Molecular Characterization of Extended Spectrum β-lactamase and Carbapenemase Producing *Klebsiella pneumoniae* from a Tertiary Care Hospital

Beena Hosdurg Bhaskar¹, Shalini Shenoy Mulki², Sangeetha Joshi³, Ranjeeta Adhikary⁴, Bhavana Malavalli Venkatesh⁵

**Abstract**

**Objective:** The extended-spectrum beta-lactamase (ESBL) and carbapenemase producing gram-negative bacteria among the members of Enterobacteriaceae are of major health concern globally. The present study was carried out to determine proportion and genetic characterization of ESBL and carbapenemase producing *Klebsiella pneumoniae* strains isolated from intensive care units of a tertiary care hospital.

**Materials and methods:** A total of 250 non-duplicate *K. pneumoniae* isolates were recovered from various clinical specimens from our intensive care units from May 2014 to May 2015. Antibiotic susceptibility testing was performed as recommended by Clinical and Laboratory Standard Institute. Phenotypic identification of ESBL and carbapenemase producing isolates were confirmed by the double-disk synergy test, modified Hodge test, imipenem and imipenem-EDTA combined test, respectively. Molecular characterization of β-lactamase genes were performed by polymerase chain reaction.

**Results:** Out of 250 *Klebsiella pneumoniae*, 84% isolates were ESBL producers, 66% were carbapenem resistant based on their reduced susceptibility to imipenem, meropenem and ertapenem. Among these 165 carbapenem resistant isolates, 9.7% were positive for *bla*<sub>NDM-1</sub>, and these isolates were also found to be positive for one or more *bla* genes. Co-carriage of AmpC in ESBL and carbapenem resistant isolates were 7.8% and 3.6%, respectively and were negative for *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes.

**Conclusion:** The study indicated the prevalence of ESBLs and *bla*<sub>NDM-1</sub> with additional *bla* genes and AmpC among the *K. pneumoniae* isolates in our intensive care units. NDM-1 producing Enterobacteriaceae is a growing health care problem. Detection of the prevalence of antibacterial resistance pattern helps towards improved antibiotic policy and empirical antibiotic treatment.

**Keywords:** AmpC, Carbapenemase, Extended spectrum β-lactamase, Metallo β-lactamase

**Indian Journal of Critical Care Medicine** (2019): 10.5005/jp-journals-10071-23118

**Introduction**

Infections due to multidrug resistant (MDR) Enterobacteriaceae are an important cause of morbidity and mortality worldwide. Carbapenems are most often used in this treatment. The emergence of resistance to these agents has become a serious health concern globally.

*Klebsiella pneumoniae* is one of the most common Gram-negative bacteria showing resistance to multiple antibiotics. The development of extended-spectrum cephalosporins in the early 1980s was regarded as a major addition to our therapeutic armamentarium in the fight against beta-lactamase mediated bacterial resistance. The emergence of enzymes that have the ability to hydrolyze this cephalosporin’s seriously compromised the efficacy of these lifesaving antibiotics. These enzymes were called extended spectrum beta lactamases. Extended spectrum beta-lactamases are plasmid-mediated enzymes that are capable of conferring bacterial resistance to the penicillins, first, second third, fourth generation cephalosporins and aztreonam.

They do this by hydrolysis of these antibiotics but they are inhibited in vitro by beta-lactamase inhibitors.

ESBL is predominantly found in *Klebsiella* spp. and *Escherichia coli*, and other members of the Enterobacteriaceae. The most prevalent ESBLs are included in three groups: TEM, SHV and CTX-M. CTX-M type ESBLs show only 40% identity to TEM or SHV ESBLs, but they are closely related to β-lactamases of the *Kluvyvera* spp.

Carbapenems were the drug of choice for the treatment of multidrug resistant gram-negative bacterial infections. Emergence of carbapenem resistant bacteria left limited options in the choice of antibiotics to treat the infections caused by them. These bacteria have the potential to spread rapidly within the hospital environment and also across the continents. Resistance to carbapenem is mostly due to production of enzymes-carbapenemases that hydrolyze carbapenems and other β-lactams. Acquired carbapenemases belong to group A (IMI, NMC, SME GES, and *Klebsiella pneumoniae* carbapenemase (KPC), group B metallo-β-lactamase (MBLs of VIM, IMP, GIM, NDM, SIM, and DIME) series), and group D (carbapenem hydrolyzing oxacillinases e.g. OXA). NDM-1 producing bacteria are important because the gene encoding this enzyme is located on a transmissible plasmid (of varying size). It is also associated with other resistant determinants.

© The Author(s). 2019 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and non-commercial reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
leading to extensive drug resistance which is usually exhibited by a majority of the NDM-1 producing enterobacteriaceae leaving only a few therapeutic options.

Therefore NDM-1 producing organisms are also named as “Super bugs”. NDM-1 was first identified and reported in 2009 in Klebsiella pneumoniae and Escherichia coli. It was isolated from Swedish patient of Indian origin who was previously hospitalized in New Delhi, India10. Indian investigators identified E. coli and Klebsiella species containing the gene for NDM-1 in multiple geographic regions in India, Pakistan and Bangladesh11.

The objective of this study was molecular characterization of the enzymatic mechanisms of resistance to β-lactam antibiotics in K. pneumoniae. A multiplex polymerase chain reaction (PCR) was setup for the detection of CTX-M, TEM and SHV genes. The reduced susceptibility to carbapenems by disk diffusion test prompted us to determine the molecular assay on these isolates to detect KPC and NDM-1 genes and also to analyze coexistence of AmpC producers among Klebsiella pneumoniae isolates at a tertiary care hospital.

Materials and Methods

Bacterial Isolates

A total of 250 nonrepetitive clinical isolates of K. pneumoniae were recovered over a period of one year (2014–2015) from our intensive care units (ICUs), i.e. medical ICU (MICU), neurosurgery ICU (NSICU), intensive thoracic unit (ITU), neonatal ICU (NICU), pediatric ICU (PICU), coronary care unit (CCU), and renal ICU (RICU). These isolates obtained from various clinical samples such as endotracheal aspirate (n = 103), blood (n = 56), urine (n = 31), pus (n = 22), sputum (n = 3), bronchoalveolar lavage (n = 11), central nervous catheter tips (n = 13), and sterile body fluids (n = 11). The present study was carried out in a tertiary care hospital of Karnataka, South India, with bed strength of 618.

Antimicrobial Susceptibility Testing

The susceptibilities of the different β-lactam and non-β-lactam antibiotics were tested and the results were interpreted as per the Clinical and Laboratory Standards Institute guidelines12. Escherichia coli ATCC 25,922 was used as a quality control. The antibiotics were procured from Hi Media, Mumbai, Maharashtra, India.

Minimum Inhibitory Concentration (MIC)

MIC determination was performed for all the isolates by agar dilution method (CLSI)12. Among NDM-1 producers, the MIC of meropenem and colistin ranged between 4–32 μg/mL and 0.25–256 μg/mL, respectively.

Detection of Extended Spectrum β-lactamase Producers

Isolates resistant or intermediately resistant to aztreonam, cefotaxime and/or ceftazidime were phenotypically detected for the presence of ESBL by the Double Disk Synergy test using cefotaxime (30 μg) and cefotaxime + clavulanic acid (30/10 μg) and (30/10 μg)12. K. pneumoniae ATCC 700603 was used as the ESBL positive control and E. coli ATCC 25,922 was used as the negative control.

Detection of Carbapenemase Producers

Isolates resistant or intermediately resistant to imipenem, ertapenem and/or meropenem were phenotypically detected for the production of carbapenemases by the modified Hodge test using ertapenem (10 μg) as an indicator disc and by comparing the zone diameter surrounding ertapenem discs supplemented with and without 0.5M EDTA (750 μg), an increase of zone diameter by ≥4 mm suggested the production of metallo-carbapenemase13.

MICs of meropenem and colistin (Sigma-Aldrich Corporation, St. Louis, US) were determined by the agar dilution method according to the guidelines from the CLSI13. The colistin breakpoint was evaluated using breakpoints for Enterobacteriaceae recommended by the European Committee on Antibiotic Susceptibility Testing. (Resistant: >2 μg/mL; sensitive: ≤2 μg/mL).

K. pneumoniae ATCC 700603 was used as a quality control.

Phenotypic Detection of AmpC Production

AmpC phenotype was detected by means of combined disc method using cefoxitin disc (30 μg) (Hi-media Laboratories, Mumbai), alone and in combination with 400 μg of phenylboronic acid (BA) (Sigma-Aldrich, Fluka, China)14.

DNA Extraction and Amplification

Total DNA was extracted as described by Lee J H15. The extracted DNA was subjected to multiplex PCR for the detection of ESBLs and uniplex PCR for NDM and KPC genes16, 17, 18.

The Universal primers used for PCR amplification were as follows:

- **bla**<sub>CTXM</sub> F-5’-CGCTTTGGATGCGAG-3’
- **bla**<sub>CTXM</sub> R-5’-GGTTTGGCGATCTGGTTTTC-3’
- **bla**<sub>TEM</sub> F-5’-ACCGGATATCGTTG-3’
- **bla**<sub>TEM</sub> R-5’-CTATGCATCATTTGCGAT-3’
- **bla**<sub>SHV</sub> F-5’-GTTCAATGTTGCGCTGAC-3’
- **bla**<sub>SHV</sub> R-5’-GGTTTGGCGATCTGGTTTTC-3’
- **bla**<sub>NDM</sub> F-5’-GAATGGCTCATACGATC-3’
- **bla**<sub>NDM</sub> R-5’-GAATGGCTCATACGATC-3’
- **bla**<sub>KPC</sub> F-5’-ATGTCACTGTATCGCCGCTC-3’
- **bla**<sub>KPC</sub> R-5’-TTTTCAGACCTTACTGCCC-3’

Results

A total of 250 K. pneumoniae were isolated from different clinical samples. The distribution of these isolates in clinical specimens and in ICUs is shown in Graphs 1 and 2.

Out of 250 K. pneumoniae isolates, 210 were screened positive for ESBL producers. The distribution of **bla** genes among ESBL positive K. pneumoniae and the gel picture showing the multiplex PCR are given in Graphs 3 and 4.
The susceptibility of ESBL producers found to be, amikacin (42%), netilmicin (36.5%), imipenem (35%), meropenem (34%), ertapenem (34%), ceftaperazone/sulbactum (31%) and piperacillin/tazobactam (28%), respectively.

Out of 250 *K. pneumoniae* isolates, a total of 165 (66%) isolates were carbapenem resistant. The distribution of carbapenem resistant *K. pneumoniae* isolates in clinical samples and in ICUs is given in Graphs 5 and 6.

### Prevalence of Carbapenemase Activity Based on Phenotypic tests

The phenotypic detection of carbapenemase production was performed as mentioned in Table 1.

### Table 1: Carbapenem resistant *Klebsiella pneumoniae* isolates positive for phenotypic tests

| Test     | Number | Percentage (%) |
|----------|--------|----------------|
| MHT      | 96     | 58.2           |
| EDTA*    | 28     | 16.9           |
| AmpC     | 6      | 3.6            |
| Total    | 130    | 78.7           |

Lane-1 = 100 bp ladder (Thermo scientific, Made in (EU) Lithuania). Lane -2, 5 and 14 = *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> positive amplicons (800 bp and 713 bp, respectively). Lane-3: Negative control (no template DNA added).
Molecular Characterization of Extended Spectrum β-lactamase and Carbapenemase Producing Klebsiella pneumoniae

Indian Journal of Critical Care Medicine, Volume 23 Issue 2 (February 2019)

Prevalence and Distribution of Carbapenemase Genes
Plasmid DNA was extracted from all the screened positive isolates15. The extracted plasmid DNA of each isolate was subjected to PCR detection of the blaNDM-1 and blaKPC genes by using target specific primers17,18, which is given in Table 217,18.

Agarose gel showing PCR amplified product of blaNDM-1 genes and susceptibility pattern of blaNDM-1 isolates is shown in Figure 1 and Graph 7.

**Table 2: Distribution of carbapenemase encoding genes in carbapenem resistant K. pneumoniae isolates**

| bla Encoding Genes | Number | Percentage (%) |
|--------------------|--------|----------------|
| blaNDM-1           | 16     | 9.7            |
| blaKPC             | nil    | 0              |

**Fig. 1:** Agarose gel showing PCR amplified product of blaNDM-1 gene. Lane-1 = 100 bp ladder (Thermo scientific, Made in (EU) Lithuania). Lane -2, 5 and 15 = blaNDM-1 positive amplicons (621 bp). Lane-3: Negative control (no template DNA added)

**Graph 7:** Susceptibility pattern of blaNDM-1 isolates by disk diffusion

In our study, the prevalence of ESBL production was 84% which was higher when compared to other studies. Sharma et al found 67.04%, Hoda Hassan et al 66.7%, and Mohammed et al 30%29,30. It was similar to a study by Feizabadi et al, with 72.1% prevalence22. None of the isolates were positive for CTX M gene in our study.

Among these 210 ESBL positive K. pneumoniae isolates, 14 (7.8%) of them found to be AmpC producers. A study by Hemalatha et al found 7 (9.2%) isolates positive for AmpC23. Two other Indian studies reported 8% and 43% AmpC producers24,25.

*K. pneumoniae* have acquired carbapenemases, the enzymes capable of breaking down most β-lactams including carbapenems, and confer resistance to these drugs. Reports indicate that carbapenemase producing enterobacteriaceae isolates seem to be increasing in number in the last few years. In our study, we found 66% of the isolates were carbapenemase producers. Gupta et al., from All India Institute of Medical Sciences, New Delhi in 2006 found that resistance to meropenem was 6.9%26. Nagaraj et al observed 75% of the *K. pneumoniae* isolates were carbapenem resistant in their study9. Aseem et al stated that 35.3% of the isolates in their study were resistant to carbapenems27.

Genotypic analysis of these carbapenem resistant isolates revealed the prevalence of the blaNDM-1, as 9.7% and none of the isolates were positive for blaKPC in our study. The epidemiology of *K. pneumoniae* producing KPCs varies geographically. Globally, the highest rate of carbapenem resistance has been reported in Greece with 68% resistance (KPC, OXA-48-like, NDM) followed by India (NDM, OXA-48-like, KPC) and eastern Mediterranean regions (NDM, OXA-48-like) with 54% resistance. USA, China (KPC, NDM, OXA-48-like) and Africa (OXA-48-like, NDM) have low resistance rates with 11, 11 and 4% respectively28.

The predominant enzymatic mechanism of resistance in Europe is KPC followed by OXA-48-like and NDM, while in USA; it is KPC followed by NDM and minimal due to OXA-48-like29. Several studies in Spain showed that most carbapenemase-producing *K. pneumoniae* harbored OXA-48-like or class B carbapenemases, and that of KPC-producing *K. pneumoniae* was very low (2–3%)30,31. Several studies in Spain showed that most carbapenemase-producing *K. pneumoniae* isolates in the country harbored OXA-48-like or class B carbapenemases, and that of KPC-producing *K. pneumoniae* was very low (2–3%)30,31.

Lascos et al reported 34.8% prevalence of blaKPC in CRE and Aseem et al observed only 3.7% of blaKPC out of 35.2% carbapenem resistant Klebsiella pneumoniae isolates1,30.

However, in our study, none of the isolates were positive for blaKPC. This correlates with findings of Nagaraj et al, who has observed 75% of blaNDM but not detected blaKPC from any of the carbapenem resistant isolates5. A study from Vellore, by Veeraraghavan et al showed the coexpression of blaNDM and blaOXA48 in 28%, blaNDM 19%, blaOXA48 in 13% and blaKPC was absent among the carbapenem resistant isolates31. The endemic spread of NDM-producing *K. pneumoniae* has also been reported in the UK, which has close relationships with India and Pakistan32.

The Balkan states, the Arabian Peninsula, and North African countries have also been recently considered as an additional reservoir of NDM producers33–36. In the Arabian Peninsula, NDM-1 was the most frequently encountered carbapenemase 46.5%, followed by OXA-48-like carbapenemases 32.5%36. In India, a study by Deshpande et al, reported NDM-1 was the most common carbapenemase type detected and accounted for 75.2% of the carbapenemase-producing isolates37. In another study, an incidence of blaNDM-1 in a single *K. pneumoniae* isolate was reported from a surgical site infection38. In our study we found 9.7% of NDM-1 which is similar to a study from tertiary care hospital of Northeast India where they found 8.67% of NDM possessing *Klebsiella pneumoniae* isolates39.
It was observed that the coexistence of bla gene along with NDM producers found to be 69% and 50% of SHV and TEM respectively. Previous studies from India have reported the coexistence of bla gene along with NDM producers\textsuperscript{39-41}. Similar studies from abroad also showed the presence of bla genes along with NDM producers\textsuperscript{42,43}. We found 78.7% of the CRKP were positive for the production of one or more carbapenemase mechanisms phenotypically. It has been observed that among the screened CRKP, 9.7% isolates found to harbor bla\textsubscript{NDM-1} gene. Rest of the CRKP isolates did not possess any of the resistant mechanisms tested. The important contributing factors leading to carbapenem resistance in these isolates might be due to the hyper production of ESBL, or other enzymatic mechanisms of carbapenem resistance like, IMP, VIM, and OXA, porin loss or efflux pumps\textsuperscript{44,45}.

**CONCLUSION**

In conclusion, carbapenem resistant *K. pneumoniae* have been considered as one of the greatest threats to the global health care in this century. The prevalence of KPC gene i.e., bla\textsubscript{KPC} in carbapenem resistant isolates from our geographical area (South Western India) seems to be very less. Pathogens that produce carbapenemases along with an ESBL or AmpC \( \beta \)-lactamases are particularly challenging for clinicians and are a major threat worldwide. Moreover, the widespread dissemination of the new NDM-1 metallo-\( \beta \)-lactamase requires particular attention as the genetic background demonstrates extreme mobility and versatility. The dissemination of such plasmids between different clinically important bacterial species may lead to serious public health issues, as *K. pneumoniae* accounts for one of the important bacterial species in the dissemination of antibiotic resistance genes; particularly in hospital environments. Therefore, the early detection of the bla\textsubscript{NDM-1} possessing *K. pneumoniae* isolates is necessary with any reduced susceptibility to the carbapenems. Timely intervention in the form of rapid detection, good infection control practices and judicious use of antibiotics will ensure that the spread of drug resistance among bacteria can be kept under control.

**REFERENCES**

1. Giske CG, Monnet DL, et al. ReAct-Action on antibiotic resistance. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob Agents Chemother. 2008;52:813–821.
2. Perez F, Endimiani A, et al. The continuing challenge of ESBLs. Curr Opin in Pharmacol.2007;7:459–469.
3. Johann DD, Pitout DK, et al. Extended spectrum beta lactamases producing Enterobacteriaceae: an emerging public health concern. Lancet Infect Dies 2008;8:159–166.
4. Kang CI, Kim SH, et al. Bloodstream infections due to extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*:risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob Agents Chemother 2004;48:4574–4581.
5. Lin CF, Hsu SK, et al. Genotypic detection and molecular epidemiology of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a regional hospital in central Taiwan. J Med Microbiol. 2010;59:665-671.
6. BabyPadmini S, Appala Raju B, et al. Detection of Enterobacteriaceae producing CTX-M extended spectrum beta-lactamasas from a tertiary care hospital in south India. Indian J Med Microbiol.2008;26:163-166.
7. Walsh TR. Emerging carbapenemases. A Global perspective.Int J Antimicrob agents 2010;36; SB-14.
8. Nagaraj S, Chandran SP, et al. Carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital, South India. Indian J Med Microbiol 2012; 30:93-95.
9. Nordmann P, Naas T, et al. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis.2011;17:1791-1798.
10. Yong D, Toleman MA, et al. Characterization of a new metallo-beta-lactamase gene, bla\textsubscript{NDM-1}, and a novel erythromycin esterase gene which was carried on a unique genetic structure in *Klebsiella pneumoniae*, sequence type 14 from India. Antimicrob Agents Chemother 2009;53:5046-5054.
11. Kumaraswamy KK, Toleman MA, 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.
12. Clinical and Laboratory Standards Institute. Performance standards for antimiicrobial susceptibility testing. Twenty-First informational supplement. CLSI document M100-S21. Wayne, PA: CLSI; 2011.
13. Yong D, Lee K, et al. Imipenem-EDTA disk method or differentiation of metallo-\( \beta \)-lactamase-producing clinical isolates of *Pseudomonas* spp and *Acinetobacter* spp. J Clin Microbiol 2002; 40:3798-3801.
14. Coudron PE. Inhibitor based methods for detection of plasmid-mediated AmpC beta-lactamases in *Klebsiella spp.*, *Escherichia coli*, and *Proteus mirabilis*. J Clin Microbiol 2005; 43:4163-4167.
15. Lee J H. Methicillin (Oxacillin)-Resistant Staphylococcus aureus strains isolated from Major food animals and their potential transfer to Humans. Appl Environ Microbiol 2003; 69:6489-6494.
16. Manoharan A, Premalatha K, et al. SARI study group India. Correlation of TEM, SHV and CTX-M extended-spectrum beta-lactamases among Enterobacteriaceae with their in vitro antimicrobial susceptibility. Indian J Med Microbiol 2011; 29:161-164.
17. Nordmann P, Poirel L, et al. How to detect NDM-1 producers? J.Clin. Microbiol. 2011; 49:718-721.
18. S. Vered, SR Keren, et al. Evaluation of PCR-Based testing for surveillance of KPC-producing Carbapenem-Resistant members of the Enterobacteriaceae family. J. Clin. Microbiol 2009; 47:3261-3265.
19. Sharma M, Pathak S, et al. Prevalence and antibiogram of Extended Spectrum \( \beta \)-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella spp*. J Clin Diagn Res 2013;7: 173-177.
20. Hoda Hassan, B.A. Molecular characterization of extended-spectrum beta-lactamase-Producing Enterobacteriaceae in a Saudi Arabian tertiary hospital. J Infect Dev Ctries 2014;8: 282-288.
21. Mohammed Y, Gadzama GB, et al. Characterization of Extended-Spectrum beta-lactamase from *Escherichia coli* and *Klebsiella* Species from North Eastern Nigeria Journal of Clinical and Diagnostic Research 2016;10:7-10.
22. Feizabadi MM, Raji N, et al. Distribution of bla(TEM), bla(SHV), bla(CTX-M) genes among clinical isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran.Iran Microb Microb Res 2013; 16:49-53.
23. Hemalatha V, Padma M, et al. Detection of AmpC beta lactamases production in *Escherichia coli* and *Klebsiella* by an inhibitor based method. Indian J Med Res 2007;126:220-223.
24. Manchanda V, Singh NP, et al. Molecular epidemiology of clinical isolates of AmpC producing *Klebsiella pneumoniae*. Indian J Med Microbiol 2006; 24:177-181.
25. Black JA, Thomson KS, et al. Evaluation of beta-lactamase inhibitors in disk tests for detection of plasmid-mediated AmpC beta-lactamases in well characterized clinical strains of *Klebsiella spp*. J Clin Microbiol 2005;43:4166-4171.
26. Gupta E, Mohanty S, et al. Emerging resistance to carbapenems in a tertiary care hospital in north India. *Indian J Med Res*. 2006;124:95-8.
27. Aseem R, Shenoy S, et al. Approach to carbapenemase detection in *Klebsiella pneumoniae* in routine diagnostic laboratories. J Clin Diagn Res 2016; 10:24-27.
28. Oteo J, Saenz D, et al. Carbapenemase-producing entero bacteriaceae in Spain in 2012. Antimicrob Agents Chemother 2013;57:6344 –47.
29. Palacios-Baena, ZR, et al. Comprehensive clinical and epidemiological assessment of colonisation and infection due to carbapenemase-producing Enterobacteriaceae in Spain. J. Infect. 2016;72:152–60.
30. Lascols C, Hackel M, et al. Increasing prevalence and dissemination of NDM-1 metallo-beta-lactamase in India: data from the SMART study (2009). J Antimicrob Chemother 2011;66:1992-7.
31. Veeraraghavan B, Shankar C, et al. Carbanapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. Pathogens and Global Health. 2017;111:240–46.
32. Voulgari E, Gartzonika C, et al. The balkan region: NDM-1-producing Klebsiella pneumoniae ST11clonal strain causing outbreaks in Greece. J. Antimicrob. Chemother 2014;69: 2091–97.
33. Dortet L, Brechard L, et al. Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures. Clin. Microbiol. Infect 2014;20:340–44.
34. Sonnevend A, Ghazawi A, et al. Characterization of KPC-type carbapenemase-producing Klebsiella pneumoniae strains isolated in the Arabian Peninsula. J. Antimicrob. Chemother 2015;70:1592–93.
35. Kazi M, Drego L, et al. Molecular characterization of carbapenem-resistant Enterobacteriaceae in tertiary care laboratory in Mumbai. Eur. J. Clin. Microbiol. Infect. Dis 2015;34:467–72.
36. Sarma JB, Bhattacharya PK, et al. Multidrug-resistant Enterobacteriaceae which include metallo-β-lactamase producers are the predominant pathogens of healthcare-associated infections in an Indian teaching hospital. Indian J Med Microbiol 2011;29:22-7.
37. Deshpande P, Rodrigues C, et al. New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: the treatment options with the carbapenems are compromised. J Assoc Physicians India 2010;58:147-9.
38. Chacko B, Peter JV, et al. The New Delhi Metallo-Beta-Lactamases: Their origins and implications for the intensivists. J Global Infect Dis 2011;3:319.
39. Ahmed N, Khalid S, et al. Occurrence of blaNDM Variants Among Enterobacteriaceae From a Neonatal Intensive Care Unit in a Northern India Hospital. Front Microbiol 2018;9:1-10.
40. Samuelsen Ø, Thilesen CM, et al. Identification of NDM-1-producing Enterobacteriaceae in Norway. J Antimicrob Chemother 2011;66:670-2.
41. Poirel L, Revathi G, et al. Detection of NDM-1-producing Klebsiella pneumoniae in Kenya. Antimicrob Agents Chemother 2011;55:934-36.
42. Kanj SS, Kanafani ZA. Current concepts in the antimicrobial therapy against resistant gram-negative organisms: extended-spectrum beta-lactamase-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae, and multidrug-resistant Pseudomonas aeruginosa. Mayo Clin Proc 2011;86:250-59.
43. Jeremiah S, Balaji V, et al. A possible alternative to the error prone modified Hodge test to correctly identify the carbapenemase producing Gram-negative bacteria. Indian J Med Microbiol. 2014;32(4):414.
44. Shanthi M, Sekar U, et al. OXA-181 beta lactamase is not a major mediator of carbapenem resistance in Enterobacteriaceae.
45. Li H, Zhang J, et al. Molecular characteristics of carbapenemase-producing Enterobacteriaceae in China from 2008 to 2011: predominance of KPC-2enzyme. Diagn. Microbiol. Infect. Dis 2013;78:63-65.