ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 8 | August - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

DETECTION OF BLANDM-1 GENE IN MULTIDRUG RESISTANT ESCHERICHIA COLI IN A TERTIARY CARE HOSPITAL OF PUNJAB-NORTH WEST INDIA

Microbiology

Shaveta Kataria*	Senior Resident G.G.S. M.C.H. Faridkot, Punjab*Corresponding Author
Shivani	Senior Resident G.G.S. M.C.H. Faridkot, Punjab.
Shipra Galhotra	Assistant Professor G.G.S. M.C.H. Faridkot, Punjab.
Neerja Jindal	Professor G.G.S. M.C.H. Faridkot, Punjab.
Deepak Arora	Professor G.G.S. M.C.H. Faridkot, Punjab.
Shilpa Arora	Associate Professor G.G.S. M.C.H. Faridkot, Punjab.
Vishal Sharma	Associate Professor G.G.S. M.C.H. Faridkot, Punjab.
Pragati Grover	Assistant Professor G.G.S. M.C.H. Faridkot, Punjab.
Nitika Dhuria	Assistant Professor G.G.S. M.C.H. Faridkot, Punjab.
Hobinder Arora	Assistant Professor Social and Preventive Medicine G.G.S. M.C.H. Faridkot, Punjab.
Trimaan Kaur Bians	Junior student G.G.S. M.C.H. Faridkot, Punjab.
Anato K Swu	Junior student G.G.S. M.C.H. Faridkot, Punjab.

ABSTRACT

INTRODUCTION- Escherichia coli, a common pathogen usually associated with urinary tract infections and diarrhoea, is becoming a major concern among hospital acquired infections. The exact proportion of NDM-1 E. coli in different parts of our country is still not known. Hence, the present study was undertaken to determine the occurrence of multidrug resistant and blaNDM-1 positive E. coli among the hospitalised patients and to study the demographic and clinical characteristics of the patients infected with blaNDM-1 positive E. coli. **METHODOLOGY**- The present hospital based descriptive study was conducted on 260 gram negative bacilli isolated from 5307 consecutive clinical specimens. The brief history and sociodemographic profile of the patients was recorded. The antibiotic susceptibility testing of E. coli isolates was performed by Kirby Bauer disk diffusion method and MIC values were obtained by VITEK2 Compact system. All the multidrug resistant E. coli which showed reduced susceptibility to carbapenems were subjected to Modified Hodge test (the confirmation of carbapenemase production), Combined disk synergy test (metallo-β-lactamase production) and blaNDM-1 gene by PCR. RESULTS- Of 260 gram negative bacilli, 129 were E. coli and 77(59.7%) were multidrug resistant. Eleven(8.5%) isolates were screened as resistant to carbapenem on Kirby Baeur Disc Diffusion method and 8(6.2%) demonstrated the presence of blaNDM-1 gene on PCR, while 8 were positive in MHT and 7 in CDST. Sociodemographic and clinical characteristics of patients having blaNDM-1 positive E. coli infections showed that these isolates infects patients of all age groups and factors like gender, geographical distribution, education status of the patients play statistically insignificant role in the acquisition of this infection. **CONCLUSIONS-** The present study underscores the need of early detection which would help to prevent the dissemination of NDM-1 positive E. coli by implementation of strict infection control measures, formulation of antibiotic policy and use of antibiotic stewardship program.

KEYWORDS

Gram negative bacilli, multidrug resistant, New Delhi metallo-beta-lactamases, Combined disk synergy test, Modified hodge test.

INTRODUCTION

Escherichia coli, a common pathogen usually associated with urinary tract infection and diarrhoea, is becoming a major concern among hospital acquired infections. Till recently this organism could be treated effectively with the empirical antimicrobial therapies and among these third generation cephalosporins were quite effective with a rare inclusion of carbapenems. However, recent reports of resistance to carbapenems due to carbapenems hydrolyzing b-lactamases (carbepenemases) have poded a serious challenge to the treatment of these infections1. The carbapenemsases found in Enterobacteriacae can be metallo-b-lactamases, extended spectrum-b-lactamases, oxacillins or clauvulanic acid inhibitor b-lactamases 2. New Delhi metallo-b-lactamase-1 (NDM-1) is a novel type of metallo-b-lactamases (MBLs) which was first described in 2009 in a strain of Klebsiella pneumoniae isolated from the urine sample of a Swedish patient of Indian origin (who had been hospitalized in New Delhi)3. NDM-1 is able to inactivate all b-lactam antibiotics except aztreonam. This, it produces resistance against all compounds that contain b-lactam ring such as penicillins, cephalosporins and carbapenems. The gene that encodes for NDM-1 is called blaNDM-1 gene, that is reported to be mostly carried on transmissible plasmids ranging between 140 to 400 Kb. These plasmids also carry the genes for upto 14 other antibiotic resistant determinants and can transfer this resistance to other bacteria, resulting in the rapid dissemination of bacteria having multidrug resistance 4,5. the genes for upto 14 other antibiotic resistant determinants and can transfer this resistance to other bacteria, resulting in the rapid dissemination of bacteria having multidrug resistance[4,5].

Recently, there are several reports of NDM-1 producing *E. coli* from different parts of the World including India[5-7]. It has been observed in 30% of E. coli isolates in Chennai and in 13% in Haryana (with E. coli and *Klebsiella pneumoniae* in majority)[1]. In a study conducted in Bangalore, 14.7% of *E. coli* isolates were found to be NDM-1 positive[8]. Since, the exact proportion of NDM-1 carrying E. coli from our region is not known, the present study was undertaken to determine the occurrence of multidrug resistance and *bla*NDM-1 *E. coli* among the hospitalised patients and to study the sociodemographic and clinical characteristics of the patients infected with *bla*NDM-1 positive *E. coli*. This would help to review the strategies to limit the spread of these organisms.

MATERIAL AND METHODS

The present hospital based descriptive study was conducted on 260 gram negative bacilli (GNB) isolated from 5307 consecutive clinical specimens (blood, urine, pus, sputum, tracheal aspirate, endotracheal secretions, catheter tips, broncho-alveolar lavage and body fluids) received and processed in the department of Microbiology of a tertiary care hospital during the period of one and a half years. Out of 260 gram negative bacilli, 129 were *E. coli*. Standard microbiological techniques were used for isolation and identification of these isolates[9]. The brief history and sociodemographic profile of the patients was recorded. The

study was carried out with the consent of the Institutional Ethics Committee.

The antibiotic susceptibility testing of *E. coli* isolates was performed by Kirby Bauer disk diffusion method as per CLSI guidelines[10]. The antibiotics used were (concentration/ disk in microgram)- Ampicillin (10µg), Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Imipenem (10µg), Meropenem (10µg), Ertapenem (10µg), Piperacillin-tazobactam (100/10µg), Aztreonam (30µg), Cefipime (30µg), Colistin (300µg) and Norfloxacin (10µg) and Nitrofurantoin (300µg) for urine isolates (Hi-Media Ltd., Mumbai, India).

MIC values were obtained by VITEK 2 Compact system[11].

All the multidrug resistant E. coli (MDR) which showed reduced susceptibility to carbapenems (imipenem/ meropenem/ertapenem) (diameter of zones of inhibition ≤ 21mm) were subjected to Modified Hodge test (MHT) for the confirmation of carbapenemase production and Combined disk synergy test (CDST) for metallo-β-lactamase production.

MHT[10]- A 0.5 McFarland dilution of the *E. coli* ATCC 25922 was prepared in 5ml of broth or saline. A 1:10 dilution was streaked as lawn culture on Muller Hinton Agar (MHA) plate and a 10µg meropenem disk was placed in the centre. Using an inoculation loop, 3-5 colonies of test isolate along with the negative and positive controls were streaked in a straight line, atleast 25mm out from the edge of the disc. The plate was incubated overnight at 35±2°C for 16-24 hours. Following incubation, MHA plate was examined for enhanced growth around the test and quality control organism at the intersection of the streaks and the zone of inhibition. The presence of enhanced growth suggested that the test was positive for carbapenemase production.

CDST[12]- A 0.5 McFarland standard inoculum of the test strain in 1 in 10 dilution was streaked as lawn culture on Muller Hinton Agar (MHA) plate. Two imipenem (IMP) disks, one containing anhydrous EDTA and other containing only IMP (10µg) were placed 25 mm apart and an increase in zone diameter of more than 4mm around the IMP-EDTA disk compared to that of IMP alone was considered positive for MBL production.

Molecular detection of NDM-1 gene[13,14]- All the MDR *E. coli* which had shown resistance to carbapenems was further subjected to Polymerase chain reaction to identify NDM-1 gene. For this purpose Plasmid DNA was extracted by alkaline lysis method. 1µl of the extracted DNA was subjected to PCR with specific forward primer (5'-GGGCAGTCGCTTCCAACGGT) and reverse primer (5'-GTAGTGCTCAGTGTCGGCAT). DNA amplification was carried out in thermocycler (Quanta Biotech), with the following thermal cycling profile. This included initial denaturation at 94°C for 3 minutes (30 cycles for 30 sec each), annealing and elongation at 60°C and 72°C respectively for 30 seconds each and the final extension step for 3 minutes at 72°C. DNA fragments was visualized by electrophoresis on 2 percent agarose gel at 100 V for 1hour in 1X Tris acetate EDTA (TAE) containing 0.05 mg/l ethidium bromide.

STATISTICALANALYSIS

The data pertaining to sociodemographic and other clinical variables was entered in the form of data matrix in Microsoft ® Excel ® and analysed using IBM® SPSS® v 20.0.0. The descriptive statistics for categorical variables was represented in the form of frequencies and percentages and as means and standard deviations for continuous variables. The Goodness-of-Fit test for Discrete Multivariate data was accomplished using Exact multinomial test in R v [3.6.2].

RESULTS

The antibiogram of 129 *E. coli* isolates showed maximum resistance (97.7%) against 3rd generation cephalosporins, followed by 89.1% against ampicillin, 86% against ciprofloxacin and 84.5% against aztreonam. While resistance against aminoglycosides and piperacillin-tazobactam was 29.5% and 23.5% respectively, all the isolates were found to be sensitive to tigecycline and colistin. Out of 129 isolates, 77 (59.7%) were MDR and 11 (8.5%) were resistant to carbapenems.

The 11 carbapenem resistant isolates were further subjected to various phenotypic and genotypic assays for the detection of *bla*NDM-1 gene. Table 1 shows that while MHT demonstrated the presence of carbapenemases in 9, CDST showed the presence of metallo-β-lactamases in 7. 8 of these isolates were positive for *bla*NDM-1 gene in PCR assay. While all the 8 *bla*NDM-1 positive isolates showed positive results in MHT, only 7 were positive in CDST. There was one isolate (EC-137), which was both MHT and CDST positive but negative for *bla*NDM-1 gene on PCR (Table 1).

The analysis of MIC values of the 3 carbapenems (i.e. imipenem, meropenem and ertapenem) of the 8 *bla*NDM-1 positive *E. coli* isolates showed that all the 8 isolates had MIC values >1µg/ml but there was variation in their MIC values (2-16µg/ml) to different carbapenems (Table 2).

Sociodemographic and clinical characteristics of patients having *bla*NDM-1 positive *E. coli* infections were studied. These patients were aged between 19-65 years and their mean age was 38.37 years. The significance of other factors like gender, geographical distribution, religion, educational status of the patients having *bla*NDM-1 positive E. coli showed no statistically significance difference from all those having carbapenem resistant *E. coli* infections (p value ≥1) (Table 3). Study of various clinical characteristics showed that 75% (6/8) of NDM-1 carrying isolates had previous history of surgical intervention, mechanical ventilation and/or other medical instrumentation. As many as 62.5% (5/8) isolates were from the patients with previous history of ICU stay and 75% (6/8) had history of prior exposure to antibiotics for ≥14 days and of long duration of hospitalisation. All the eight isolates were from indoor patients. While 3 were from patients of orthopaedics department having bone and skeletal tissues infections, 2 were from surgery department with urinary tract infection and peritonitis (one each). The rest of 2 patients were from ICU having septicaemia and septic shock. The maximum (50%) isolates were from the specimens of pus, followed by urine and blood (25% each) and there was no statistically significant difference between these specimens for isolation of *bla*NDM-1 positive *E. coli* (p value = 0.295).

Table 1-The different characteristics of carbapenemase resistant *E. coli* (n=11)

Isolate	Source of infection	Department of isolation	Carbapenem resistant by Kirby Baeur disk diffusion test	Carbapenem resistant by Vitek 2 Compact system	MHT	CDST	PCR
EC-7	Blood	O & G	Pos	Pos	Neg	Neg	Neg
EC-8	Urine	Med	Pos	Pos	Pos	Neg	Pos
EC-55	Pus	Ortho	Pos	Pos	Pos	Pos	Pos
EC-129	Blood	MICU	Pos	Pos	Pos	Pos	Pos
EC-132	Pus	Ortho	Pos	Pos	Pos	Pos	Pos
EC-136	Blood	Surg	Pos	Pos	Pos	Pos	Pos
EC-137	Blood	Surg	Pos	Pos	Pos	Pos	Neg
EC-194	Pus	Ortho	Pos	Pos	Pos	Neg	Pos
EC-244	Urine	Surg	Pos	Pos	Pos	Pos	Pos
EC-247	Pus	Surg	Pos	Pos	Pos	Pos	Pos
EC-256	Blood	ENT	Pos	Pos	Neg	Neg	Neg
Total positive			11	11	9	7	8

O & G- Obstetrics and Gynaecology, Med- Medicine, Ortho- orthopaedics, MICU- medicine ICU, Surg- surgery, ENT-, ENT- Otorhinolarynology, Pos- positive, Neg- Negative.

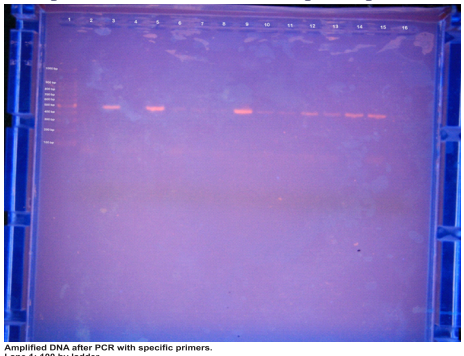
Table 2- MIC vales of NDM-1 positive *E. coli* (n=8)

Isolate	Imipenem	Meropenem	Ertapenem
EC-8	2	2	≥8
EC-55	8	8	8
EC-129	8	16	≥8
EC-132	≥16	≥16	8
EC-136	≥16	≥16	8
EC-194	16	8	8
EC-244	8	≥16	≥8
EC-247	≥8	8	8

Table 3 Sociodemographic and clinical characteristics of patients infected with *bla*NDM-1 positive *E. coli*.

Factors	Carbapenem resistant (n=11)	NDM-1 positive (n=8)	p value (Exact multinomial test) (<0.05 statistically significant)
Mean age	38 years	38.37 years	-
Gender			0.7266
Male	5 (45.4)	5 (62.5)	
Female	6 (54.5)	3 (37.5)	
Geographical distribution			1
Rural	4 (36.4)	4 (50)	
Urban	7 (63.6)	4 (50)	
Religion			0.2891
Hindu	3 (27.15)	2 (25)	
Sikh	8 (14.8)	6 (75)	
Educational Status			0.871
Illiterate	5 (45.4)	3 (37.5)	
Primary	2 (18.1)	2 (25)	
High	1 (9.1)	1 (12.5)	
Intermediate	1 (9.1)	1 (12.5)	
Graduate	2 (18.1)	1 (12.5)	
Department			0.2333
Surgery	4 (36.4)	3 (37.5)	
Ortho	3 (27.3)	3 (37.5)	
Medicine	1 (9.1)	1 (12.5)	
ENT	1 (9.1)	0 (0)	
Obs-gynae	1 (9.1)	0 (0)	
MICU	1 (9.1)	1 (12.5)	
Site of infection/clinical disease			0.4434
Bone and skeletal	3 (27.27)	3 (37.5)	
GIT	2 (18.18)	1 (12.5)	
Sepsis	2 (18.18)	2 (18.18)	
UTI	2 (18.18)	2 (25)	
RTI	1 (9.09)	0 (0)	
Genital	1 (9.09)	0 (0)	
Specimens			0.295
Pus	6 (54.54)	4 (50)	
Urine	2 (18.18)	2 (25)	
Blood	2 (18.18)	2 (25)	
ETT sec	1 (9.09)	0	

Figure1- Amplified DNA after PCR with specific primers



Amplified DNA after PCR with specific primers.
Lane 1: 100 by ladder.
Lane 2: Negative control.
Lane 3: Positive control (475 bp).
Lanes 5,9,10,11,12,13,14 and 15: Clinical isolates showing positive result (475 bp).
Lanes 4,6,7,8,16: Clinicial isolates showing negative result.

DISCUSSION

The spread of NDM-1, a metallo-β-lactamase in *E. coli* is a major challenge in the field of infectious diseases since its presence makes the organism highly resistant to most of the currently available antibiotics including carbapenems, thus puts an end to the current pharmacopoeia[8]. The present study was therefore undertaken to detect *bla*NDM-1 in clinical isolates of MDR E. coli in a tertiary care hospital of Malwa region of Punjab (Northwest India) from where no such study has been conducted so far (to the best of our knowledge).

The study of 129 *E. coli* strains isolated from 5307 clinical specimens showed that almost 60% (59.68%) were MDR with more than 80% resistance against ampicillin, 3rd gen cephalosporins, ciprofloxacin and even aztreonam. The reported rates of MDR GNB from different centres vary widely and falls between 46.5% to 70.13% in different studies[15-17]. This could be because multidrug resistant phenotypes are not the result of single genetic event and have probably been selected by the empirical and frequent use of various high level broad spectrum antibiotics during the prolong period of hospitalization of the patients[18,19].

In the present study, out of 129 *E. coli* isolates, 11 (8.5%) were screened as carbapenem resistant on the basis of Kirby Bauer Disk diffusion method and 8 (6.2%) had shown the presence of NDM-1 gene on PCR (Table 1). All these 8 isolates had given positive result in MHT. This corroborates the findings of Bora et al who had reported 100% positivity of MHT in NDM-1 positive *E. coli* isolates[20]. Similar results for MHT positivity have also been observed among NDM-1 producing Enterobacteriaceae in a study from Pakistan[21]. This indicates that MHT is highly sensitive in the detection of NDM-1 carrying *E.coli*. CLSI also recommends MHT to detect carbapenemase production in Enterobacteriaceae isolates although; the sensitivity and specificity of the test for the detecting low level metallo-β-lactamase production are not known[10]. However, a previous study had reported weakly positive MHT results in 11 of 15 NDM-1 producing Enterobacteriaceae isolates including those of *E.coli*[22]. Other phenotypic methods like combined disk synergy test and double disk synergy test have also been used for enhanced detection of blaNDM-1 gene[23-25]. We performed CDST and 7 isolates were found to be positive for MBL production. However, this test failed to detect two (25%) of the 8 NDM-1 positive isolates. These two isolates had given positive result for MHT. Thus, in the present study the isolates which were missed by CDST were detected as producer of carbapenemase by MHT and VITEK 2 Compact system. This shows that while phenotypic assays (which are easy to perform) have significance in the preliminary screening of NDM-1, molecular diagnostic techniques are essential to fully identify and characterise NDM-1 positive strains.

In the present study, *bla*NDM-1 gene was detected in 6.2% (8/129) of the *E. coli* isolates. This is almost similar to the findings of Bora et al from Northeast India. They reported *bla*NDM-1 gene *E. coli* in 5.18% (14/270) of their isolates[20]. A study from Sir Ganga Ram hospital New Delhi demonstrated slightly higher (8.1%) prevalence of *bla*NDM-1 gene in the *E. coli* isolates of their indoor patients[26]. NDM-1 producing *E. coli* also been reported from other parts of the country[1,8]. This shows the rapid dissemination of NDM-1 *E. coli* in various Indian hospitals although the exact data of its prevalence needs further studies and exploration.

In the present study, 8 isolates of *E. coli* which were *bla*NDM-1 gene positive showed variability in their MIC values to different carbapenems. Similar findings have also been reported by Bora et al[20]. The variations could be explained by considering the fact that MIC values for different carbapenems depends on the expression of carbapenemase enzymes, the bacterial species and the presence of other resistance mechanisms such as ESBLs and AmpC-β-lactamases, reduced permeability and/ or efflux pumps.

The sociodemographic profile and clinical characteristics of patients infected with *bla*NDM-1 positive *E. coli* was studied. Their age ranged between 19-65 years and mean age was 38.37 years. Bora et al had reported patients even with wider age range (3 days to 72 years), having infections caused by these organisms[20]. The role of the factors like gender, geographical distribution and educational status of the patients with *bla*NDM-1 positive *E. coli* infections in comparison with patients having carbapenem resistant infections was not found to be statistically significant. However, what was important was that more than 60% of patients had the history of previous surgeries, admission in ICUs, artificial ventilation, prior exposure of antibiotics, presence of concomitant infections and longer duration of hospitalisation.

In the present study, all the *bla*NDM-1 gene positive *E. coli* isolates were obtained from the patients admitted in hospital, while Bora et al obtained these isolates from both IPD (in-patients department) and OPD (out-patients department) patients[20]. In other hospitals, NDM-1 positive isolates have been more frequently isolated from ICU[27,28]. We observed higher incidence of NDM-1 producing *E. coli* in non ICU patients (6/8), than that in ICU patients (2/8)[27,28]. Which is similar to the study of Bora et al20. Our study also showed that NDM-1 positive E. coli could be isolated from patients of any department, any site of infection and any clinical specimen (Table 3). This suggests that there is high potential of spread of NDM-1 organisms which is facilitated by conditions like overcrowding, low level of hygiene, weak hospital

antibiotic policies and over the cover availability of antibiotics. Hence, early detection of this gene could help in limiting the spread of these organisms.

An important limitation of our study was that we could not perform gene sequencing of the NDM-1 carrying isolates because of certain constrains. This would have helped in their phylogenetic analysis and to know the variation in the *bla*NDM-1 gene of our isolates with respect to *bla*NDM-1 possessing *E. coli* from other parts of India and abroad.

CONCLUSIONS

The high proportion of infections caused by MDR *E. coli* and the presence of NDM-1 gene 6.2% (8/129) is quite worrisome as it leaves the clinician with the only option of the use of colistin and polymyxin B (highly costly and toxic drugs) for the treatment of these infections. The situation could only be saved by the timely detection and prevention of spread of NDM-1 related infections by implementing the hygienic measures and isolation of the carriers. For the early detection of NDM-1 positive isolates, both the phenotypic and molecular could be employed along with antimicrobial susceptibility testing. The present study also highlights the importance of regular monitoring of drug resistance and the need of implementation of infection control practices, formulation of antibiotic policy and use of antibiotic stewardship program to decrease the rate of drug resistance in our clinical isolates.

REFERENCES

1. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm. Clin Microbiol Rev. 2005;18:306-25.
2. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase- producing bacteria. Lancet Infect Dis. 2009;9:228-36.
3. 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 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.
4. 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;11:355-62.
5. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, 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(9):597-602.
6. Fomda BA, Khan A, Zahoor D. NDM-1 (New Delhi metallo-beta-lactamase-1) producing gram negative bacilli: emergence and clinical implications. Indian J Med Res. 2014;140(5):672-8.
7. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi metallo-beta-lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. J Assoc Physicians India. 2010;58:147-9.
8. Shenoy KA, Jyothi EK, Ravikumar R. Phenotypic identification and molecular detection of blaNDM-1 gene in multidrug resistant gram negative bacilli in a tertiary care centre. Indian J Med Res. 2014;139: 625-31.
9. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. The Enterobacteriaceae. In: Color atlas and textbook of diagnostic microbiology. 7th ed. Philadelphia: Wolters Kluwer 2017.p.214-80.
10. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 26th informational supplement. Wayne, PA: CLSI;2016.
11. Funke G, Monnet D, Debernardis C, Graevenitz VA, Freney J. Evaluation of the VITEK 2 system for rapid identification of medically relevant gram negative rods. J Clin Microbiol. 1998;36:1948-52.
12. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem susceptible metallo-beta-lactamase producing gram negative bacilli in the clinical laboratory. J Clin Microbiol. 2006;44:3139-44.
13. MACHEREY-NAGEL Genomic DNA from microorganisms user's manual NucleoSpin® Microbial DNA 2015.
14. Manchanda V, Rai S, Gupta S, Rautela RS, Chopra R, Rawat DS, et al. Development of Taq Man real time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of Escherichia coli, Klebsiella pneumoniae and Acinetobacter baumannii. Indian J Med Microbiol. 2011;29:249-53.
15. Aliyu S, Smaldone A, Larson E. Prevalence of multidrug resistant gram negative bacteria among nursing home residents: a systematic review and meta-analysis. Am J Inc Control. 2017;45(5):512-8.
16. Maraki S, Mavros MN, Kofteridis DP, Samonis G, Falagas ME. Epidemiology and antimicrobial sensitivities of 536 multi drug resistant gram negative bacilli isolated from patients treated on surgical wards. Surg Infect. 2012;13(5):326-31.
17. Khanal S, Joshi DR, Bhatta DR, Devkota U, Pokhrel BM. β-lactamase producing multidrug resistant bacterial pathogens from tracheal aspirates of intensive care unit patients at National institute of neurological and allied sciences, Nepal. ISRN Microbiol. 2013;847569:1-5.
18. Kahvecioglu D, Ramiah K, McMaughan D, Garfinkel S, McSorley VE, Nguyen QN, et al. Multidrug resistant organism infections in US nursing homes: a national study of prevalence, onset and transmission across care settings, October 1, 2010-December 31, 2011. Infect Control Hosp Epidemiol. 2014;35(3):S48-55.
19. Pop-Vicas A, Strom J, Stanley K, D'Agata EM. Multidrug resistant gram negative bacteria among patients who require chronic hemodialysis. Clin J Am Soc Nephrol. 2008;3:752-8.
20. Bora A, Ahmed GU, Hazarika NK, Prasad KN, Shukla SK, Randhawa V. Incidence of blaNDM-1 gene in Escherichia coli isolates at a tertiary care referral hospital in Northeast India. Indian J Med Microbiol. 2013;31(3):250-6.
21. Perry JD, Naqvi SH, Mirza IA, Alizai SA, Hussain A, Ghirardi S, et al. Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan and evaluation of two chromogenic media. J Antimicrob Chemother. 2011;66:2288-94.
22. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1 and OXA-181 producing Enterobacteriaceae in Indian hospital: reports from the SENTRY antimicrobial surveillance program, 2006-2007. Antimicrobial Agents Chemother. 2011;55:1274-8.
23. Girlich D, Poirel L, Nordmann P. Value of the modified hodge test for detection of emerging carbapenemases in Enterobacteriaceae. J Clin Microbiol. 2012;50:477-9.
24. Pasteran F, Mendez T, Rapoport M, Guerriero L, Corso A. Controlling false positive results obtained with the hodge and masuda assays for detection of class A carbapenemase in species of Enterobacteriaceae by incorporating boronic acid. J Clin Microbiol. 2010;48:1323-32.
25. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Microbiol. 2011;49:718-21.
26. Ganga Ram study finds high levels of superbug NDM-1. [Internet]. [Last accessed 2019 Apr 20]. Available from: .http://www.articles.timesofindia.indiatimes.com/2011-10-05/Delhi/30246456_1_1_ndm-1-antibiotic-resistant-superbug-mark-toleman.
27. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi metallo-beta-lactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. J Assoc Physicians India. 2010;58:147-9.
28. Seema K, Ranjan-Sen M, Upadhyay S, Bhattacharjee A. Dissemination of the New Delhi metallo-beta-lactamase-1 (NDM-1) among Enterobacteriaceae in a tertiary referral hospital in north India. J Antimicrob Chemother. 2011;66:1646-7.