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Short Communication

Prevalence of New Delhi Metallo Beta-Lactamase (NDM) Producing Gram-Negative Bacteria from Different Tertiary Care Hospitals in Lahore, Pakistan

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MF, FR designed the study and interpreted data. RM collected the samples and performed experimental work. MF drafted the manuscript.

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ABSTRACT

Carbapenem resistance conferred by metallo-β-lactamase (MBL) has increasingly being reported worldwide among Enterobacteriaceae. In this study, 240 non repetitive gram negative clinical isolates were studied for antimicrobial resistance. Presence of bla NDM was determined by polymerase chain reaction. Among 240 strains, prevalence of MBL in K. pneumoniae, P. aeruginosa, E. coli and Acinetobacter Species was 26%, 52%, 27% and 5% respectively. Prevalence of blaNDM-1 gene was 51% identified in 14 strains of P. aeruginosa, 2 strains of A. Baumanni, 29 strains of E. coli and 18 strains of K. pneumoniae. In conclusion, high prevalence of blaNDM-1 in our strains is a serious concern. A careful use of carbapenems is important to prevent the spread of these organisms.

Carbapenems are the most potent agents among β-lactams for treatment of serious gram-negative bacterial infections. The use of these antibiotics is well-suited in hospital settings because of their broad spectrum activity and resistance to hydrolysis by most β-lactamases, including the extended-spectrum β lactamases (ESBL) (Bush *et al*., 1995). These properties have led to an increase use of carbapenems, especially in hospitals in which ESBLs are highly prevalent, and Pakistan is not an exception. Bacterial resistance to carbapenems is due to the production of carbapenem hydrolyzing enzymes called carbapenemases. These bacteria have the potential to spread rapidly within the hospital environment and also across continents. (Cornaglia and Rossolini, 2010). The main mechanism of carbapenem resistance is production of Metallo-β-lactamase (MBL) coded by bla_{KPC}, bla_{VIM} and bla_{IMP} (Nordmann *et al*., 2009). Chromosomal MBL was first detected in environmental and opportunistic pathogenic bacteria such as *Bacillus cereus*, *Aeromonas* spp., and *Stenotrophomonas maltophilia.* However, a dramatic increase in the detection and spread of acquired and transferable families of these metallo-enzymes (IMP, VIM, SPM, GIM, SIM and AIM enzymes) was reported in different studies (Nordmann *et al*., 2009). New Delhi metallo-β lactamase-1 (NDM-1) is newly identified transportable class B (zinc) MBL, which was initially detected in *Klebsiella pneumonia*e isolate. NDM-1 positive *Enterobacteriaceae ha*ve been widespread in India and also have been found in over 40 countries on every continent except Central and South America (Walsh *et al*., 2011)

Antibiotic resistance is one of the major health issues in Pakistan. Resistance in Gram-negative organisms was increasingly recognized with extended spectrum beta lactamases (ESBLs) being a major concern. Similarly, increase in multidrug-resistant organisms (MDR) and an alarmingly high resistance in *Enterobacteriaceae* against 3rd generation cephalosporins and carbapenems among *Pseudomonas* and *Acinetobacter* isolates has been reported. Like many other developing countries, prescription and dispensing practices are not satisfactory in public sector health facilities of Pakistan with prescribing rates of antimicrobials as high as 52% (Hafeez *et al*., 2004). *NDM*-1 producing bacteria are strong agents of serious therapeutic and public health hazard due to the bla_{NDM-1} gene. Growing numbers of infections has been reported in people from India, Pakistan, and the United Kingdom due to this enzyme (Yong *et al*., 2009). Carbepenam resistance in health care settings of Pakistan has also been reported (Kaleem *et al*., 2010; Ashraf and Altaf, 2015). In view of

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the increasing reports of *NDM-1* producing strains, this work was conducted to examine the occurrence of MBL in carbapenem-resistant isolates as well as prevalence of *NDM*-1 gene in gram negative bacterial isolates collected from different tertiary care hospitals of Lahore, Pakistan.

Materials and methods

A total number of 240 clinically significant, non-duplicate strains were collected from different tertiary care referral hospitals in this study during November 2017 to January 2018. The isolated strains included *Pseudomonas aeruginosa* (n= 90), *Klebsiella pneumonia* (n=70), *Acinetobacter baumannii* (n=12) and *Escherichia coli* (n=68). These strains were isolated from various samples (urine, pus, blood, sputum, cerebrospinal fluid, human vaginal swab etc. of patients. Gram negative strains were identified by gram staining and by standard biochemical tests.

Antimicrobial susceptibility testing for different classes of antimicrobials such as cephalosporins (cefepime, ceftazidime, cefotaxime); β-lactam/β-lactamase inhibitors (piperacillin/tazobactam); carbapenems (imipenem, meropenem); fluoroquinolones (ciprofloxacin) was performed for all the isolates by Kirby Bauer disk diffusion method and interpreted according to Clinical Laboratory Standards Institute guidelines 2009 (CLSI, 2009). Isolates showing resistance to at least one agent in each of these three or four groups (cephalosporins, carbapenems, fluoroquinolones and/or aminoglycosides) were considered as MDR and included in the study for further characterization.

ESBL production was determined in all bacterial isolates by double disk synergy test (DDST) as explained elsewhere (Jarlier *et al*., 1988). The phenotypic detection of the carbapenemase production was performed by the modified Hodge test by using a imipenem disc (10 µg) as described by CLSI (CLSI, 2009). DNA extraction was performed from the screened positive bacterial isolates through standard alkaline lysis method. PCR assays were performed to amplify $bla_{\text{NDM-1}}$ gene from the extracted DNA using the target specific primer set Forward: (5′-CAGCGCAGCTTGTCG-3′) Reverse: (5′-TCGCGAAGCTGAGCA-3′) (Poirel *et al*., 2010).

Results

We determined the antibiotic resistance rates of *E. coli, P. aeruginosa* and *K. pneumoniae* and *Acinector spp* isolated from different patients against different antibiotics that we evaluated in this study. Among the 240 bacterial isolates, 70% (168/240) were ESBL producing strains whereas 52% (124/240) were found to be MBL positive as determined by phenotypic methods. The percentage of MBL positive *K. pneumoniae, P. aeruginosa, E. coli* and *Acinetobacter Species* were as 26%, 52%, 27% and 5% respectively. Among MBL positive strains, most of the isolates were recovered from urine and pus. MBL positive bacterial strains (*E coli, K. pneumoniae*, *P. aeruginosa*) were found highly resistant to third generation cephalosporins and flouroquinones (Fig. 1A, B, C). The same holds true for carbapenms. All MBL producing strains showed higher resistance levels (>50%) against meropenem and imipenem.

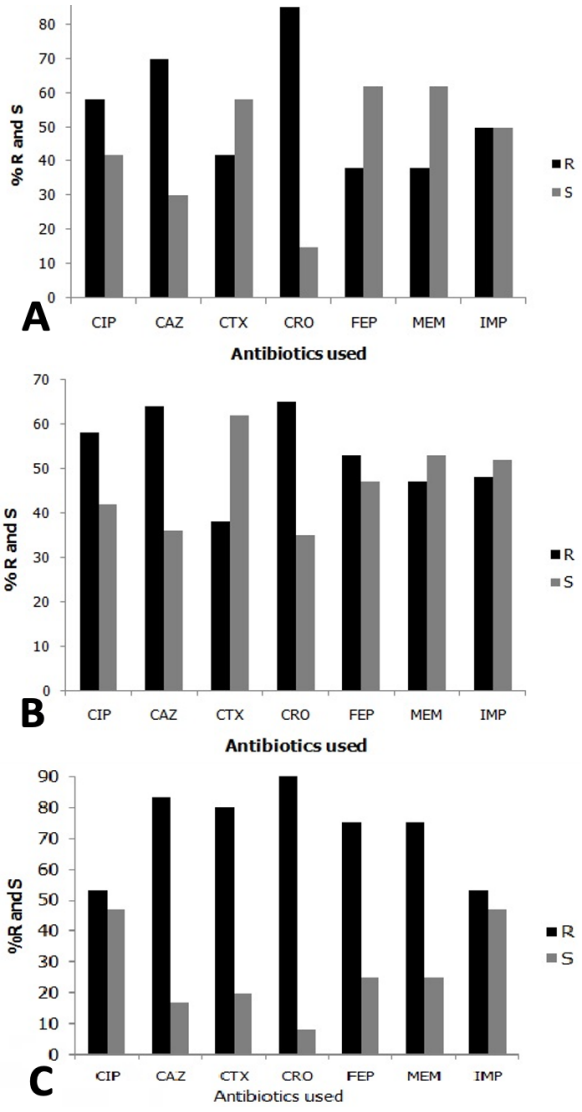

Fig. 1. Resistance pattern of MBL production *E. Coli* (A), *K. pneumonia* (B) and *P. aeruginosa* (C) strains against various antibiotics; R: Resistance; S: Sensitive; MBL: Metallo-β-lactamases; IPM: Imipenem; MEM: Meropenem; FEP: Cefepime; CIP: Ciprofloxacin; CAZ: Caftazidime; CTX: Cefotaxime; CRO: Ceftriaxone.

The PCR amplification of bla_{NDM-1} gene among MBL positive gram negative bacterial strains were shown in Figure 2. Out of 124 MBL producers, 63 (51%) isolates carried *bla* NDM gene. The isolates displaying bla_{NDM-1} gene identified through PCR included 14 strains of *P. aeruginosa*, 2 strains of *A. Baumanni,* 29 strains of *E. coli* and 18 strain of *K. pneumoniae.* Highest number of MBL gene (bla_{NDM-1}) was found in *E. coli.*

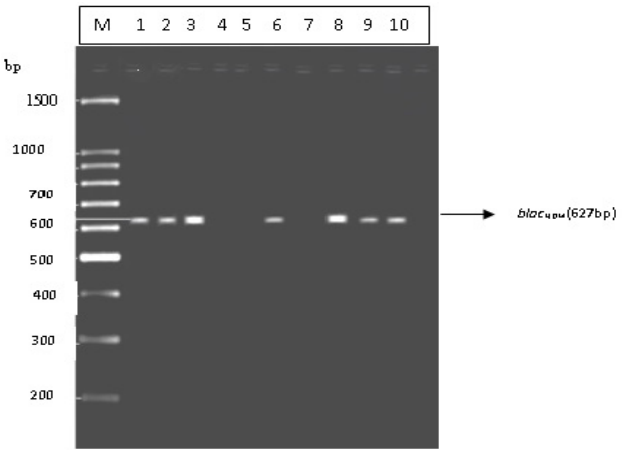

Fig. 2. PCR amplification of bla_{NDM-1} gene in different bacterial strains. M: DNA ladder 100bp. Lane 1,3,6: Amplified PCR product of *P. aeruginosa* bla_{NDM-1} gene. lANE 2: Amplified PCR product of *K. pneumoniae* bla_{NDM-1} gene. Lane 8,9,10: Amplified PCR product of *E. coli* bla_{NDM-1} gene.

Discussion

In the last few years, numerous reports indicated that infections due to *Enterobacteriaceae* are an important cause of morbidity and mortality worldwide. Carbapenems, e.g. imipenem and meropenem are the last choice to treat multidrug- resistant (MDR) Gram-negative bacteria infections (Nordman *et al*., 2011). However, resistance to carbapenemases producing Enterobacteriaceae isolates seems to be increasing worldwide. (Poirel *et al*., 2010; Nordmann *et al*., 2011). New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance has been identified in Enterobacteriaceae from numerous countries including those of the Indian subcontinent (Jarlier *et al*., 1988) and is a serious concern in Pakistan. In the present study, a high number of metallolactamases producing bacterial strains (52%) was observed. A previous study from Pakistan reported that 26% of isolates harbor ndm gene from two hospitals (Nahid *et al*., 2013). Similarly, among the metallo-lactamases strains, frequency of bla_{NDM-1} gene was found to be (51%) indicating substantial number of studied strains harboring bla_{NDM-1} gene. The present data and previous reports showed the need to control the distribution of carbapenemase producing Enterobacteriaceae in hospital as well as in the community (Yong *et al*., 2009; Jarlier *et al*., 1988). This control will require the improvement of fast, inexpensive and easy-to handle diagnostic methods for the recognition of carbapenemase producers. Because of the multi drug resistant nature of these bacteria, the worldwide distribution of Enterobacteriaceae producing NDMs will have serious implications for the treatment of hospital acquired infections. Moreover, there are small numbers of antibiotics available with activity against these gram-negative bacteria.

This study was restricted mainly to the detection of metallo beta lactamases and the resistance mediated by bla_{NDM-1} type gene in bacterial strains. Further research is required to evaluate the phylogenetic analysis about possible variations with respect to bla_{NDM-1} possessing bacterial isolates from other parts of the world. In conclusion, *NDM-1*-producing *Enterobacteriaceae* is a growing healthcare problem with increasing prevalence in Pakistan, especially in hospitalized patients, leaving few therapeutic options. A high prevalence of NDM-1 necessitates the implementation of strict infection control to prevent the spread of these organisms. In addition, careful use of carbepenem is important to prevent the spread of these organisms.

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Statement of conflicts of interest

There are no conflicts of interest.

References

Bush, K., Jacoby, G.A. and Medeiros, A.A., 1995. *Antimicrob. Agents. Chemother*., **39**: 1211-1233. https://doi.org/10.1128/AAC.39.6.1211

Cornaglia, G. and Rossolini, G.M., 2010. The emerging threat of acquired carbapenemases in Gram negative bacteria. *Clin. Microbiol. Infect*., **16**: 99-101. https://doi.org/10.1111/j.1469-0691.2009.03114.x

Nordmann, P., Cuzon, G. and Naas, T., 2009. *Lancet Infect. Dis*., **9**: 228-236. https://doi.org/10.1016/S1473-3099(09)70054-4

Queenan, A.M. and Bush, K., 2007. *Clin. Microbiol. Rev*., **20**: 440-458. https://doi.org/10.1128/CMR.00001-07

Walsh, T.R., Weeks, J., Livermore, M. and Toleman, M.A., 2011. *Lancet Infect. Dis*., **11**: 355-362.

https://doi.org/10.1016/S1473-3099(11)70059-7

Hafeez, A., Kiani, A.G., Din, S., Muhammad, W., Butt, K., Shah, Z. and Mirza, Z., 2004. *J. Pak. med. Assoc*., **54**:187–191

Kaleem, F., Usman, J., Hassan, A. and Khan, A., 2010. *J. Infect. Devel. Count*., **4**: 810-813. https://doi.org/10.3855/jidc.1050

Ashraf, W. and Ahmed, A., 2015. Am. J. Infect. Dis., **11**: 98-101. https://doi.org/10.3844/ajidsp.2015.98.101

Yong, D., Toleman, M.A., Giske, C.G., Cho, H.S., Sundman, K., Lee, K. and Walsh, T.R., 2009. *Antimicrob. Agents Chemother*., **53**: 5046-5054. https://doi.org/10.1128/AAC.00774-09

Clinical and Laboratory Standards Institute (CLSI). 2009. *Performance standards for antimicrobial susceptibility testing*. Nineteenth informational supplement. CLSI document; M100-S19, Wayne, PA, (USA).

Jarlier, V., Carlet, J., McGowen, J., Goossens, H., Voss, A. and Harbath, S., 1988. *Antimicrob. Resist. Infect. Contr*., **1**: 101-106.

Poirel, L., Lagrutta, E., Taylor, P., Pham, J. and Nordmann, P., 2010. *Antimicrob. Agents Chemother*., **54**: 4914-4916. https://doi.org/10.1128/AAC.00878-10

Nordmann, P., Naas, T. and Poirel, L., 2011. *Emerg. Infect. Dis*., **17**: 1791–1798. https://doi.org/10.3201/eid1710.110655

Nahid, F., Amir, A.K., Sana, R. and Rabaab, Z., 2013. *J. Infect. Publ. Hlth.,* **6**: 487-490.