Original Article

Emergence of carbapenemase-producing urinary isolates at a tertiary care hospital in Dhaka, Bangladesh

Nurjahan Begum*, S.M. Shamsuzzaman

Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh

A R T I C L E   I N F O

Article history:
Received 15 February 2016
Received in revised form 14 March 2016
Accepted 6 April 2016
Available online 27 May 2016

Keywords:
Antibiotic resistance
Bangladesh
blaNDM-1
Carbapenemases
Uropathogens

A B S T R A C T

Objectives: A growing incidence of pathogens producing carbapenemases has been observed in many countries including Bangladesh. The present study was carried out to determine the presence of carbapenemase producers among uropathogens.

Materials and Methods: A total of 138 Gram-negative uropathogens were isolated and identified by conventional methods and were screened for carbapenemase production using imipenem discs. Phenotypic identification of carbapenemase production was done by the double disc synergy test, combined disc assay, and modified Hodge test. The minimum inhibitory concentration of imipenem was determined by the agar dilution method. Genes encoding blaNDM-1, blaIMP, blaVIM, blaKPC and blaOXA-48/blaOXA-181 were identified by polymerase chain reaction.

Results: Twenty (14.49%) imipenem resistant strains were detected among 138 Gram-negative uropathogens. The most common isolates were Escherichia coli and Klebsiella spp. Among 20 imipenem resistant strains, 16 (80%) carbapenemase producers were detected by polymerase chain reaction, 13 (65%) by double disc synergy, 15 (75%) by combined disc assay, and seven (35%) by modified Hodge test. The blaNDM-1 gene was most prevalent (55%), followed by blaOXA-48/OXA-181, blaKPC (20%), blaVIM (15%), and blaIMP (10%). More than one carbapenemase gene was present in nine (45%) of the isolates. The minimum inhibitory concentration of imipenem of the carbapenemase producers ranged from ≥128 μg/mL to 4 μg/mL. Overall, carbapenemase encoding genes were detected in 11.6% (16/138) of the studied Gram-negative uropathogens. All (100%) of the carbapenemase-producing organisms were resistant to all tested antibiotics apart from colistin.

Conclusion: The study shows a significant rate of urinary isolates were carbapenemase producers, including a high prevalence of blaNDM-1, in Bangladesh.

Copyright © 2016, Buddhist Compassion Relief Tzu Chi Foundation. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Carbapenems are the mainstay of therapy for patients with serious and life threatening infections caused by Enterobacteriaceae, which produce extended spectrum β-lactamases (ESBL) [1]. However, the widespread emergence and dissemination of carbapenem resistance in this family has resulted in a public health crisis [2]. This resistance is mediated by production of carbapenemases [either serine based carbapenemase or metallo-β-lactamases (MBLs)], by modification of membrane permeability (i.e., porin loss) or by production of carbapenem hydrolyzing β-lactamases (i.e., hyperproduction of AmpC β-lactamase, certain ESBLs with increased capacity to hydrolyze carbapenems) and upregulation of efflux pumps [3]. Carbapenem-hydrolyzing β-lactamases, which belong to Ambler classes A, B, and D have been reported worldwide among Enterobacteriaceae. The most clinically significant ones are KPC-type (class A); IMP, VIM, and NDM-1 types (class B); and OXA-48 (class D). The genes encoding them are located on mobile genetic elements, which allow them to spread [1,4]. Class A and D enzymes have a serine-based hydrolytic mechanism for cleaving the β-lactam ring in antibiotics. MBLs, by contrast, are class B carbapenemases containing zinc at the active site [5]. Carbapenemase-producing pathogens are considered a serious nuisance as they have the ability to hydrolyze penicillins, cephalosporins, and monobactams as well as carbapenems [6].

* Corresponding author. Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh. Tel.: +8802 9665518; fax: +8802 8615919.
E-mail address: nurjahan.begum.akhi@gmail.com (N. Begum).

http://dx.doi.org/10.1016/j.tcmj.2016.04.005
1016-3190/© 2016, Buddhist Compassion Relief Tzu Chi Foundation. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Under such circumstances of extreme resistance towards antibiotics, the pathogens remain susceptible only to colistin and tigecycline combination therapy [7]. However, with its increased use, we may actually trigger resistant mechanisms against these combinations leading to the end of the current era of pharmacopoeia [8]. The objective of our study was to isolate and study the occurrence of blaNDM-1 and other carbapenemase genes among urinary isolates in Bangladesh, along with the antimicrobial resistance patterns of these organisms. This knowledge will greatly help reduce the morbidity and mortality rates associated with urinary tract infections caused by these organisms.

2. Materials and methods

2.1. Bacterial isolates

The study was conducted after obtaining due approval from the institutional ethical committee. From January to December 2014, a total of 138 consecutive nonduplicate (one isolate per patient) Gram-negative isolates recovered from urine cultures of patients with clinically suspected urinary tract infection in the Department of Microbiology of Dhaka Medical College, Dhaka were included in the study. Bacterial identification was performed by routine conventional microbial cultures and biochemical tests using standard recommended techniques [9].

2.2. Antimicrobial susceptibility testing

All bacterial isolates were subjected to antimicrobial susceptibility testing using the disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) [10]. Escherichia coli ATCC 25922 was used as the quality control strain. The antibiotics tested were as follows (potency in μg/disc): ceftazidime (30), ceftriaxone (30), imipenem (10), meropenem (10), cefoxitin (30), cefotaxime (30), amoxiclav (20/10), ciprofloxacin (5), gentamicin (10), co-trimoxazole (1.25/23.75), azithromycin (15), nitrofurantoin (300), and colistin sulfate (10).

2.3. Screening for carbapenemases by the disc diffusion technique

Screening for carbapenem-resistance was determined using the Kirby–Bauer disc diffusion method with a 10 μg imipenem disc. Three to five well isolated colonies of test organisms were emulsified into 3 mL of sterile normal saline. The turbidity of the suspension was incubated on Mueller–Hinton agar plates. The test isolates were streaked in a straight line from the edge of the disc to the edge of the plate and were incubated overnight. A positive test was indicated by a cloverleaf-like indentation at the intersection of the test organism and the standard strain, within the zone of inhibition of the carbapenem antibiotic [10]. The detection of MBL production was performed by the double-disc synergy (DDS) test and combined disc (CD) assay as described previously [11,12].

2.5. Detection of carbapenemase producers by the minimum inhibitory concentration of imipenem

The minimum inhibitory concentration (MIC) of imipenem against all carbapenemase producers was determined using the agar dilution method according to CLSI guidelines. For detection of carbapenem resistance among Enterobacteriaceae, an MIC of imipenem ≥ 4 μg/mL was considered resistant, 2 μg/mL intermediate, and ≤ 1 μg/mL sensitive [10]. For preparation of the imipenem stock solution, a vial of a 500 mg base of commercially available imipenem injection was added to 50 mL distilled water to a concentration of 10 mg/mL. For each plate, 50 mL Mueller–Hinton medium was prepared and impregnated with 5 μL, 10 μL, 20 μL, 40 μL, 80 μL, 160 μL, 320 μL, 640 μL, or 1280 μL of the imipenem stock solution to achieve concentrations of 1 μg/mL, 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL, 64 μg/mL, 128 μg/mL, or 256 μg/mL per plate, respectively.

2.6. Molecular characterization of carbapenem resistance genes

Polymerase chain reaction (PCR)-based detection of Ambler class B MBLs (blaIMP, blaVIM and blaNDM-1), Ambler class D (blaOXA-48/OXA-181), and serine carbapenemases (blaKPC) was carried out on imipenem resistant isolates. Coexistence of carbapenemase encoding genes, namely, NDM-1, VIM, IMP, OXA-48/OXA-181 and KPC were also evaluated by PCR. Genomic DNA was extracted by the boiling method. The following pairs of previously used primers were used to yield PCR products: for blaNDM-1: ACCGCC TGG ACC GAT GAC CA (forward), GCC AAA GTT GGG CCC GGT TG (reverse); for blaIMP: GGA ATA GAG TGG CTT AAY TCT C (forward), CCA AAC YAC TAS GTT ATC T (reverse); for blaVIM: GAT GGT TGG GTC GCA TA (forward), CAA ATG AGC ACC AG (reverse); for blaKPC: CTT CTA GTT CTG TCG TCT TG (forward), CTT GTC ATC CTT GTT AGG GG (reverse); for blaOXA-48: GCC TGG TTA AGG ATC AAC AC (forward), CAT CAA GTT CAA CCC AAC CG (reverse); and for blaOXA-181: ATG GGT GTA TTA GGC TTA TCG (forward), AAC TAC AAG CGG ATC GAG CA (reverse) [13–16]. The following cycling parameters were used: initial denaturation at 95°C for 10 minutes, then 30 cycles of denaturation at 95°C for 1 minute, annealing at 63°C (for blaNDM-1 and blaOXA-48), 52°C (for blaIMP and blaVIM), or 55°C (for blaOXA-181) for 45 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. The amplified DNA was analyzed by 1.5% agarose gel-electrophoresis at 100 volts for 35 minutes, stained with 1% ethidium bromide and visualized under UV light.

2.7. Statistical analysis

Data were analyzed using Excel (2007) software (Microsoft, Redmond, WA, USA).

3. Results

Twenty of the total 138 Gram-negative urinary isolates (14.49%) were found to be imipenem resistant by the disk diffusion test and by the MIC of imipenem. Thirteen E. coli, five Klebsiella spp., one Citrobacter spp. and one Pseudomonas spp. were isolated from the imipenem-resistant organisms. The MIC of imipenem of these isolates ranged from ≥ 128 μg/mL to 4 μg/mL. Among 20 imipenem-resistant strains, 16 (80%) carbapenemase producers were detected by PCR. Eleven (84.62%) of the 13 imipenem-resistant E. coli, four (80%) of the five Klebsiella spp., and the only Citrobacter spp. were
positive for carbapenemase-encoding genes by PCR. The one (100%) imipenem resistant *Pseudomonas* spp. was negative for carbapenemase-encoding genes. Out of 20 imipenem-resistant strains, 11 (55%) were positive for *bla*NDM-1, 8 (40%) for *bla*OXA-48/*bla*OXA-181, 4 (20%) for *bla*KPC, 3 (15%) for *bla*VIM, and 2 (10%) for *bla*IMP (Table 1).

Representative PCR amplified NDM-1, VIM, IMP, OXA-48/OXA-181, and KPC genes are shown in Fig. 1.

Among the carbapenemase-producing organisms, nine (45%) contained two or more carbapenemase genes, seven (35%) contained a single carbapenemase gene, and four (20%) had no carbapenemase gene. Among the 16 carbapenemase producers, *bla*NDM-1 + *bla*OXA-48/OXA-181 was the predominant combination, which was present in four (25%) of the isolates (Table 2).

The DDS test, CD assay, and modified Hodge test detected 13 (65%), 15 (75%), and seven (35%) carbapenemase producers, respectively, among the 20 imipenem-resistant isolates. Out of the four negative amplified PCR products, three were positive by the DDS test and all were positive by CD assay and modified Hodge test. Considering PCR as the gold standard, the sensitivity of the DDS test, CD assay, and modified Hodge test was 62.5%, 68.75%, and 18.75%, respectively. All (100%) of the carbapenemase-producing organisms showed resistance to other β-lactam antibiotics, aminoglycosides and quinolones tested but were found to be sensitive to colistin.

### 4. Discussion

The global emergence and dissemination of acquired carbapenemases among Gram-negative bacteria are considered a major public health problem. The carbapenemase-encoding genes are often located on plasmids along with other resistance genes, resulting in multidrug-resistant, extremely drug-resistant and pandrug-resistant bacteria [17]. Continuous monitoring and rapid detection of these virulent organisms may check their spread and play a vital role in infection control. To address this rising resistant determinant, we have observed the occurrence of carbapenemase-encoding genes among carbapenem-resistant Gram-negative uropathogens.

The present study identified 16 (80%) carbapenemase producers out of the 20 imipenem-resistant bacteria, which included 84.62% of the imipenem-resistant *E. coli* and 80% of the imipenem-resistant *Klebsiella* spp. Although acquired carbapenemase-encoding genes are frequently found in *Pseudomonas* spp. and *Acinetobacter* spp., the existence of carbapenemase-encoding genes in the species of *E. coli* and *Klebsiella pneumoniae* in this study suggests that plasmid-mediated horizontal transfer of carbapenemase-encoding genes occurs continuously among Gram-negative bacilli, as reported previously [2].

In the present study, out of 20 imipenem-resistant Gram-negative bacteria, 11 (55%) *bla*NDM-1 producers were detected by PCR, including seven (53.85%) of the imipenem-resistant *E. coli* and four (80%) of the imipenem-resistant *Klebsiella* spp. The prevalence of *bla*NDM-1 producers is increasing in Bangladesh, which is reflected by several studies conducted in Bangladesh [18,19]. In Indian studies, the prevalence of *bla*NDM-1 producers among carbapenem-resistant Enterobacteriaceae ranged between 31.2% and 91.6% [20]. An editorial by Abdul Ghafor highlights the widespread nonprescription use of antibiotics in this subcontinent, leading to huge selection pressure, and predicts that the NDM-1 problem is likely to get substantially worse in the foreseeable future [21]. This scenario is of great concern because there are few new anti-Gram-negative antibiotics in the pharmaceutical pipeline and none that are active against NDM-1 producers [7]. A study by Bushnell et al reported that urine was the most common specimen source and *K. pneumoniae* and *E. coli* were the most frequently detected bacteria detected in 60 cases of *bla*NDM-1 producing bacteria [22]. Rapid dissemination of *bla*NDM-1 producing

### Table 1

| Distribution of carbapenemase-encoding genes among different species of imipenem resistant organisms. |
|--------------------------------------------------------------------------------------------------|
| Imipenem-resistant organisms | Carbapenemase-encoding genes |
|-----------------------------|-------------------------------|
|                            | NDM-1 (%) | VIM (%) | IMP (%) | OXA-48/OXA-181 (%) | KPC (%) |
| *Escherichia coli* (n = 13) | 7 (53.85) | 2 (15.38) | 2 (15.38) | 5 (38.46) | 2 (15.38) | 3 (23.08) |
| *Klebsiella spp.* (n = 5)   | 4 (80.00) | 1 (20.00) | 0 (0.00) | 2 (40.00) | 1 (20.00) |
| *Citrobacter spp.* (n = 1)  | 0 (0.00)  | 0 (0.00)  | 0 (0.00) | 1 (100.00) | 0 (0.00)  |
| *Pseudomonas spp.* (n = 1)  | 0 (0.00)  | 0 (0.00)  | 0 (0.00) | 0 (0.00)  | 0 (0.00)  |
| Total (n = 20)              | 11 (55.00) | 3 (15.00) | 2 (10.00) | 8 (40.00) | 4 (20.00) |

The total of last row is more than 100% as most of the isolates had two or more carbapenemase genes.

### Table 2

| Distribution of *bla*VIM, *bla*IMP, *bla*NDM-1, *bla*OXA-48/OXA-181, *bla*KPC among imipenem-resistant organisms. |
|---------------------------------------------------------|
| Genotype | n (%) |
|-----------|-------|
| NDM-1 + OXA-48/OXA-181 + KPC | 1 (5) |
| NDM-1 + IMP + VIM | 2 (10) |
| NDM-1 + OXA-48/OXA-181 | 4 (20) |
| NDM-1 + KPC | 2 (10) |
| NDM-1 | 2 (10) |
| VIM | 1 (5) |
| OXA-48/OXA-181 | 3 (15) |
| KPC | 1 (5) |

Fig. 1. Photograph of amplified NDM-1, OXA-48, OXA-181, KPC, VIM and IMP genes. Lane 4: 100-bp DNA ladder; Lane 2: amplified DNA of 390 bp for the VIM gene; Lane 3: 888 bp for the OXA-181 gene; Lane 5: 438 bp for the OXA-48 gene; Lane 6: 264 bp for the NDM-1 gene; Lane 7: 188 bp for the IMP gene; Lane 8: and 498 bp for KPC gene; Lane 1: negative control (*Escherichia coli* ATCC 25922).
organisms might be facilitated by conditions such as overcrowding, over-the-counter availability of antibiotics, low levels of hygiene, and weak hospital antibiotic policies [23].

Among 20 imipenem-resistant isolates, four (20%) were positive for the blaKPC gene. So far there are no reports of blaKPC positive Enterobacteriaceae in Bangladesh. In the current study, three (23.08%) of the imipenem-resistant E. coli and one (20%) of the imipenem-resistant Klebsiella spp. were positive for the blaKPC gene. The highest prevalence of KPC producing organisms to date were identified mostly in the USA, Israel, and Greece [24]. Shanmugam et al in India reported that 31 (67.4%) of 46 carbapenem-resistant Enterobacteriaceae isolates harbored the blaKPC gene [25]. The blaKPC genes that encode KPCs are present on transferable plasmids and are flanked by transposable elements, thus allowing for the gene to move from plasmid to the bacterial chromosome and back [26]. The presence of this gene suggests the possibility of horizontal transmission, as this carbapenemase has been associated with mobile genetic elements (transposons), which can be transferred from one bacterium to another [27].

A point mutant analog of OXA-48, OXA-181, with similar carbapenemase activity, has been identified in strains from India and strains of Indian origin [16]. In the current study, out of 20 imipenem-resistant Gram-negative isolates, 8 (40%) were positive for blaOXA-181 and OXA-48 detected by PCR (using specific primer). These included five (38.46%) of the imipenem-resistant E. coli, two (40%) of the imipenem-resistant Klebsiella spp., and the one (100%) imipenem-resistant Citrobacter spp. PCR screening results need to be validated by sequencing to ascertain the presence of either OXA-48 or OXA-181, which was not performed in the present study, although OXA-181 is more common in the Indian subcontinent than OXA-48. In September 2012, the CDC reported isolation of blaOXA-181 positive K. pneumoniae in two patients from Bangladesh who were admitted to separate hospitals in Singapore within a short period [28]. A study by Castanheira et al in India reported that 10 of 39 (25.64%) carbapenem-resistant strains harbored the blaOXA-181 gene [16]. The findings in the present study revealed that blaOXA-48/OXA-181 carbapenemase-producing organisms appear to be an emerging cause of carbapenem resistance in Gram-negative bacteria in Bangladesh in addition to blaNDM-1-producing organisms.

Combinations of different genes in single strains were observed, with a combination of blaNDM-1 and blaOXA-48/blaOXA-181 being predominant (20%). A study by Khajuria et al. in India reported 55% of carbapenemase-producing urinary isolates of E. coli co-harbored blaNDM-1 and blaOXA-48 [29]. Plasmids carrying the blaNDM-1 gene are diverse and can harbor a high number of resistance genes associated with other carbapenemase genes (OXA-48 types, VIM types), plasmid-mediated cephalosporinase genes, ESBL genes, aminoglycoside resistance genes (16S RNA methylases), macrolide resistance genes (esterase), and rifampin (rifampin-modifying enzymes) and sulfamethoxazole resistance genes as sources of multidrug resistance and pandrug resistance [20].

The actual prevalence of carbapenemase producers among Gram-negative bacteria is still unknown because many countries that are likely to be their main reservoirs have not established any search protocol for their detection [30]. In the present study, 16.2% (16/103) of Gram-negative uropathogens showed the occurrence of carbapenemase encoding genes, which is almost four times higher than other data reported in Bangladesh, where only 4.8% isolates were found to be carbapenemase producers [31]. The discrepancy in the findings between the latter and present studies might be due to the increased use of carbapenem in Dhaka, Bangladesh.

Imipenem discs were used for screening carbapenemase production. However, we observed that four (20%) imipenem-resistant strains showed negative amplification by PCR. Gram-negative bacteria have the capacity to elude the action of carbapenems through modification of outer membrane permeability (i.e. porin loss), upregulation of efflux systems, production of carbapenem-hydrolyzing β-lactamases (i.e. hyperproduction of AmpC β-lactamases, certain ESBLs with increased capacity to hydrolyze carbapenems) and production of carbapenemases (either serine based carbapenemases or MBLs) [3]. The plethora of imipenem resistance among the noncarbapenemase producers in this study might be due to resistance mechanisms other than carbapenemase production.

In addition to showing resistance to β-lactams, all the carbapenemase-producing organisms were highly resistant to most other antibiotic classes, including aminoglycosides and fluoroquinolones. The higher antibiotic resistance in the present study might be due to the fact that common antibiotics are sold over the counter in Bangladesh and anybody can buy them without a doctor’s advice. By contrast, the co-resistance of MBL producers to non-β-lactam antibiotics might be due to simultaneous presence of other drug resistance mechanisms in addition to MBL genes [32].

5. Conclusion

This study shows a significant rate of carbapenemase producing Gram-negative uropathogens. This is extremely worrisome, as dissemination of plasmids carrying resistant determinant genes from one species to another makes organisms refractory to the common antibiotics used in clinical practice. The need of the hour is a strong antimicrobial stewardship program, which is followed by all concerned doctors, with further emphasis on better, cost-effective, logical infection control measures to prevent the dissemination of these multidrug resistant bacteria.

Acknowledgments

The authors gratefully acknowledge the technical support provided by the Department of Microbiology, Dhaka Medical College, Bangladesh.

References

[1] Kalpoe JS, Naiemi NA, Poirel L, Nordmann P. Detection of an Ambler class D OXA-48 type β- lactamase in a Klebsiella pneumoniae strain in The Netherlands. J Med Microbiol 2011; 60:677–8.
[2] Lascols C, Hackel M, Marshall SH, Hujer AM, Bouchillon S, Badal R, et al. Increasing prevalence and dissemination of NDM-1 metallo-β-lactamase in India: data from the SMART study (2009). J Antimicrob Chemother 2011; 66:1992–7.
[3] Frere JM. β-lactamases and bacterial resistance to antibiotics. Mol Microbiol 1995; 16:385–95.
[4] Poirel L, Potron A, Nordmann P. OXA-48 like carbapenemases: the phantom menace. J Antimicrob Chemother 2012;67:1597–606.
[5] Ambler RP. The structure of β-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995;8:557–84.
[6] Cobo J, Morosini MI, Pinto V, Tato M, Samaranch N, Baquero F. Use of tigecycline for the treatment of prolonged bacteremia due to multi-resistant VIM-1 and SHV-12 beta lactamase producing Klebsiella pneumoniae epidemic clone. Diagn Microbiol Infect Dis 2008;60:319–22.
[7] Cohen SJ, Dierickx C, Al Naiemi N, Kaczynska R, Van Hoek AH, Vos P, et al. Rapid detection of TEM, SHV and CTX-M extended-spectrum β-lactamases in Enterobacteriaceae using ligation mediated amplification with microarray analysis. J Antimicrob Chemother 2010;65:1377–81.
[8] Collie JC, Miles RS, Wan B. Tests for the identification of bacteria. In: Collie JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 131–30.
[9] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement. CLSI document M100-S23. CLSI: Wayne, PA.
[10] Lee K, Lim YS, Yong D, Yum JH, Cheong Y. Evaluation of the Hodge Test and the Imipenem-EDTA Double-Disk Synergy Test for differentiating metallo-beta-
lactamase producing isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2003;41:6623–6.

[12] 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;53:5046–54.

[13] Poirel L, Walsh TR, Cuvelier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119–23.

[14] Zarfel G, Hoenigl M, Leitner E, Salzer H, Feierl G, Masoud L, et al. Emergence of New Delhi metallo-β-lactamase, Austria. Emerg Infect Dis 2011;17:125–30.

[15] Mendes RE, Kyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, et al. Rapid detection and identification of metallo-β-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. J Clin Microbiol 2007;45:544–7.

[16] Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1 and OXA-181-producing Enterobacteriaceae in Indian hospitals: report from the sentry antimicrobial surveillance program, 2006–2007. Antimicrob Agents Chemother 2011;55:1274–8.

[17] Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 2012;25:682–707.

[18] Islam MA, Talukdar PK, Hoque A, Huq M, Nabi A, Ahmed D, et al. Emergence of multidrug-resistant NDM-1-producing Gram-negative bacteria in Bangladesh. Eur J Clin Microbiol Infect Dis 2012;31:2593–600.

[19] Farzana R, Shamsuzzaman SM, Mamun KZ. Isolation and molecular characterization of New Delhi metallo-β-lactamase-1 producing superbug in Bangladesh. J Infect Dev Ctries 2013;7:161–8.

[20] Kumarasamy KR, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010;10:597–602.

[21] Ghafur AK. An obituary on the death of antibiotics! J Assoc Physician India 2010;58:143–4.

[22] Bushnell G, Mitram-Gold F, Mundy LM. Emergence of New Delhi metallo-β-lactamase type-1 producing Enterobacteriaceae and non-Enterobacteriaceae: global case detection and bacterial surveillance. Int J Infect Dis 2013;17:e325–33.

[23] Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother 2011;66:689–92.

[24] Poirel L, Hombrouck-Alet C, Freneaux C, Bernabeu S, Nordmann P. Global spread of New Delhi metallo-β-lactamase 1. Lancet Infect Dis 2010;10:832.

[25] Shanmugam P, Meenakshisundaram J, Jayaraman P. BlaKPC gene detection in clinical isolates of carbapenem-resistant Enterobacteriaceae in a tertiary care hospital. J Clin Diag Res 2013;7:2736–8.

[26] Raghunathan A, Samuel L, Tibbetts RJ. Evaluation of a real-time PCR assay for the detection of the Klebsiella pneumoniae carbapenemase genes in microbiological samples in comparison with the modified Hodge test. Am J Clin Pathol 2011;135:566–71.

[27] Hoss T, Cuzon G, Villegas MV, Cartigue MF, Quinn JP, Nordmann P. Genetic structures at the origin of acquisition of the beta-lactamase blbKPC gene. Antimicrob Agents Chemother 2008;52:1257–63.

[28] Koh TH, Cao Delphine YH, Chan KS, Wijaya L, Low SBG, et al. BlaOXA-181-positive Klebsiella pneumoniae, Singapore. Emerg Infect Dis 2012;18:1524–5.

[29] Khajuria A, Prahararaj AK, Kumar M, Grover N. Emergence of Escherichia coli, co-producing NDM-1 and OXA-48 carbapenemases, in urinary isolates, at a tertiary care centre at Central India. J Clin Diag Res 2014;8:DC01–4.

[30] Jean SS, Hsueh PR. High burden of antimicrobial resistance in Asia. Int J Antimicrob Agents 2011;37:291–5.

[31] Hayder N, Hasan Z, Afrin S, Noor R. Determination of the frequency of carbapenemase producing Klebsiella pneumoniae isolates in Dhaka city, Bangladesh. Stamford J Microbiol 2012;2:28–30.

[32] Amudhan MS, Sekar U, Kanalanathan A, Balaraman S. BlaIMP and blaVIM mediated carbapenem resistance in Pseudomonas and Acinetobacter species in India. J Infect Dev Ctries 2012;6:757–62.