac F, Bearzi C, *et al*. Endemic carbapenem-resistant *Pseudomonas aeruginosa* with acquired metallo-β-lactamase determinants in European Hospital. *Emerg Infect Dis* 2004: 10(3): 535-538.
12. Peleg AY, Franklin C, Bell J and Spelman DW. Emergence of IMP-4 metallo-beta-lactamase in a clinical isolate from Australia. *J Antimicrob Chemother* 2004; 54(3):699-700.
13. Gales AC, Menezes LC, Silbert S and Sader HS. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM metallo-β-lactamase. *J Antimicrob Chemother* 2003; 52(4):699-702.
14. Toleman MA, Rolston K, Jones RN and Walsh TR. $bla_{VIM-7}$, an evolutionarily distinct metallo-β-lactamase gene in a *Pseudomonas aeruginosa* isolate from the United States. *Antimicrob Agents Chemother* 2004; 48(1):329-332.
15. Cheesbrough M. Microscopical techniques used in Microbiology, culturing bacterial pathogens, biochemical tests to identify bacteria. In Cheesbrough M, editor. *District Laboratory Practice in Tropical Countries*, Part-2. UK: Cambridge University Press; 2006: p. 65, 67-70, 80-84, 132-143, 180,194.
16. Collee JG and Marr W. Pseudomonas, Stenotrophomonas, Burkholderia. In: Collee JG, Frased AG, Marmion BP, Simons A, eds. Mackie & McCartney Practical Medical Microbiology, 14th ed. USA: Churchill Livingstone, 1996:413-424.
17. Clinical and laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fifth Informational Supplement. CLSI document 2015; M100-S25.
18. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM and Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2002; 40(10):3798-3801.
19. Payne DJ, Bateson JH, Gasson BC, Proctor D, Khusi T, Farmer TH, *et al*. Inhibition of metallo-β-lactamases by a series of mercaptoacetic acid thiol ester derivatives. *Antimicrob Agents Chemother* 1997; 41:135-40.
20. Gladstone P, Rajendran P, Brahamanand KN. Incidence of carbapenem resistant nonfermenting gram-negative bacilli from patients with respiratory infections in the intensive care unit. Ind J Med Microbiol 2005; 23:189-91.
21. Saxena N, Dadhich D and Maheshwari D. Aerobic bacterial isolates from burn wound infection patients and their antimicrobial susceptibility pattern in Kota, Rajasthan. Journal of Evolution of Medical and Dental Sciences. 2013; 2(23):4156-4160.
22. Parsa H. and Samani S. Microbiological features and risk factors in patients with diabetic foot ulcers. Wounds. 2015; 27(11):308-312.
23. Farzana A. Imipenem resistant *bla* NDM1 producing *Pseudomonas* isolated from burn unit of DMCH with their antimicrobial susceptibility pattern and effective combination therapy [master's thesis]. Dhaka Medical College, Dhaka; 2013. 56,57p.

24. Raza MS, Chander A and Ranabhat A. Antimicrobial susceptibility pattern of the bacterial isolates in post-operative wound infections in a tertiary care hospital, Kathmandu, Nepal. Open Journal of Medical Microbiology. 2013; 3:159-163.
25. Abedin J. In vitro and in vivo evaluation of effect of antibiotic combination against imipenem resistant Pseudomonas aeruginosa isolated from burn wound of DMCH [master's thesis]. Dhaka Medical College, Dhaka; 2016. 76p.
26. Yassin NA, Khali HM, Hassan AO. Prevalence of Metallo-β-lactamase producing *Pseudomonas aeruginosa* in wound infections in Duhok city. Iraq Int J Res Med Sci 2014: 2:1576-9.