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ORIGINAL ARTICLE

Phenotypic detection and molecular characterization of beta-lactamase genes among *Citrobacter* species in a tertiary care hospital

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ABSTRACT

Objective: To examine the distribution, emergence, and spread of genes encoding beta-lactamase resistance in *Citrobacter* species isolated from hospitalized patients in a tertiary care hospital. **Methods:** A prospective study was conducted in a 1000-bed tertiary care center in Pune, India from October 2010 to October 2013. A total of 221 *Citrobacter* spp. isolates were recovered from clinical specimens from different patients (one isolate per patient) admitted to the surgical ward, medical ward and medical and surgical Intensive Care Units. Polymerase chain reaction (PCR) assays and sequencing were used to determine the presence of beta-lactamase encoding genes. Conjugation experiments were performed to determine their transferability. Isolate relatedness were determined by repetitive element based-PCR, enterobacterial repetitive intergenic consensus-PCR and randomly amplified polymorphic DNA. **Results:** Among 221 tested isolates of *Citrobacter* spp. recovered from various clinical specimens, 179 (80.9%) isolates showed minimum inhibitory concentration (MIC) >4 μg/ml against meropenem and imipenem. One hundred and forty-five isolates with increased MICs value against carbapenems were further processed for molecular characterization of beta-lactamase genes. Susceptibility profiling of the isolates indicated that 100% retained susceptibility to colistin. Conjugation experiments indicated that bla_{NDM-1} was transferable via a plasmid. **Conclusion:** The ease of NDM-1 plasmid transmissibility may help their dissemination among the *Citrobacter* species as well as to others in *Enterobacteriaceae*. Early detection, antimicrobial stewardship and adequate infection control measures will help in limiting the spread of these organisms.

Key words: bla_{NDM-1}, bla_{VIM-2}, combined-disc synergy test, *Citrobacter freundii, Citrobacter koseri*, double-disc synergy tests, metallo-beta-lactamase, modified Hodge test

INTRODUCTION

Citrobacter species are an important cause of nosocomial infections, particularly involving the urinary and respiratory tracts of hospitalized patients and are inhabitants of the human gastrointestinal tract, often found in human feces and hospital environment.[1,2] In recent years, *Citrobacter* species have been commonly isolated from various clinical specimens such as urine, pus, and blood. A significant increase in nosocomial infections caused by *Citrobacter* species has been reported, especially in Neonatal Intensive Care Units (NICUs).[3-5] It has been reported to cause neonatal sepsis, brain abscess, urinary tract infections (UTIs), bloodstream infections, skin and surgical site infections, burns infections, intra-abdominal sepsis, meningitis, and pneumonia.[3-5] Fatality in *Citrobacter* septicemia ranges

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from 33% to 48%[6] Infant survivors may have significant damage to the central nervous system, including profound mental retardation, seizures, and hemiparesis.[7] There is very little data dealing with *Citrobacter* isolates in India: Neither its antibiotic sensitivity pattern nor the molecular characterization of its resistance genes. This study focused on determining the antibiotic resistance pattern and prevalence of metallo-beta-lactamase (MBL) genes in carbapenem-resistant *Citrobacter* spp. isolated in a tertiary care center.

MATERIALS AND METHODS

The bacterial isolates

A prospective study was conducted in a 1000-bed tertiary care center in Pune, India from October 2010 to October 2013. A total of 221 *Citrobacter* spp. isolates were recovered from clinical specimens of hospitalized patients admitted to the medical and surgical ICUs. Samples were collected from patients, using strict aseptic precautions and in accordance with standard protocols[8] and immediately processed without delay. The isolates were obtained from various clinical specimens such as urine, blood, pus, respiratory secretions (sputum, endotracheal secretions, broncho-alveolar lavage (BAL), and bronchial wash), and other sterile body fluids. Bacterial identification was performed by routine conventional microbial culture and biochemical tests using standard recommended techniques.[8] The organism was identified up to the species level using VITEK-GNI cards (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

The antimicrobial susceptibility test was performed by the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) guidelines.[9] The antibiotics tested were as follows (potency in μg/disc): Ampicillin (10), cefuroxime (30), cefpodoxime (CPD) (30), ceftazidime (30), cefepime (30), cefotaxime (30), piperacillin (100), ticarcillin (75), piperacillin-tazobactam (100/10), ticarcillin-clavulanic acid (75/10), aztreonam (30), imipenem (IP) (10), meropenem (10), ertapenem (10), colistin (10), gentamicin (10), tobramycin (10), amikacin (30), netilmicin (30), ciprofloxacin (5), levofloxacin (5), lomefloxacin (10), and ofloxacin (5) (Hi-Media Laboratories Pvt., Ltd., Mumbai, India). *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains.

Minimum inhibitory concentration determination

Minimum inhibitory concentrations (MICs) of antibiotics were determined by VITEK-2 AST-GN25 and AST-GN280 susceptibility cards in accordance with the CLSI recommendations and manufacturer's instructions, except that the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used for tigecycline and colistin.[9,10] MICs were further determined by the E-test (bioMérieux, Marcy l'Etoile, France).

Phenotypic screening for carbapenemase production

Isolates with reduced susceptibility to meropenem and IP (diameter of zones of inhibition ≤13 mm) by disc diffusion method and showed higher MICs as determined by the E-test were further screened for the production of carbapenemase. The phenotypic detection of the carbapenemase production was performed by the modified Hodge test (MHT) using ertapenem and meropenem discs (10 μg) for each isolate as per CLSI guidelines.[9] For MHT *K. pneumoniae* ATCC BAA-1705 and BAA-1706 were used as positive and negative controls, respectively. MBL production detected by double-disc synergy tests (DDST) with both IP and meropenem discs (10 ug) plus ethelenediaminetetraacetic acid (EDTA) (750 ug) for all the carbapenem resistant isolates, as described earlier by Lee *et al*. and combined-disc synergy test (CDST) as described previously by Franklin *et al*. using IP and meropenem discs (10 μg) and 0.1 M EDTA (292 μg).[11,12] *K. pneumoniae* ATCC BAA-2146 and *P. aeruginosa* ATCC 27853 were used as positive and negative controls, respectively. MBL (IP/IP-inhibitor [IPI]) E-test was carried out to detect MBL as per manufacturer's instructions.

DNA extraction and molecular detection

DNA was extracted from the bacterial isolates using the spin column method (QIAGEN; GmbH, Hilden, Germany) as per manufacturer's instructions. Polymerase chain reaction (PCR)-based detection of beta-lactamase (extended-spectrum beta-lactamase [ESBL]) genes (bla_{CTXM}, bla_{SHV}, bla_{TEM} and bla_{OXA}), Ambler class B MBLs (bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{GIM}, bla_{SIM} and bla_{NDM-1}), Ambler class D (bla_{OXA-23}, bla_{OXA-24} and bla_{OXA48}) and serine carbapenemases (bla_{KPC}, bla_{GES} and bla_{NMC}) were carried out on the isolates using Gene Amp 9700 PCR System (Applied Biosystems, Singapore).[13-16] PCR products were run on 1.5% agarose gel, stained with ethidium bromide visualized under ultraviolet light and photographed. The amplicons were purified using QIAquick PCR purification kit (QIAGEN; GmbH, Hilden, Germany).

DNA sequencing and sequence analysis

Automated sequencing was performed on an ABI 3730XL DNA analyzer using the Big Dye system (Applied Biosystems Foster City, CA, USA). Sequences were compared with

known sequences using the BLAST facility (http://blast.ncbi.nlm.nih.gov).

Conjugation experiments

Transfer of resistance genes by conjugation was assayed by mating experiments in Luria-Bertani broth using the clinical *Citrobacter* isolates (parental strains) as donors and an azide-resistant *E. coli* J53 as the recipient strain using 1:10 ratio. The transconjugants were selected on Luria-Bertani agar with selection based on growth on agar in the presence of ceftazidime (30 μg/ml) and sodium azide (100 μg/ml).[16] Plasmids were separated and compared by co-electrophoresis with plasmid of known sizes from *E. coli* (V517 and 39R861) on a horizontal 0.5% agarose gel at 50 volts for 3 h. Bands were visualized with UV transilluminator after staining with 0.05% ethidium bromide.

Strain molecular typing

Repetitive element based-PCR (REP-PCR), enterobacterial repetitive intergenic consensus (ERIC-PCR) and randomly amplified polymorphic DNA (RAPD) assays were performed to characterize *Citrobacter* spp. recovered from patients.[17,18]

Plasmid analysis

Plasmids from each parental strain and its transconjugants were extracted by using Qiagen plasmid mini kit (GmbH, Hilden, Germany) as per manufacturer's Instructions. Extracted plasmid DNA were subjected to plasmid-based replicon incompatibility (Inc.) typing by using eighteen pairs of primers to perform five multiplex and three single PCRs which recognized F, FIA, FIB, FIC, B/O, X, Y, N, P, W, T, A/C, HI1, HI2, I1-Ic, L/M, K, and FII replicons as described previously.[19] Plasmid replicons were determined for the ESBL and carbapenemase-producing clinical isolates.

RESULTS

A total of 221 *Citrobacter* spp. isolates were recovered from clinical specimens from different patients (one isolate per patient) admitted to the surgical ward, medicinal ward and medical and surgical ICUs of a tertiary care center. Distribution of *Citrobacter* spp. isolates from various samples is shown in Figure 1 and Table 1.

The largest proportion of specimens were from UTI (98 or 44%), followed by 19% (43) in skin and soft tissue infections (SSTIs), 13% (29) in blood stream infections (BSIs), 14% (30) in Intra-abdominal infections (IAIs) and miscellaneous and 10% (21) in Respiratory tract infections (RTIs), respectively. Among 221 tested isolates, 179 (80.9%) isolates showed MIC >4 μg/ml against IP and meropenem. The majority of Carbapenem-resistant *Citrobacter* spp. were from urine 48% (87), followed by 21% (37) in wound swabs and pus, 12% (21) in IAIs and miscellaneous, 11% (20) in blood and endo-tracheal aspirate (09), BAL (05) both together constitute 08% (14), respectively [Table 1].

One hundred and ninety-eight out of 221 isolates, showed resistance to penicillins and third generation cephalosporins by the disc diffusion method, among them 179 (80.99%) were found to exhibit reduced susceptibility to IP and meropenem (diameter of zones of inhibition ≤15 mm) and 145 were found to have MIC values for IP, meropenem and ertapenem ranging from 8 to 32 μg/ml as per CLSI breakpoints. All the 221 isolates were found to be susceptible to colistin while (167/221) 75.56% were susceptible to tigecycline *in vitro* as per EUCAST MIC breakpoints. Of 221 isolates, 179 were found carbapenem-resistant as MICs was >4 μg/ml against IP and meropenem as determined by the E-test and VITEK-2, MHT for carbapenemase production was positive for 34.84% (77), DDST in 51.58% (114), CDST in 50.67% (112) isolates and MBL (IP/IPI) E-test was positive for 58.37% (129) isolates. Results of different phenotypic tests of *Citrobacter* spp. recovered from various clinical specimens are shown in Tables 2 and 3.

In these phenotypic tests from different infection sites among 130 *Citrobacter freundii* tested, carbapenem resistance was detected in 82.30% (107) isolates. MBL E-test was found positive for 78.64% (81), followed by CDST in 54.6% (71), DDST in 53.8% (70), and MHT in 39.2% (51) Table 2. Among 91 *Citrobacter koseri* tested, carbapenem resistance was detected in 79.1% (72) isolates MBL E-test found positive for 52.74% (48) isolates, followed by CDST in 47.3% (43), DDST in 46.15% (42) and MHT in 28.57% (26) [Table 3].

Of 221 isolates, 179 (80.99%) were found to exhibit reduced susceptibility to IP and meropenem and were ESBL

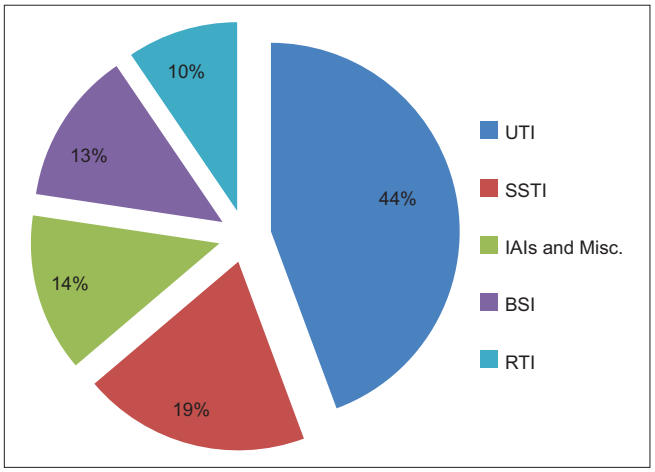

Figure 1: Distribution of *Citrobacter* spp. from various sites of infection

Table 1: The distribution of carbapenem resistant *Citrobacter* spp. from total isolated

Specimen	Wound and Pus *n* (%)	Blood *n* (%)	Endo tracheal aspirate *n* (%)	BAL *n* (%)	Urine *n* (%)	Drain tip, other fluids *n* (%)	Miscellaneous *n* (%)	Total
Total isolated *Citrobacter* spp.	43 (19.45)	29 (13.12)	13 (5.88)	8 (3.6)	98 (44.34)	21 (9.5)	9 (4)	221
Resistant *Citrobacter* spp.	37 (20.7)	20 (11.2)	9 (5)	5 (2.8)	87 (48.60)	16 (8.9)	5 (2.8)	179
C. frundii	23 (21.5)	12 (11.2)	6 (5.7)	4 (3.7)	49 (45.8)	11 (10.3)	2 (1.8)	107
C. koseri	14 (19.4)	8 (11.1)	3 (4.2)	1 (1.4)	38 (52.8)	5 (6.9)	3 (4.2)	72

C. freundii: *Citrobacter freundii*, *C. koseri*: *Citrobacter koseri*, BAL: Broncho-alveolar lavage

Table 2: Percentage and result of different phenotypic tests of *C. freundii recovered* from various infection sites

Infection site	Total	Carbapenem resistant by MIC[a]	MBL E-Test[b]	MHT[c]	CDST[d]	DDST[e]
SSTI	25	23	19	15	18	18
BSI	17	12	8	5	7	7
UTI	58	49	36	20	28	28
IAIs and others	18	13	10	7	10	10
RTI	12	10	8	4	7	8
Total (%)	130	107 (82.30)	81 (78.64)	51 (39.2)	70 (53.8)	71 (54.6)

[a]MIC values for imipenem, meropenem, and ertapenem ≥4 µg/ml, [b]MBL (IP/IPI) E-test, [c]MHT: Modified Hodge test, [d]CDST: Combined-disc synergy test, [e]DDST: Double-disc synergy tests. *C. freundii*: *Citrobacter freundii*, MBL: Metallo-beta-lactamase, SSTI: Skin and soft tissue infection, BSI: Blood stream infection, UTI: Urinary tract infection, IAIs: Intra-abdominal infections, RTI: Respiratory tract infection, MIC: Minimum inhibitory concentration, IP: Imipenem, IPI: Imipenem inhibitor

Table 3: Percentage and result of different phenotypic tests of *C. koseri* recovered from various infection sites

Infection site	Total	Carbapenem resistant by MIC[a]	MBL[b] E-test	MHT[c]	CDST[d]	DDST[e]
SSTI	18	14	12	8	11	11
BSI	12	8	3	2	2	2
UTI	40	38	24	12	22	22
IAIs and others	12	8	6	2	5	6
RTI	9	4	3	2	2	2
Total (%)	91	72 (79.12)	48 (52.74)	26 (28.57)	42 (46.15)	43 (47.25)

C. koseri: *Citrobacter koseri*, SSTI: Skin and soft tissue infection, BSI: Blood stream infection, UTI: Urinary tract infection, IAIs: Intra-abdominal infections, RTI: Respiratory tract infection, MIC: Minimum inhibitory concentration, MBL: Metallo-beta-lactamase, MHT: Modified Hodge test, CDST: Combined-disc synergy test, DDST: Double-disc synergy tests, a- MIC values for imipenem, meropenem, and ertapenem ≥4 µg/ml, b- MBL (IP/IPI) E-test, c- MHT: Modifi ed Hodge test, d- CDST: Combined-disc synergy test, e- DDST: Double-disc synergy tests

producers and among them 145 were found to have MIC values for IP, meropenem, and ertapenem ranging from 8 to 32 µg/ml as per CLSI breakpoints. The presence of bla_{NDM-1} was detected in 55.30% (99/179) while bla_{VIM} was present in 17.87% (32/179) of carbapenem-resistant strains. Based on Automated sequencing the genes were characterized and known sequences were compared using the BLAST facility (http://blast.ncbi.nlm.nih.gov). The sequences of bla_{NDM-1} from *C. freundii and C. koseri* determined in this study have been assigned GenBank accession no. KR816561 and KR816562.

From UTIs, a single NDM-1 gene was present in 26 *C. freundii* isolates. NDM-1, TEM-1 and CTXM-15 altogether were found in 13 isolates while SHV, CTXM-15, and NDM-1 gene were present in 15 isolates. SHV, CTXM-15 and VIM-2 gene were present in 12 isolates whereas VIM-2, TEM-1, and CTXM-15 were found in 10 isolates.

In *C. koseri*, a single NDM-1 gene was present in 21 isolates, NDM-1, TEM-1, SHV, and CTXM-15 together were found in 18 isolates while CTXM-15 and NDM-1 gene were present in 18 isolates. VIM-2, CTXM-15, and TEM-1 altogether were present in 03 isolates [Figure 2].

From BSIs, NDM-1, SHV, TEM-1, and CTXM-15 were found in 5 *C. freundii* isolates while VIM-2, TEM-1, SHV, and CTXM-15 were altogether detected in 3 isolates whereas In *C. koseri* NDM-1 along with TEM-1, CTXM-15, and SHV genes was present in 03 isolates [Figure 3].

From RTIs, NDM-1, CTXM-15, SHV, and TEM-1, genes altogether were present in 06 *C. freundii* isolates while one isolate had the co-presence of VIM-2, TEM-1, CTXM-15, and SHV-12 gene. In *C. koseri* co-presence of NDM-1, TEM-1, CTXM-15, and SHV genes was detected in 03 isolates [Figure 4].

From SSTIs, *C. freundii* NDM-1, CTXM-15, TEM-1, and SHV genes altogether were present in 11 isolates, while copresence of VIM-2, CTXM-15, TEM-1, and SHV gene were detected in 5 isolates, 08 isolates, 05 isolates with

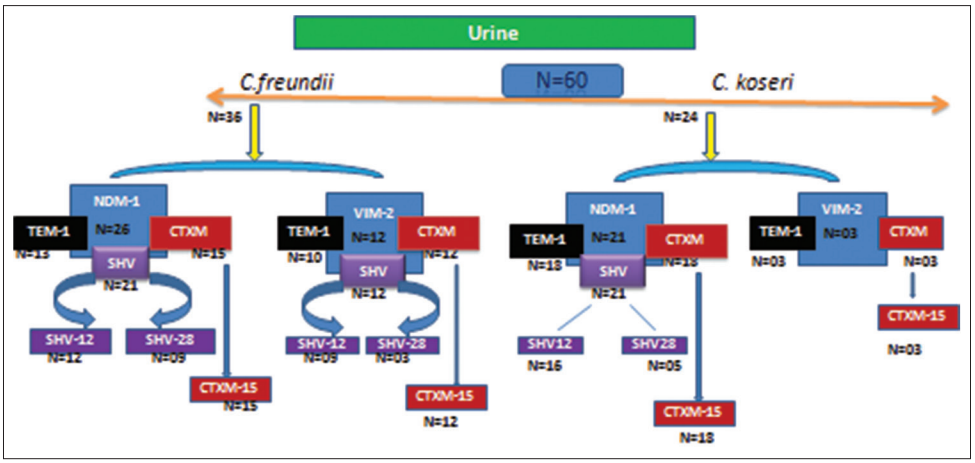

Figure 2: Distribution of beta-lactamase genes in *Citrobacter* spp. isolated from urine

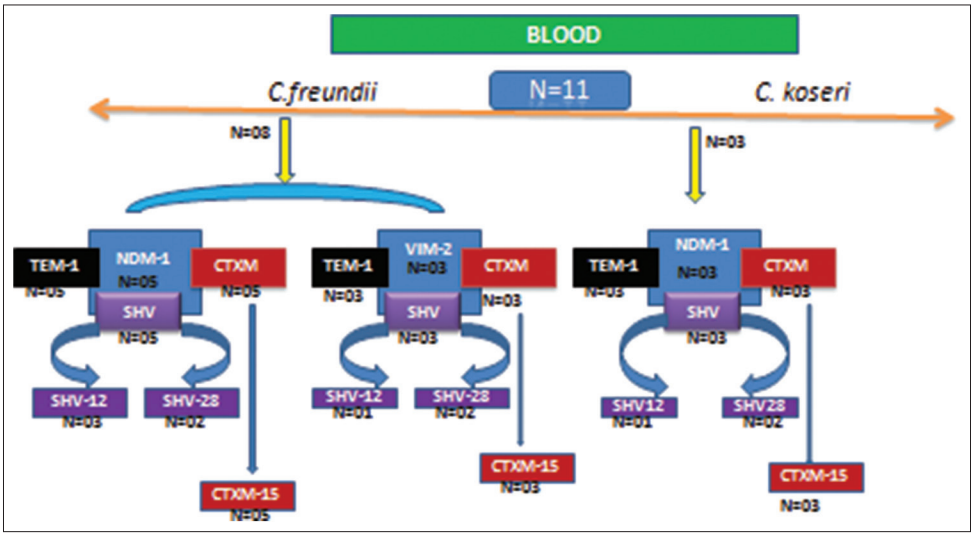

Figure 3: Distribution of beta-lactamase genes in *Citrobacter* spp. isolated from blood stream infections

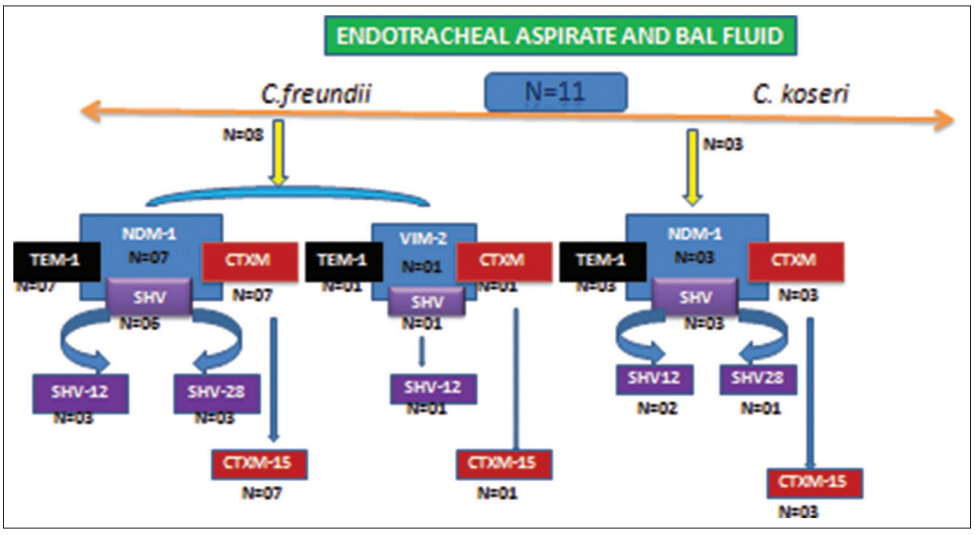

Figure 4: Distribution of beta-lactamase genes in *Citrobacter* spp. isolated from respiratory tract infections

VIM-2 also had and 05 isolates with also had CTXM-15 whereas in *C. koseri* NDM-1, SHV, TEM-1, and CTXM-15 genes were present in 7 isolates while copresence of VIM-2, CTXM-15, and TEM-1 was detected in 3 isolates [Figure 5].

From IAIs and miscellaneous in *C. freundii* NDM-1, CTXM-15, TEM-1, and SHV altogether were present in 8 isolates while VIM-2, CTXM-15. Moreover, TEM-1 were detected in 02 isolates whereas in *C. koseri* 6 isolates had co presence of NDM-1, SHV, CTXM-15, and TEM-1 genes [Figure 6].

Strain molecular typing

Genotypic analysis by molecular typing of 81 strains of *C. freundii* (MBL producers) using RAPD PCR produced an average of 14–18 fragments per *C. freundii* strains. There were all together 10 RAPD pattern assigned as CF-A to CF-J [Figure 7].

As per ERIC PCR and REP PCR banding pattern, the isolates showed a genotypic diversity with 08 clonal clusters exhibited by 81 isolates. Genotypic analysis using REP PCR produced an average of 6–8 fragments per *C. freundii* strains [Figure 8].

Genotypic analysis by molecular typing of 48 strains of *C. koseri* using RAPD PCR produced an average of 10–12 fragments per *C. koseri* strains. There were all together 6 RAPD pattern assigned as CK-A to CK-F [Figure 9].

As per ERIC PCR and REP PCR banding pattern, 06 clonal clusters were exhibited by 48 isolates (MBL producers). Genotypic analysis using ERIC PCR produced an average of 12–18 fragments per *C. koseri* strains [Figure 10].

RAPD PCR distinguishes the various clones from one another better than REP PCR and ERIC PCR [Figures 7-10]. In molecular strain typing RAPD types distributed between various REP and ERIC types.

Plasmid replicon typing, transferability and conjugation studies

Conjugation experiments revealed that bla_{NDM-1} was transferable via a plasmid along with other beta-lactamase genes carried on other plasmids. Plasmid profiling of the

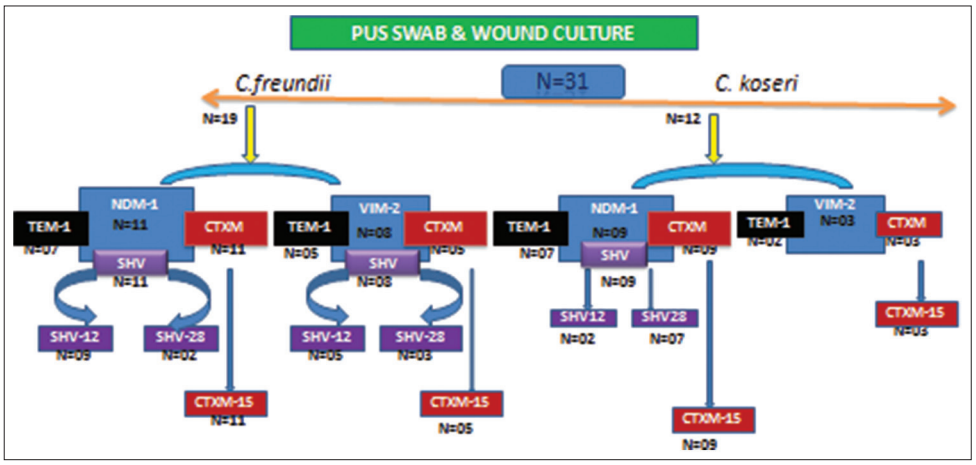

Figure 5: Distribution of beta-lactamase genes in *Citrobacter* spp. isolated from skin and soft tissue infections

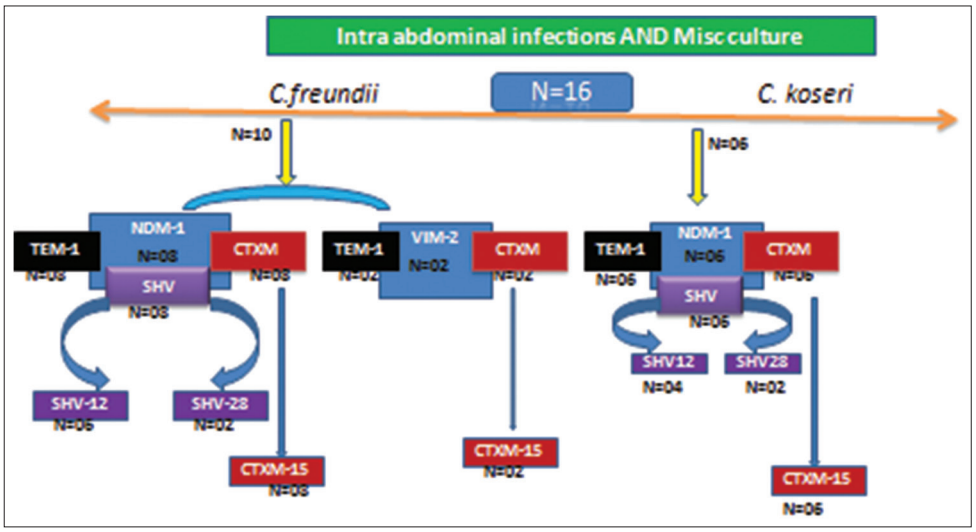

Figure 6: Distribution of beta-lactamase genes in *Citrobacter* spp. isolated from intra-abdominal infections and miscellaneous culture

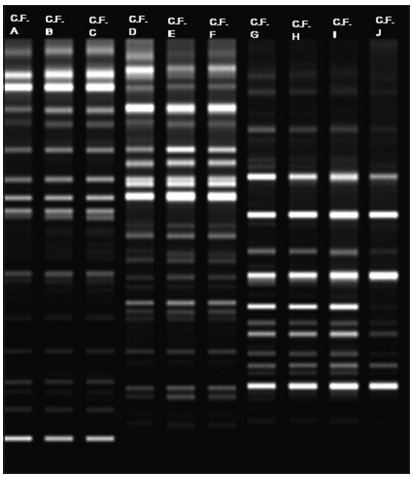

Figure 7: Randomly amplified polymorphic DNA polymerase chain reaction banding pattern among 10 clonal clusters of *C. freundii*

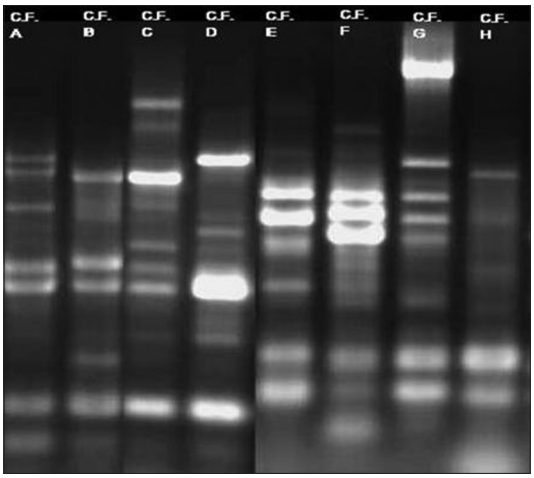

Figure 8: Repetitive element based-polymerase chain reaction banding pattern among 8 clonal cluster of *Citrobacter freundii*

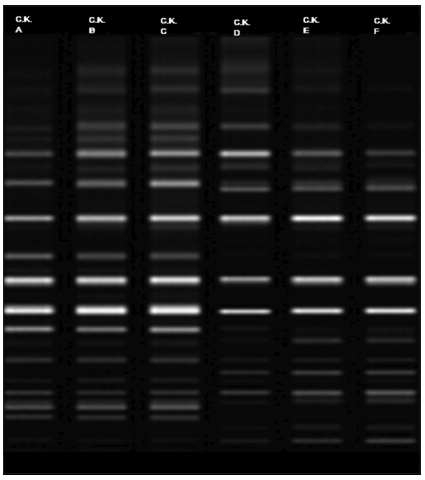

Figure 9: Randomly amplified polymorphic DNA polymerase chain reaction banding pattern among 6 clonal clusters of *Citrobacter koseri*

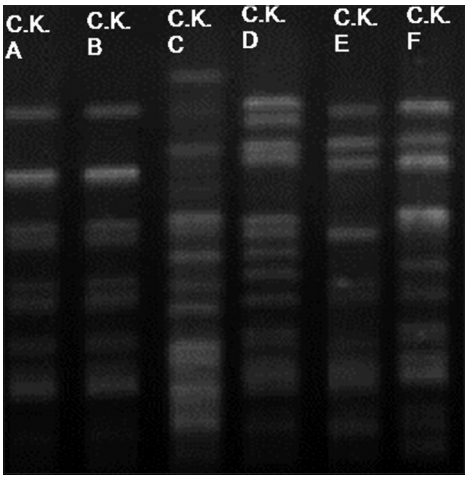

Figure 10: Enterobacterial repetitive intergenic consensus-polymerase chain reaction banding pattern among 6 clonal cluster of *Citrobacter koseri*

isolates showed that $bla_{\text{NDM-1}}$ was carried on plasmids ranging in sizes from 35 to 130 kb and bla_{VIM} was carried on 50 to 200 kb size plasmids. All of the plasmid types were transferable. From UTI 50% (n = 20), SSTIs, BSIs, RTIs and IAIs and others 50% (N = 23) of multidrug resistant *C. freundii* were randomly selected as a donor *Citrobacter* spp. strains for conjugation studies and plasmid typing [Table 4].

From SSTIs, BSIs, UTIs, RTIs, and IAIs and others 50% (N = 24) of multidrug resistant *C. koseri* were randomly selected as a donor *Citrobacter* spp. strains for conjugation studies and plasmid typing [Table 5].

MIC values for IP, meropenem and ertapenem among transconjugants are ranging from 8 to 32 µg/ml as per CLSI breakpoints. Both *bla*TEM-1 and *bla*SHV were associated with Inc. FIA, Inc. FIB, Inc. FIC multiple replicons. The $bla_{\text{NDM-1}}$ gene was located on Inc. A/C, Inc. FII and Inc. N plasmids. $Bla_{\text{VIM-2}}$ was carried on plasmids belonging to Inc. FII replicons, Inc. B/O replicons and Inc. nreplicons. Majority of *bla*CTX-M-15 was associated with multiple replicons either (Inc. FIA, Inc. FIB) OR (Inc. FIIB, Inc. FIB) type [Tables 4 and 5].

DISCUSSION

Citrobacter is an opportunistic pathogen causing outbreaks where there are local or systemic breaches to host defenses. Common infections caused by *Citrobacter* spp. are UTI, bacteremia, meningitis, pneumonia, osteomyelitis, peritonitis, and endocarditis.[3,6,7,20-25] It has been a cause of neonatal sepsis,[4-7] and IAI.[26] *Citrobacter* bacteremia is associated with a high mortality rate between 33% and 48%.[6,7,27] *C. freundii* and *C. koseri* are the two most common pathogens and infections can be acquired from exogenous as well as endogenous

Table 4: Transferability of MBL and ESBL gene present along with plasmid typing of *C. freundii* isolates

Isolate	MBL gene	Plasmid type	Transferability	Other ESBL gene present			Plasmid type			Transferability
UC324	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIA	FIA, FIB	Transferable
UC384	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIC	FIA	FIA, FIB	Transferable
UC641	NDM-1	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIB	FIC	FIA, FIB	Transferable
UC729	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIC	FIB	FIA, FIB	Transferable
UC899	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIA	FIA, FIB	Transferable
UC1013	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIB	FIC	FIA, FIB	Transferable
UC1117	NDM-1	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FIA, FIB	Transferable
UC1209	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIC	FIA	FIA, FIB	Transferable
UC1303	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIB	FIC	FIA, FIB	Transferable
UC1495	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIC	FIB	FIA, FIB	Transferable
UC1532	VIM-2	N	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIA	FII, FIB	Transferable
UC1583	VIM-2	B/O	Transferable	TEM-1	SHV-12	CTXM-15	FIB	FIA	FII, FIB	Transferable
UC1681	VIM-2	B/O	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIC	FII, FIB	Transferable
UC1805	VIM-2	FII	Transferable	TEM-1	SHV-28	CTXM-15	FIC	FIA	FII, FIB	Transferable
UC2620	VIM-2	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIC	FII, FIB	Transferable
UC3030	VIM-2	B/O	Transferable	TEM-1	SHV-12	CTXM-15	FIC	FIB	FII, FIB	Transferable
UC3786	VIM-2	B/O	Transferable	TEM-1	SHV-12	CTXM-15	FIB	FIA	FII, FIB	Transferable
UC4423	VIM-2	B/O	Transferable	TEM-1	SHV-28	CTXM-15	FIB	FIC	FII, FIB	Transferable
UC4503	VIM-2	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FII, FIB	Transferable
UC4522	VIM-2	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIC	FII, FIB	Transferable
ETB 127	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIA	FIC	FIA, FIB	Transferable
ETB 273	VIM-2	N	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FIA, FIB	Transferable
ETB 487	NDM-1	N	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FIA, FIB	Transferable
ETB561	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIB	FIC	FIA, FIB	Transferable
BACT49	VIM-2	N	Transferable	TEM-1	SHV-28	CTXM-15	FIB	FIC	FIA, FIB	Transferable
BACT301	VIM-2	N	Transferable	TEM-1	SHV-28	CTXM-15	FIA	FIC	FIA, FIB	Transferable
BACT437	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FIA, FIB	Transferable
BACT78	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIB	FIC	FIA, FIB	Transferable
PC39	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FIA, FIB	Transferable
PC46	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIC	FIC	FIA, FIB	Transferable
PC71	NDM-1	A/C	Transferable	TEM-1	SHV-28	CTXM-15	FIB	FIC	FIA, FIB	Transferable
PC89	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FIA, FIB	Transferable
PC103	NDM-1	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIA	FIA, FIB	Transferable
PC148	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIC	FIB	FIA, FIB	Transferable
PC195	NDM-1	N	Transferable	TEM-1	SHV-12	CTXM-15	FIB	FIA	FIA, FIB	Transferable
PC201	VIM-2	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FII, FIB	Transferable
PC242	VIM-2	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIB	FIA	FII, FIB	Transferable
PC312	VIM-2	FII	Transferable	TEM-1	SHV-28	CTXM-15	FIA	FIC	FII, FIB	Transferable
DTP27	NDM-1	FII	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIB	FIA, FIB	Transferable
DTP41	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIA	FIC	FIA, FIB	Transferable
DTP69	NDM-1	A/C	Transferable	TEM-1	SHV-12	CTXM-15	FIB	FIB	FIA, FIB	Transferable
DTP75	VIM-2	N	Transferable	TEM-1	ND	CTXM-15	FIA	ND	FIA, FIB	Transferable
DTP93	VIM-2	N	Transferable	TEM-1	ND	CTXM-15	FIA	ND	FIA, FIB	Transferable

C. freundii: *Citrobacter freundii*

sources, being ubiquitous in nature as a saprophyte in soil and sewage and as a commensal in human gastrointestinal tract.

In our study, carbapenem-resistant *C. freundii* was the most prominent species isolated 59.78% (107/179) followed by *C. koseri* 40.22% (72/179) and our finding [Table 1] were similar to others as reported earlier.[28,29] These isolates showed a high level of resistance to the beta-lactam antibiotics as well as to the beta-lactam/beta-lactamase inhibitor combination which were tested in the study. Sixty-five percentage (145/221) isolates were found to be multi drug resistant, the resistance being to penicillins, cephalosporins, fluoroquinolones, and aminoglycosides using disc diffusion method. The majority of specimens were from urine 44%, followed by SSTI 19%, Drain tip, tissue, other body fluids, and miscellaneous culture constitute 14%, blood 13% and respiratory secretions 10%, respectively.[25,30] CPD resistance can be used as a phenotypic marker for ESBL detection in cases of UTI. The worldwide prevalence of ESBLs available at PubMed in *Citrobacter* spp. was reported to be 0.5–36%.[31,32] In our study, 80.9% (179/221) of *Citrobacter* isolates were ESBL producers and this study correlates well with another study by Khanna *et al.* from India.[25] $Bla_{\text{CTX-M-15}}$ was the only CTX-M reported in our study while others have reported $bla_{\text{CTX-M-35}}$, $bla_{\text{CTX-M-30,}}$ $bla_{\text{CTX-M-14}}$ $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-3}}$ from USA,[33] Canada,[34] China,[35] UK,[36] France,[37] Poland,[38] Korea,[39] and Spain.[40] There are very few studies in Medical literature, regarding MBL detection among *Citrobacter* spp. in India and abroad as compared to other members of family *Enterobacteriaceae*. In our study,

Table 5: Transferability of MBL and ESBL gene present along with plasmid typing of *C. koseri* isolates

Isolate	MBL gene	Plasmid type	Transferability	Other ESBL gene present			Plasmid type			Transferability
UC69	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIA	FIA, FIB	FIB	Transferable
UC145	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-28	FIB	FIA, FIB	FIC	Transferable
UC218	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIA	FIA, FIB	FIB	Transferable
UC356	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-28	FIB	FIA, FIB	FIC	Transferable
UC378	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIC	FIA, FIB	FIB	Transferable
UC615	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA, FIB	FIB	Transferable
UC719	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIC	FIA, FIB	FIB	Transferable
UC861	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIC	FIA, FIB	FIB	Transferable
UC937	A/C	A/C	Transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA, FIB	FIC	Transferable
UC1148	VIM-2	FII	Transferable	TEM-1	CTXM-15	ND	FIC	FII, FIB	ND	Transferable
UC1361	VIM-2	FII	Transferable	TEM-1	CTXM-15	ND	FIB	FII, FIB	ND	Transferable
UC1417	VIM-2	FII	Transferable	TEM-1	CTXM-15	ND	FIA	FII, FIB	ND	Transferable
DTP43	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA, FIB	FIC	Transferable
DTP81	NDM-1	FII	Transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA, FIB	FIC	Transferable
DTP97	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA, FIB	FIB	Transferable
PC21	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIA	FIA, FIB	FIB	Transferable
PC52	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-28	FIC	FIA, FIB	FIC	Transferable
PC98	NDM-1	FII	Transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA, FIB	FIB	Transferable
PC112	NDM-1	N	Transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA, FIB	FIC	Transferable
PC131	VIM-2	N	Transferable	TEM-1	CTXM-15	ND	FIB	FII, FIB	ND	Transferable
PC157	VIM-2	N	Transferable	TEM-1	CTXM-15	ND	FIA	FII, FIB	ND	Transferable
BACT64	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA, FIB	FIB	Transferable
BACT58	NDM-1	N	Transferable	TEM-1	CTXM-15	SHV-28	FIB	FIA, FIB	FIC	Transferable
ETB 375	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-12	FIA	FIA, FIB	FIB	Transferable

ESBL: Extended-spectrum beta-lactamase, MBL: Metallo-beta-lactamase, *C. koseri: Citrobacter koseri*

58.37% (129/221) of *Citrobacter,* were producing MBL genes. A study from Kolkata, India[41] have reported 41.67% of MBL production among *Citrobacter* spp. [Tables 2 and 3]. Their lower frequency might be due to the sample size and geographical region or to timing of the studies as the prevalence of these resistance genes in increasing with time.[30,41] Emergence of bla_{NDM-1} producing *Citrobacter* isolates reported from Bangladesh,[42] Turkey,[43] Thailand,[44] France,[45] South Africa,[46] United Arab Emirates,[47] Canada,[48,49] and India.[50] We detected presence of bla_{NDM-1} in 55.30% (99/179) while bla_{VIM} was present in 17.87% (32/179) of carbapenem resistant strains. The presence of bla_{IMP} [51,52] and bla_{GIM} [53] has been reported in *Citrobacter* isolates in other countries, but we did not find any of these MBL in our study. Likewise, we found no bla_{KPC-2} and bla_{KPC-3} as has been reported in *Citrobacter* spp. by Deshpande *et al.*[54] and Mavroidi *et al.*[55] PBRT of purified plasmids from the clinical isolates of *Citrobacter* spp. revealed Inc. N, Inc. A/C and Inc. FII type plasmids associated with NDM-1 carriage which correlates well with previous studies.[47,48,50] Carriage of NDM-1 has also been reported on plasmid Inc. HII, Inc. X-type and Inc. L/M.[47,48,50-52] Inc. FII, Inc. B/O and Inc. N replicon type plasmids were associated with bla_{VIM} carriage suggesting that MBL genes are carried on multiple plasmids. RAPD PCR was better as compared to REP PCR and ERIC PCR [Figures 7-10]. This study has shown that the MBL genes are transmissible by conjugation, which suggests that the presence of plasmid-borne MBL genes among the organisms making up the gut flora may facilitate transmission of resistance genes from one organism to another.

CONCLUSION

A high prevalence of carbapenem resistance was reported among *Citrobacter* isolates investigated in this study. This indicates spread of NDM-1 producing *Citrobacter* in central India. Early detection is important as the simultaneous presence of other resistance genes makes the organisms refractory to most of the common antibiotics used in clinical practice. Furthermore, the presence of these genes on plasmids that are transmissible to other species. Thus, the detection of genes for carbapenem resistance should be a major focus of infection control to prevent transmission of MBL genes to other patients and to other bacterial species within the same patient.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Washington WC Jr, Allen SD, Janda WM, Koneman EW, Gary PW, Schreckenberger PC, *et al*. The *Enterobacteriaceae*. Color Atlas and Textbook of Diagnostic Microbiology. 6th ed., Ch. 6. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 211-302.

2. Abbott SL. *Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas,* and Other *Enterobacteriaceae*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Landry ML, editors. Manual of Clinical Microbiology. 9th ed. New York: ASM Press; 2007. p. 698-715.
3. Holmes B, Aucken HM. *Citrobacter*, *Enterobacter*, *Klebsiella*, *Serratia* and other members of the *Enterobacteriaceae*. In: Collier L, Balows A, Sussman M, editors. Microbiology and Microbial Infections: Systematic Bacteriology. 9th ed. London: Arnold; 1998. p. 999-1033.
4. Khadka SB, Thapa B, Mahat K. Nosocomial *Citrobacter* infection in neonatal intensive care unit in a hospital of Nepal. J Nepal Paediatr Soc 2010;31:105-9.
5. Thapa B, Tribuddharat C. Molecular characterization of *Citrobacter freundii* isolated from neonates in Neonatal Intensive Care Unit of Nepal. J Nepal Paediatr Soc 2012;32:132-5.
6. Pepperell C, Kus JV, Gardam MA, Humar A, Burrows LL. Low-virulence *Citrobacter* species encode resistance to multiple antimicrobials. Antimicrob Agents Chemother 2002;46:3555-60.
7. Doran TI. The role of *Citrobacter* in clinical disease of children: Review. Clin Infect Dis 1999;28:384-94.
8. Collee JG, Miles RS, Wan B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 131-50.
9. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty Second Informational Supplement M100-S22. Wayne, PA, USA: CLSI; 2012.
10. European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for Interpretation of MICs and Zone Diameters (Version 2); 2012. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCASTfiles/Breakpointtables/Breakpointtablev2.0120221.pdf. [Last accessed on 2014 Feb 23].
11. Lee K, Lim YS, Yong D, Yum JH, Chong 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;41:4623-9.
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. Oliver A, Weigel LM, Rasheed JK, McGowan JE Jr, Raney P, Tenover FC. Mechanisms of decreased susceptibility to cefpodoxime in *Escherichia coli*. Antimicrob Agents Chemother 2002;46:3829-36.
14. Villalón P, Valdezate S, Medina-Pascual MJ, Carrasco G, Vindel A, Saez-Nieto JA. Epidemiology of the *Acinetobacter*-derived cephalosporinase, carbapenem-hydrolysing oxacillinase and metallo-ß-lactamase genes, and of common insertion sequences, in epidemic clones of *Acinetobacter baumannii* from Spain. J Antimicrob Chemother 2013;68:550-3.
15. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: The phantom menace. J Antimicrob Chemother 2012;67:1597-606.
16. Hong SS, Kim K, Huh JY, Jung B, Kang MS, Hong SG. Multiplex PCR for rapid detection of genes encoding class A carbapenemases. Ann Lab Med 2012;32:359-61.
17. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 1991;19:6823-31.
18. Vogel L, Jones G, Triep S, Koek A, Dijkshoorn L. RAPD typing of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia marcescens* and *Pseudomonas aeruginosa* isolates using standardized reagents. Clin Microbiol Infect 1999;5:270-6.
19. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 2005;63:219-28.
20. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin Infect Dis 2001;33:947-53.
21. Lipsky BA, Hook EW 3rd, Smith AA, Plorde JJ. Citrobacter infections in humans: Experience at the Seattle Veterans Administration Medical Center and a review of the literature. Rev Infect Dis 1980;2:746-60.
22. Kline MW. *Citrobacter* meningitis and brain abscess in infancy: Epidemiology, pathogenesis, and treatment. J Pediatr 1988;113:430-4.
23. Gupta N, Yadav A, Choudhary U, Arora DR. *Citrobacter* bacteremia in a tertiary care hospital. Scand J Infect Dis 2003;35:765-8.
24. Drelichman V, Band JD. Bacteremias due to *Citrobacter diversus* and *Citrobacter freundii*. Incidence, risk factors, and clinical outcome. Arch Intern Med 1985;145:1808-10.
25. Khanna A, Singh N, Aggarwa AI, Khanna M. The antibiotic resistance pattern in *Citrobacter* species: An emerging nossocomial pathogen in a tertiary care hospital. J Clin Diagn Res 2012;6:642-4.
26. Shih CC, Chen YC, Chang SC, Luh KT, Hsieh WC. Bacteremia due to *Citrobacter* species: Significance of primary intraabdominal infection. Clin Infect Dis 1996;23:543-9.
27. Kanamori H, Yano H, Hirakata Y, Endo S, Arai K, Ogawa M, *et al.* High prevalence of extended-spectrum ß-lactamases and qnr determinants in *Citrobacter* species from Japan: Dissemination of CTX-M-2. J Antimicrob Chemother 2011;66:2255-62.
28. Khorasani G, Salehifar E, Eslami G. Profile of microorganisms and antimicrobial resistance at a tertiary care referral burn centre in Iran: Emergence of *Citrobacter freundii* as a common microorganism. Burns 2008;34:947-52.
29. Samonis G, Karageorgopoulos DE, Kofteridis DP, Matthaiou DK, Sidiropoulou V, Maraki S, *et al. Citrobacter* infections in a general hospital: Characteristics and outcomes. Eur J Clin Microbiol Infect Dis 2009;28:61-8.
30. Mohanty S, Singhal R, Sood S, Dhawan B, Kapil A, Das BK. *Citrobacter* infections in a tertiary care hospital in Northern India. J Infect 2007;54:58-64.
31. Fernandes R, Amador P, Oliveira C, Prudêncio C. Molecular characterization of ESBL-producing *Enterobacteriaceae* in northern Portugal. ScientificWorldJournal 2014;2014:782897.
32. Ali AM, Rafi S, Qureshi AH. Frequency of extended spectrum beta lactamase producing gram negative bacilli among clinical isolates at clinical laboratories of Army Medical College, Rawalpindi. J Ayub Med Coll Abbottabad 2004;16:35-7.
33. Tian GB, Adams-Haduch JM, Qureshi ZA, Wang HN, Doi Y. CTX-M-35 extended-spectrum beta-lactamase conferring ceftazidime resistance in *Citrobacter koseri*. Int J Antimicrob Agents 2010;35:412-3.
34. Abdalhamid B, Pitout JD, Moland ES, Hanson ND. Community-onset disease caused by *Citrobacter freundii* producing a novel CTX-M beta-lactamase, CTX-M-30, in Canada. Antimicrob Agents Chemother 2004;48:4435-7.
35. Zhang R, Yang L, Cai JC, Zhou HW, Chen GX. High-level carbapenem resistance in a *Citrobacter freundii* clinical isolate is due to a combination of KPC-2 production and decreased porin expression. J Med Microbiol 2008;57(Pt 3):332-7.
36. Munday CJ, Whitehead GM, Todd NJ, Campbell M, Hawkey PM. Predominance and genetic diversity of community- and hospital-acquired CTX-M extended-spectrum beta-lactamases in York, UK. J Antimicrob Chemother 2004;54:628-33.
37. Lartigue MF, Fortineau N, Nordmann P. Spread of novel expanded-spectrum beta-lactamases in *Enterobacteriaceae* in a university hospital in the Paris area, France. Clin Microbiol Infect 2005;11:588-91.
38. Baraniak A, Fiett J, Sulikowska A, Hryniewicz W, Gniadkowski M. Countrywide spread of CTX-M-3 extended-spectrum beta- lactamase-producing microorganisms of the family *Enterobacteriaceae* in Poland. Antimicrob Agents Chemother 2002;46:151-9.
39. Kim J, Lim YM. Prevalence of derepressed ampC mutants and extended-spectrum beta-lactamase producers among clinical isolates

of *Citrobacter freundii*, *Enterobacter* spp. and *Serratia marcescens* in Korea: Dissemination of CTX-M-3, TEM-52, and SHV-12. J Clin Microbiol 2005;43:2452-5.
40. Miró E, Mirelis B, Navarro F, Rivera A, Mesa RJ, Roig MC, *et al*. Surveillance of extended-spectrum beta-lactamases from clinical samples and faecal carriers in Barcelona, Spain. J Antimicrob Chemother 2005;56:1152-5.
41. Kumar S, Bandyopadhyay M, Mondal S, Pal N, Ghosh T, Bandyopadhyay M, *et al*. Tigecycline activity against metallo-ß-lactamase-producing bacteria. Avicenna J Med 2013;3:92-6.
42. 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.
43. Yanik K, Emir D, Eroglu C, Karadag A, Güney AK, Günaydin M. Investigation of the presence of New Delhi metallo-beta-lactamase-1 (NDM-1) by PCR in carbapenem-resistant gram-negative isolates. Mikrobiyol Bul 2013;47:382-4.
44. Rimrang B, Chanawong A, Lulitanond A, Wilailuckana C, Charoensri N, Sribenjalux P, *et al*. Emergence of NDM-1- and IMP-14a-producing *Enterobacteriaceae* in Thailand. J Antimicrob Chemother 2012;67:2626-30.
45. Denis C, Poirel L, Carricajo A, Grattard F, Fascia P, Verhoeven P, *et al*. Nosocomial transmission of NDM-1-producing *Escherichia coli* within a non-endemic area in France. Clin Microbiol Infect 2012;18:E128-30.
46. Rubin JE, Peirano G, Peer AK, Govind CN, Pitout JD. NDM-1-producing *Enterobacteriaceae* from South Africa: Moving towards endemicity? Diagn Microbiol Infect Dis 2014;79:378-80.
47. Sonnevend A, Al Baloushi A, Ghazawi A, Hashmey R, Girgis S, Hamadeh MB, *et al*. Emergence and spread of NDM-1 producer *Enterobacteriaceae* with contribution of IncX3 plasmids in the United Arab Emirates. J Med Microbiol 2013;62(Pt 7):1044-50.
48. Peirano G, Ahmed-Bentley J, Fuller J, Rubin JE, Pitout JD. Travel-related carbapenemase-producing gram-negative bacteria in Alberta, Canada: The first 3 years. J Clin Microbiol 2014;52:1575-81.
49. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. J Clin Microbiol 2012;50:3877-80.
50. Poirel L, Ros A, Carricajo A, Berthelot P, Pozzetto B, Bernabeu S, *et al*. Extremely drug-resistant *Citrobacter freundii* isolate producing NDM-1 and other carbapenemases identified in a patient returning from India. Antimicrob Agents Chemother 2011;55:447-8.
51. Yan JJ, Ko WC, Chuang CL, Wu JJ. Metallo-beta-lactamase-producing *Enterobacteriaceae* isolates in a university hospital in Taiwan: Prevalence of IMP-8 in *Enterobacter cloacae* and first identification of VIM-2 in *Citrobacter freundii*. J Antimicrob Chemother 2002;50:503-11.
52. Hawkey PM, Xiong J, Ye H, Li H, M'Zali FH. Occurrence of a new metallo-beta-lactamase IMP-4 carried on a conjugative plasmid in *Citrobacter youngae* from the People's Republic of China. FEMS Microbiol Lett 2001;194:53-7.
53. Wendel AF, Brodner AH, Wydra S, Ressina S, Henrich B, Pfeffer K, *et al*. Genetic characterization and emergence of the metallo-ß-lactamase GIM-1 in *Pseudomonas* spp. and *Enterobacteriaceae* during a long-term outbreak. Antimicrob Agents Chemother 2013;57:5162-5.
54. Deshpande LM, Jones RN, Fritsche TR, Sader HS. Occurrence and characterization of carbapenemase-producing *Enterobacteriaceae*: Report from the SENTRY Antimicrobial Surveillance Program (2000-2004). Microb Drug Resist 2006;12:223-30.
55. Mavroidi A, Neonakis I, Liakopoulos A, Papaioannou A, Ntala M, Tryposkiadis F, *et al*. Detection of *Citrobacter koseri* carrying beta-lactamase KPC-2 in a hospitalised patient, Greece, July 2011. Euro Surveill 2011;16. pii: 19990.