British Microbiology Research Journal
4(9): 1035-1045, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antimicrobial Resistance Pattern of Multidrug Resistant *Enterobacteriaceae* (MDRE) Isolated from Clinical Samples with Special Reference to Carbapenemase Production and Susceptibility to Tigecycline

Joel Filgona[1], Tuhina Baneerje[1] and Shampa Anupurba[1*]

[1]Institute of Medical Science, Banaras Hindu University Varanasi, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SA and JF contributed to conception and designing of study. Author JF managed the literature searches. Authors JF and TB wrote the protocol, managed data collection, analysis and drafting of manuscript. Author SA reviewed first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 24th February 2014
Accepted 17th May 2014
Published 2nd June 2014

ABSTRACT

Aim: The study was carried out to investigate the prevalence of multidrug resistant *Enterobacteriaceae* (MDRE), their susceptibility to carbapenems and tigecycline, and subsequently carbapenemase producers among clinical isolates of *Enterobacteriaceae*.
Study Design: Investigative.
Place and Duration: The study was performed in the Microbiology Department, Institute of Medical Science, associated Sir Sunderlal hospital, Banaras Hindu University Varanasi, during January 2012 to August 2013.
Methods: Samples were collected from patients in accordance with standard practice and *Enterobacteriaceae* identified by conventional biochemical procedures. Antibiotic susceptibility of isolates and Modified Hodge test were carried out according to the CLSI guide-lines.
Results: A total of 761 isolates belonging to *Enterobacteriaceae* were obtained from the samples, dominated by 292 *E. coli*, 236 *Klebsiella pneumonia*e, 53 *Citrobacter freundii*, 51 *C. koseri*, and 36 *K. oxytoca*. Antibiogram revealed piperacillin-tazobactam as the most

**Corresponding author: Email: shampa_anupurba@yahoo.co.in;*

effective agent, with 21.9% of the isolates resistant to it, followed by amikacin (22.4%), levofloxacin (22.8%) and minocycline (23.5%). A total of 512 (67.3%) isolates were MDRE, of which 198 (38.7%) were resistant to at least one of the carbapenems, and 3(0.6%) to tigecycline. Of the isolates 322 (62.9%) were carbapenem resistant enterobacteria (CRE). Carbapenemase production was detected in 256 (50.0%) and 105 (20.0%) isolates among the MDRE by disk diffusion and Modified Hodge tests respectively.
Conclusions: High prevalence of MDRE and CRE was observed. Tigecycline showed better *in vitro* activity over carbapenems indicating an increasing loss of efficacy among these comparators. There was a relatively wide disparity among likely carbapenemase producers identified by Modified Hodge and disk diffusion tests. Findings suggest the need for prudent antimicrobial and infection control policy.

Keywords: Carbapenemase; Enterobacteriaceae; multidrug resistance; tigecycline.

1. INTRODUCTION

The past decades have witnessed the evolution and spread of bacteria in *Enterobacteriaceae* with resistance to multiple classes of antibiotics [1]. Increasing resistance to third and fourth generation cephalosporins which were designed to resist the hydrolytic action of β-lactam resulted to the development of a more stable β-lactam antibiotic referred to as carbapenem. The carbapenems play a critically important role in antibiotic armamentarium as they possess the broadest spectrum of activity and greatest potency against Gram positive and Gram negative bacteria [1]. Clinicians then depended on the use of this class of antibiotic as last resort in treating infections caused by multidrug resistant organisms. The success was however short lived with the emergence of new β-lactamases, namely the carbapenemases with worldwide dissemination and threatening the efficacy of antibiotics in this class [2,3].

Although tigecycline has indication limited to treatment of complicated skin and skin structure infection, complicated intra-abdominal infections and community acquired pneumonia, it is a valuable option for the treatment of multidrug resistant enterobacteria [4,5]. It is broad spectrum antibiotic representing a new class called the glycylcyclines with an expanded spectrum of activity against most ESBL and carbapenemase producing enterobacteria [6-8]. However tigecycline non susceptible enterobacteria has been reported and ranged between 0% and 14.5% in a recent large study conducted across different continents [9,10].

Limited prevalence data are available for carbapenemase producing enterobacteria (CPE) and since carbapenem resistant *K. pneumoniae* isolates are frequently found to be carbapenemase producing, carbapenem resistance is frequently used as a surrogate marker for the presence of carbapenemase [11]. Despite the world wide use of β-lactam antibiotic, regional differences exist in the prevalence and distribution of enzymes responsible for resistance to antimicrobial agents [12,13].

In line with the above assertion, this study was carried out to determine the prevalence of multidrug resistance among clinical isolates of enterobacteria, the susceptibility of these multidrug resistant isolates to carbapenem and tigecycline, and subsequently the prevalence of likely carbapenemase producers among the multidrug resistant enterobacteria (MDRE) in a tertiary care centre in north India.

2. METHODS

2.1 Isolation and Antimicrobial Susceptibility Test

Clinical isolates were collected and identified during January 2012 to August 2013 from samples including urine (1518), blood (907), sputum (720), endotracheal tube (313), pus aspirates (890), intravascular catheter tip (254), ascitic fluid (198) and wound swabs (415), received in the routine bacteriology section from patients attending the various outpatient and inpatient departments of the university hospital. Samples were plated on cysteine lactose electrolyte deficient (CLED) agar or blood and MacConkey agar as per nature of the specimen. Enterobacteria isolates were identified using standard bacteriological methods [14]. Antibiotic susceptibility pattern of these isolates were determine by disk diffusion on Mueller Hinton agar using Kirby-Bauer disk diffusion methods [15]. Briefly, 2 to 3 colonies of the test organism from an overnight culture was suspended in 2ml of sterile normal saline and adjusted to match 0.5 McFarland turbidity standards. Sterile cotton swab was used to make a lawn of the test organism on a Mueller Hinton agar and antibiotic disks were placed on the surface of the seeded plate with a sterile forceps. The plate was incubated at 35ºC for 16-18 hours. *E. coli* ATCC 25922 was used as control. The following disks were used; ampicillin (AMP, 10µg), gentamicin (GEN, 10µg) amikacin (AK, 30µg) amoxicillin-clavulanic acid (AMC, 20 and 10µg), piperacillin-tazobactam (PTZ, 100 and10µg), ceftriaxone (CTR, 30µg), ceftazidime (CAZ, 30µg), cefepime (CPM, 30µg), cefotaxime (CTX, 30µg), cefoperazone (CPZ, 75µg), levofloxacin (LEV, 5µg), ciprofloxacin (CIP, 5 µg), minocycline (MI, 30 µg), and aztreonam (ATM, 30µg) (HiMedia, India).

2.2 Definition and Further Antimicrobial Susceptibility Testing

Multidrug resistant *Enterobacteriaceae* (MDRE) were described as all isolates showing non susceptibility to ≥1 antibiotic in ≥3 antimicrobial classes, excluding the antibiotic an organism has intrinsic resistance to [16]. All the MDRE isolates were subjected to susceptibility test to tigecycline (TGC, 15µg), ertapenem (ETP, 10µg), meropenem (MRP, 10µg), imipenem (IPM, 10µg) and doripenem (DOR, 10µg) (HiMedia, India). Carbapenem resistant enterobacteria were defined as non susceptibility to any of the carbapenems and resistance to the following third generation cephalosporin: ceftriaxone, cefotaxime and ceftazidime [17].

2.3 Screening for Carbapenemase Production

Phenotypic carbapenemase screening by disk diffusion assay using meropenem and ertapenem disk was done based on the new interpretative criteria [15]. Modified Hodge test (MHT) was also performed with these isolates based on standard method and result interpreted as shown in Fig. 1. Briefly, a 0.5 McFarland dilution of the *E. coli* ATCC 25922 in 5 ml of saline was prepared and 1:10 dilution was streaked as lawn on to a Mueller Hinton agar plate. A 10 µg ertapenem susceptibility disk was placed in the center of the lawn made. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35±2ºC for 16–24hrs [18].

2.4 Statistical Analysis

Simple descriptive statistic and tables were used for computing prevalence and presentation of data respectively. The prevalence of resistance by specific species of bacterium to

specific antimicrobial agent was computed as the number of resistant isolates divided by the number of total isolates examined, multiplied by 100.

3. RESULTS

A total of 761 *enterobacteria* isolates comprising 292 *E. coli*, 236 *Klebsiella pneumoniae*, 36 *K. oxytoca*, 53 *Citrobacter freundii*, 51 *C. koseri*, 11 *Enterobacter aerogenes*, 7 *E. cloacae*, 16 *Morganella morganii*, 34 Proteus mirabilis and 20 *P. vulgaris* were characterized from clinical samples. The *in vitro* antimicrobial susceptibility spectrum revealed that of the 761 isolates, 420 (55.0%) were resistant to Amoxicillin-clavulanic acid, 167 (21.9%) to piperacillin-tazobactam, 174 (22.4%) to amikacin, 517 (67.8) to ciprofloxacin, 179 (23.5%) to minocycline, 593 (77.7%) to ampicillin, 432 (56.6%) to ceftazidime, 473 (62.0%) to ceftriaxone, 265 (34.7%) to cefepime, 295 (63.5%) to cefotaxime. while 288 (61.5%) were resistant to cefoperazone, 335 (43.9%) to aztreonam, 236 (34.5%) to gentamicin and 174 (22.8) to levofloxacin.

Resistance of various species of the *enterobacteria* isolates to some commonly used antibiotics is presented in Table 1. The highest resistance to amoxicillin-clavulanic acid was observed in *K. pneumoniae* (69.1%) followed by *K. oxytoca* (66.7%). All isolates showed low to moderate resistance to piperacillin-tazobactam, with the highest being *K. pneumoniae* (36.0%) and *C. freundii* (34.5%). Resistance to aminoglycosides and levofloxacin was below 40%, however *K. pneumoniae* and *K. oxytoca* expressed 48.7% and 41.0% resistance to gentamicin and levofloxacin respectively. Ampicillin and ciprofloxacin performed poorly, as resistance range of 45.0% to 81.8% was observed among the isolates except those in *E. cloacae* which showed 100% susceptibility to ciprofloxacin. Susceptibility to minocycline was better as resistance of the isolates were below 26.1% except *P. mirabilis* and *P. vulgaris* that showed resistance of 70.6% and 50.0% respectively. All isolates showed resistance of less than 50% to aztreonam, with the highest resistance being observed in *K. pneumoniae* (49.2%) (Table 1).

The third generation cephalosporin ceftazidime, ceftriaxone, cefotaxime, cefoperazone showed poor activity on *E. coli*, *K. pneumoniae*, *K. oxytoca*, *C. freundii*, *C. koseri* and *E. aerogenes*, as all expressed resistance level above 33.0% (Table 2). The highest resistance to the fourth generation cephalosporin-cefepime, was observed among *K. pneumoniae* (38.6%) followed by *E. coli* (38.0%), *C. freundii* (36.4%) and *E. aerogenes* (36.4%). While the resistance level of *K. oxytoca* and *C. koseri*, was 38.6%, and 35.3% respectively, the other isolates showed resistance level less than 18%.

A total of 512 (67.3%) of the 761 clinical isolates of enterobacteria subjected to susceptibility test were multidrug resistant, including 218 (74.7%) in *E. coli*, 173 (73.3%) in *K. pneumoniae*, 25 (64.1%) in *K. oxytoca*, 29 (52.7%) in *C. freundii*, 36 (70.6%) in *C. koseri*, 5 (45.5%) in *E. aerogenes*, 2 (28.6%) in *E. cloacae*, 2 (12.5%) in *M. morganii*, 16 (47.1%) in *P. mirabilis* and 6 (30.0%) in *P. vulgaris* (Table 2).

Resistance of the MDRE isolates to ertapenem, meropenem, imipenem doripenem and tigecycline was 131 (25.6%), 175 (34.2%), 94 (18.4%), 117 (25.4%) and 3 (0.6%) respectively. The highest resistance to ertapenem, meropenem and imipenem was observed in *E. aerogenes* (60.0%), followed by *M. morganii* (50.0%) and *C. freundii* (48.3%) respectively; while the highest resistance to doripenem and tigecycline was observed in *E. cloacae* (50.0%), followed by *K. oxytoca* (8.0%) (Table 3).

Table 1. Resistant spectrum of species of enterobacteria isolates

Bacteria (n)	Number and proportion (%) of isolates resistant to the antibiotics*													
	AMC	PTZ	AK	GEN	LEV	CIP	MI	AMP	CAZ	CTR	CPM	CTX	CPZ	AT
E. coli (292)	139(47.6)	41(14.0)	34(11.6)	86(29.5)	111(38.0)	238(81.5)	76(26.0)	236(80.8)	173(59.2)	208(71.2)	111(38.0)	146(73.7)	141(71.2)	127(43.5)
K. pneumoniae (236)	163(69.1)	85(36.0)	87(36.9)	115(48.7)	92(39.0)	144(61.0)	39(16.5)	190(80.5)	149(63.1)	160(67.8)	91(38.6)	85(67.5)	83(65.9)	116(49.2)
K. oxytoca (39)	26(66.7)	7(17.9)	9(23.1)	12(30.8)	16(41.0)	26(66.7)	6(15.4)	29(74.4)	19(48.7)	22(56.4)	13(33.3)	12(60.0)	11(52.4)	17(43.6)
C. freundii (55)	38(69.1)	19(34.5)	21(38.2)	21(38.2)	12(21.8)	29(52.7)	11(20.0)	43(78.2)	31(56.4)	29(52.7)	20(36.4)	22(57.9)	22(57.9)	26(47.3)
C. koseri (51)	34(66.7)	10(19.6)	14(27.5)	16(31.4)	19(37.3)	37(72.5)	7(13.7)	41(80.4)	30(58.8)	35(68.6)	18(35.3)	17(60.7)	18(62.1)	23(45.1)
E. aerogenes (11)	7(63.6)	2(18.2)	2(18.2)	3(27.3)	1(9.1)	5(45.5)	2(18.2)	9(81.8)	5(45.5)	7(63.6)	4(36.4)	2(66.7)	1(33.3)	4(36.4)
E. cloacae (7)	5(7)1.4	1(14.3)	1(14.3)	2(28.6)	0(0.0)	0(0.0)	0(0.0)	5(71.4)	2(28.6)	2(28.6)	1(14.3)	1(25.0)	1(25.0)	1(14.3)
M. morganii (16)	2(12.5)	0(0.0)	1(6.3)	1(6.3)	1(6.3)	12(75.0)	4(25.0)	12(75.0)	2(12.5)	2(12.5)	0(0.0)	2(14.3)	3(21.4)	1(6.3)
P. mirabilis (34)	5(14.7)	2(5.9)	4(11.8)	6(17.6)	9(26.5)	17(50.0)	24(70.6)	17(50.0)	14(41.2)	5(14.7)	6(17.6)	5(26.3)	5(26.3)	14(41.2)
P. vulgaris (20)	1(5.0)	0(0.0)	1(5.0)	1(5.0)	3(15.0)	9(45.0)	10(50.0)	10(50.0)	7(35.0)	3(15.0)	1(5.0)	3(21.4)	3(21.4)	6(30.0)

**. (AMC-amoxicillin-clavulanic acid, PTZ-piperacillintazobactam, AK-amikacin, GEN-gentamicin, LEV-levofloxacin, CIP-ciprofloxacin, MI-minocycline, AMP-ampicillin, CAZ-ceftazidime, CTR-ceftriazone, CPM-cefapime, CTX-cefotaxime, CPZ-cefoperazone, AT-aztreonam). Values in parentheses are in %*

Table 2. Distribution of antibiotic resistant isolates and likely carbapenemase producers among the test enterobacteria

Bacteria (n)	Number and proportion (%) of isolates that are *			
	MDRE	CRE	C+VE DDT	C+VE MHT
E. coli (292)	218(74.7)	137(62.8)	98(45.0)	23(10.6)
K. pneumoniae (236)	173(73.3)	112(64.7)	97(56.1)	50(28.9)
K. oxytoca (39)	25(64.1)	12(48.0)	12(48.0)	7(28.0)
C. freundii (55)	29(52.7)	20(69.0)	20(69.0)	17(58.6)
C. koseri (51)	36(70.6)	18(50.0)	15(41.7)	3(8.3)
E. aerogenes (11)	5(45.5)	4(80.0)	3(60.0)	2(40.0)
E. cloacae (7)	2(28.6)	2(100.0)	1(50.0)	1(50.0)
M. morganii (16)	2(12.5)	1(50.0)	1(50)	2(100)
P. mirabilis (34)	16(47.1)	12(75.0)	7(43.8)	5(31.3)
P. vulgaris (20)	6(30.0)	4(66.7)	2(33.3)	1(16.7)

**. MDRE-multidrug resistant enterobacteria; CRE-carbapenem resistant enterobacteria; C+VEDDT- carbapenemase producers by disk susceptibility screening test; C+VE MHT- carbapenemase producers by Modified Hodge test.*

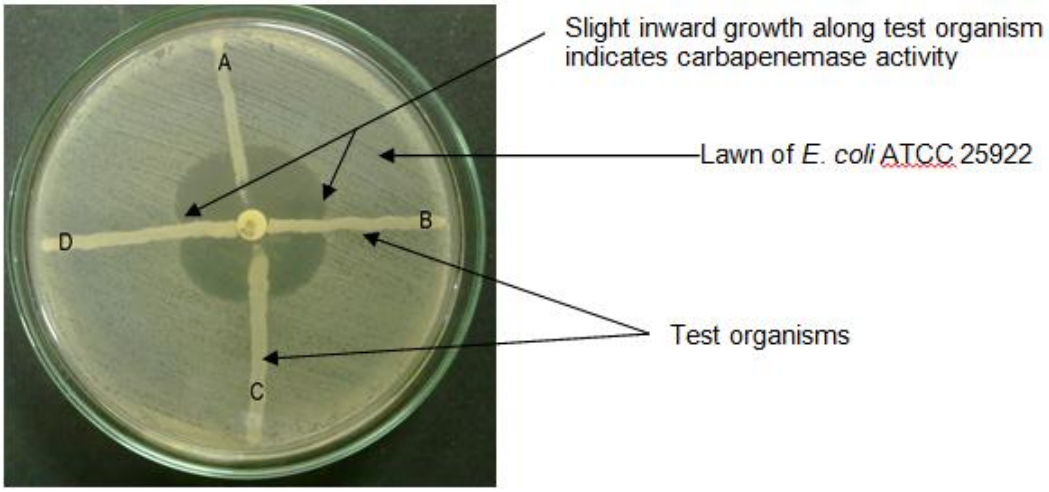

Fig. 1. Example showing the results of Modified Hodge Test. Isolates A and C were Modified Hodge Test Negative, while isolates B and D were Modified Hodge Test Positive

Of the 512 MDRE subjected to susceptibility test by carbapenems, 198 (38.7%) were resistant to at least one of the carbapenems while only 4 (0.8%) were resistant to tigecycline (Table 3). Carbapenem resistant enterobacteria (CRE) composed of 137 (62.8%) isolates in *E. coli*, 112 (64.2%) in *K. pneumoniae*, 12 (48.0%) in *K. oxytoca*, 20 (69.0%) in *C. freundii*, 18 (50.0%) in *C. koseri*, 4 (80.0%) in *E. aerogenes*, 2 (100.0%) in *E. cloacae*, 1 (50.0%) in *M. morganii*, 12 (75.0%) in *P. mirabilis* and 4 (66.7%) in *P. vulgaris*. All isolates were however susceptible to tigecycline except 0.6% and 8.0 of *K. pneumoniae* and *K. oxytoca* isolates that showed resistance to tigecycline respectively.

Table 3. Resistance spectrum of multidrug resistant enterobacteria to carbapenem and Tigecycline

Bacteria (n)	Number and proportion (%) of isolates resistant to carbapenem and tigecycline*				
	ETP	MRP	IPM	DOR	TG
E. coli (218)	26 (11.9)	60 (27.5)	17 (7.8)	27 (12.4)	0 (0.0)
K. pneumoniae (173)	67 (38.7)	79 (45.7)	46 (26.6)	61 (35.3)	1 (0.6)
K. oxytoca (25)	8 (32.0)	10 (40.0)	7 (28.6)	6 (24.0)	2 (8.0)
C. freundii (29)	15 (51.7)	12 (41.4)	14 (48.3)	14 (48.3)	0 (0.0)
C. koseri (36)	8 (22.2)	8 (22.2)	4 (11.1)	5 (13.9)	0 (0.0)
E. aerogenes (5)	3 (60.0)	1 (20.0)	2 (25.0)	1 (20.0)	0 (0.0)
E. cloacae (2)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)
M. morganii (2)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	NA
P. mirabilis (16)	3 (18.8)	4 (25.0)	3 (18.8)	2 (12.5)	NA
P. vulgaris (6)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	NA

**. ETP-ertapenem, MRP- meropenem, IPM-imipenem, DOR-doripenem,TG-tigecycline and NA – not applicable*

Carbapenemase screening by disk diffusion test against meropenem and ertapenem revealed that of the 512 MDRE isolates screened, 256 (50%) were carbapenemase

producers. However, results of Modified Hodge test revealed carbapenemase production by 105 (20.0%) isolates among the MDRE.

4. DISCUSSION

Carbapenemase production and association with multidrug resistance are emergent in *Enterobacteriaceae* [9]. The *In vitro* antibiotic disk susceptibility test conducted showed substantial and very good activity of carbapenem and tigecycline respectively on multidrug resistant isolates of enterobacteria.

The predominant isolates observed in this study were *E. coli*, followed by *K. pneumoniae*, *C. freundii*, and *C. koseri*. This finding confirms the increasing medical importance of *Citrobacter* species. Recently, several hospital settings across the globe have reported increase in the isolation of *Citrobacter* species [18]. This trend is particularly worrisome not only because as a member of Family *Enterobacteriaceae*, they have high propensity to pick and give out resistance moiety, but because they possess intrinsic resistance to ampicillin, amoxyclavulanate, ampicillin-sulbactam, piperacillin, cephamycin, and some members of the first and third generation cephalosporins [18]. The intrinsic resistance mechanism inherent in *Citrobacter* species and other members of *Enterobacteriaceae* could easily select them in the presence of such antibiotics and acquisition of other mobile resistance element could confer on them multidrug or even extensive drug resistance. In a study conducted in Maharashtra, India, the author reported increase in isolation of multidrug resistant *Citrobacter* species associated with high mortality between 30-60% [19]. In the same vein, Poirel reported the isolation of extremely drug resistant *Citrobacter freundii* producing NDM-1 and other carbapenemase from a patient returning from India [20].

High percentage of resistance to amoxyclavulanate, ciprofloxacin, ampicillin, aztreonam and the third generation cephalosporins was observed in the present study. Resistance to ampicillin was more prevalent with 77.7% while among the third generation cephalosporins, resistance ranged from 56.6% to 63.3%. Our hospital setting, being a tertiary health care center, the selection pressure exerted on microorganisms because of wide spread use of broad spectrum antimicrobial and prior exposure of patient to antibiotics, might have been the principal factor for such high level of antimicrobial resistance. In addition, the wide spread resistance to ampicillin and the third generation cephalosporin can be explained by indiscriminate use of these antibiotics in human and animals due to availability of oral formulation and over the counter unrestricted access [21]. Ampicillin and the third generation cephalosporins are used as empirical therapy in India for the management of neonatal sepsis and other health related complication like UTI, meningitis, bacteria sepsis [21]. The high prevalence of resistance to these drugs, as also observed in this study, and as reported by others raises question regarding the efficacy of these antibiotics as an empirical therapy. Thus administration of such antibiotics without proper susceptibility report could further lead to selection and dissemination of resistant strains of *Enterobacteriaceae*. Result obtained in a similar study indicated high level resistance to the third generation cephalosporin among gram negative bacilli isolates from Latin America [22].

Piperacillin-tazobactam showed superiority over amoxicillin-clavulanate; while the aminoglycoside, amikacin, showed better inhibition activity on the isolates when compared to gentamicin (Table 1). This finding is in agreement with the report that amikacin is generally regarded as the most effective agent among aminoglycosides since its chemical structure makes it less affected by enzymatic deactivation [23]. Similar observations have been reported previously [19,24,25]. Minocycline also performed well against the isolates with

resistant rate of less than 26.1% except in *P. mirabilis* and *P. vulgaris* where resistance was 70.6% and 50.0% respectively. High resistance of *Proteus* species to minocycline could be as a result of it intrinsic resistance against the tetracyclines.

In the present study multidrug resistance among the isolates of enterobacteria was high. *E. coli* showed the highest resistance to multiple drugs followed by *K. pneumoniae*, *C. koseri* and *K. oxytoca* (Table 2). Susceptibility spectrum of the multidrug resistant enterobacteria to carbapenem and tigecycline showed that, while only 0.6% of the MDRE were resistant to tigecycline, 37.1% were resistant to at least one of the carbapenems and 322 (62.9%) were carbapenem resistant enterobacteria (CRE). Imipenem exhibited the best antibiotic activity on the isolates when compared to other carbapenems, whereas a lower resistance rate to ertapenem than meropenem was observed. This finding is at variance with the report of a study on the activity of carbapenems on the bacteria in family *Enterobacteriaceae* that showed susceptibility rate of 96%, 95% and 93% for imipenem, meropenem and ertapenem respectively [26]. However, our observation could be explained by the intensive use of meropenem in our tertiary care hospital especially as empirical therapy in high risk units. In addition it has been shown that ertapenem has similar potency to meropenem and imipenem against enterobacteria and is limited in its spectrum of activity compared with other carbapenems primarily because it lacks activity against *Pseudomonas* and *Enterococcus* species [27,28]. Although carbapenem resistance among enterobacteria (CRE) seems high, it is important to note that resistance among gram negative bacteria could result not only from carbapenemase production, but also from the hyperproduction or derepression of AmpC β-lactamase or ESBLs (extended spectrum β-lactamases) with loss or alteration in outer membrane porins, augmented drug efflux, alteration in penicillin binding protein [29]. Despite wide studies on the menace of carbapenemases in clinical setting and the importance in early tracking of isolates expressing resistance due to activity of these enzymes, there still exists some gap in achieving this goal as all the definition of CRE [17,30], provides allowance for the inclusion of enterobacteria with resistance due to other resistant mechanisms that may result in over estimation of CRE phenotypically, which eventually is the common method available to most low resource laboratory.

The evaluation of tigecycline in this study demonstrated a favourable activity *in vitro* and lower resistance rate over carbapenems. Although a recent meta-analysis of pooled data from trials evaluating the use of tigecycline for variety of indications suggested excess mortality associated with its use over comparator [31,32], in the absence of other tested effective regimens, tigecycline may be an appropriate or perhaps the only therapeutic option when dealing with multidrug resistant organism. However it is advised that it should be used with caution and under strict medical supervision [31].

Given the increasing prevalence of carbapenemase-producing enterobacteria worldwide [33], simple and accurate tests are needed to detect isolates that produce carbapenemase. CLSI reviewed the recommendation on carbapenemase detection by MHT among enterobacteria (M100-S23) and informed that MHT would not be recommended, except for epidemiological or infection control purposes. In this study the disk diffusion carbapenemase screening and Modified Hodge test revealed 50.0% and 20.5% of MDRE as likely carbapenemase producers respectively. The wide difference in the result of the disk diffusion screening test and the Modified Hodge test, which is more confirmatory for phenotypic carbapenemase detection, indicated that several other mechanisms could be responsible for resistance to carbapenem besides the production of carbapenemase enzyme which the disk screening test may not discriminate [29]. Although MHT has been associated with limitations such as difficulty in the interpretation of result, varied sensitivity, inability to detect class of

carbapenemase, low specificity because CTX-M ESBL or AmpC-producing isolates with reduced or absent porin expression may give false positive results, it could complement the disk diffusion screening test in view of its advantage in determining diffusible carbapenemase production [34]. Therefore, results obtained from this study indicate that relaying on the results of just the disk diffusion screening test could overestimate the number of likely carbapenemase producing enterobacteria.

A major limitation of this study is the lack of MIC and molecular data. As a result, the multidrug resistant enterobacteria isolates might have been over estimated and likely carbapenemase producing isolate not adequately represented. However, in low resource laboratories, such a study will provide an insight on prevalence of multidrug resistance and likely carbapenemase producing *enterobacteria*.

5. CONCLUSION

Although carbapenem and tigecycline are still relevant as last line drug against MDRE, the CRE rate of 62.9% and 20% likely carbapenemase producers observed among the MDRE in this study underscore the gradual loss of efficacy of the carbapenem on this class of pathogens. However, the disk diffusion test for determination of MDRE and CRE, in spite of some limitations, is still relevant in monitoring the trend of resistance development among *enterobacteria* and can be used in settings where the other methods are not available. Therefore, we suggest routine screening of clinical isolates of *enterobacteria*, as timely identification of MDRE and CRE could improve understanding of the local emerging resistance pattern which is essential for offering targeted therapy or changing empirical treatment protocols.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Papp-Wallace KM. Endimiani A, Taracila MA, Bonomo RA. Carbapenems: Past, Present, and Future. Antimicrob Agents Chemother. 2011;55(11):4943–60.
2. Birgy A, Bidet P, Genel N, Doit C, Decre D, Arlet G, Bingen E. "Phenotypic screening of carbapenemases and associated beta-lactamases in carbapenem-resistant *enterobacteriaceae*." J Clin Microbiol. 2012;50(4):1295-1302.
3. Perez F, Van Duin D. Carbapenem-resistant *enterobacteriaceae*: A menace to our most vulnerable patients. Cleveland Clin J Med. 2013;80(4):225–33.
4. Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. Trends Microbiol. 2011;19(12):588–95.
5. Roy S, Datta S, Viswanathan R, Singh AK, Basu S. Tigecycline susceptibility in *Klebsiella pneumoniae* and *Escherichia coli* causing neonatal septicaemia (2007–10) and role of an efflux pump in tigecycline non-susceptibility. J Antimicrob Chemother. 2013;68(5):1036-1042.
6. Petersen PJ, Jacobus NV, Weiss WJ, Sum PE, Testa RT. *In vitro* and *In vivo* antibacterial activities of a novel glycylcycline, the 9-t-butylglycylamido derivative of minocycline (GAR-936). Antimicrob Agents Chemother. 1999;43(4):738–44.

7. Chen YH, Lu PL, Huang CH, Liao CH, Lu CT, Chuang YC et al. Trends in the susceptibility of clinically important resistant bacteria to tigecycline: Results from the Tigecycline In Vitro Surveillance in Taiwan study, 2006 to 2010. Antimicrob Agents Chemother. 2012;56(3):1452–7.
8. Andrasevic AT, Dowzicky MJ. *In vitro* activity of tigecycline and comparators against Gram-negative pathogens isolated from blood in Europe (2004–2009). Int J Antimicrob Agents. 2012;39(2):115–23.
9. Balode A, Punda-Polić V, Dowzicky MJ. Antimicrobial susceptibility of gram-negative and gram-positive bacteria collected from countries in Eastern Europe: results from the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) 2004-2010. Int J Antimicrob Agents. 2013;41(6):527–35.
10. Sader H, Flamm R, Jones R. Tigecycline activity tested against antimicrobial resistant surveillance subsets of clinical bacteria collected worldwide (2011). Diagn Microbiol Infect. 2013;76(2):217-221.
11. Magiorakos AP, Suetens C, Monnet DL, Gagliotti C, and Heuer OE. The rise of carbapenem resistance in Europe: just the tip of the iceberg? Antimicrob Res Infect Control. 2013;2(1):1-7.
12. Okesola AO, Makanjuola O. Resistance to third-generation cephalosporins and other antibiotics by *enterobacteriaceae* in Western Nigeria. Am J Infect Dis. 2009;5(2):17-20.
13. Okeke IN, Peeling RW, Goossens H, Auckenthaler R, Olmsted SS, de Lavison, JF et al. Diagnostics as essential tools for containing antibacterial resistance. Drug resistance updates: Reviews and commentaries in antimicrobial and anticancer chemotherapy. 2011;14(2):95–106.
14. Crichton PB. Enterobacetriaceae: *Escheriachia*, *Klebsiella*, *Proteus* and other genera. In: Collee JG, fraser AG, Marmion BP, editors. Mackie & McCartney practical medical microbiology. 14th edition. India: Elsevier. 2012;361-412.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty-third informational supplement M100-S23. CLSI, Wayne, PA, USA; 2013.
16. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG et al. Multidrug-resistant, extensively drug-resistant and pandrugresistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–281.
17. Center for Disease Control. Guidance for control of carbapenem resistant *enterobacteriaceae*. CRE toolkit; 2012.
18. Jadhav S, Mishra R, Gandham N, Sardar M, Ujagare M, Angadi K. High Prevalence of multi-drug resistant *Citrobacter* spp. from tertiary care hospital, pimpri, Pune, Indian J Pharm Biomed Sc. 2012;25(25):158-163.
19. 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(1):58-64.
20. 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 patient returning from India. Antimicrob Agents Chemother. 2011;55(1):447.
21. Kothari C, Gaind R, Singh LC, Sinha A, Kumari V, Arya S et al. Community acquisition of β-lactamase producing *Enterobacteriaceae* in neonatal gut. BMC Microbiol. 2013;13(1):136.
22. Villar HE, Baserni MN, Jugo MB. Faecal carriage of ESBL-producing *Enterobacteriaceae* and carbapenem- resistant Gram-negative bacilli in community settings. J Infect Dev Ctries. 2013;7(8):630-4.

23. Shakil S, Khan R, Zarrilli R, Khan AU. Aminoglycoside versus bacteria, a description of the cation, resistance mechanism and nosocomial battleground. J Biomed Sc. 2008;15(1):5-14.
24. Metri BC, Jyothi P, Peerapur BV. Anti–microbial resistance profile of *Citrobacter* species in a tertiary care hospital of southern India. Indian J Med Sci. 2011;65(10):429-35.
25. Shetty J, Kotigadde S. Antibiotic sensitivity pattern of *Citrobacter* isolated from various clinical specimens in a tertiary care hospital. Indian J Pathol Microbiol. 2007;50(3):666-8.
26. Behera B, Mathur P, Das A, Kapil A. Ertapenem susceptibility of extended spectrum β-lactamase producing *enterobacteriaceae* at a tertiary care center in India. Singapore Med J. 2009;50(6):628-32.
27. Sham PM, Isaacs RD. Ertapenem, the first of a group of carbapenem. J Antimicrob Chemother. 2003;52(4):538-42.
28. Zhanel GG, Wiebe R, Dilay L, Thomson K, Rubinstein E, Hoban DJ, et al. Comparative review of the carbapenems. Drugs. 2007;67(7):1027-52.
29. Patel G, Bonomo RA. Status report on carbapenemases: challenges and prospects. Expert Rev Anti Infect Ther. 2011;9(5):555–70.
30. Center for Public Health Practice Oregun. Guidance for control of carbapenem resistant *enterobacteriaceae*. Oregon Toolkit; 2013.
31. McGovern PC, Wible M, El-Tahtawy A, Biswas P, Meyer RD. All-cause mortality imbalance in the tigecycline phase 3 and 4 clinical trials. Int J Antimicrob Agents. 2013;41(5):463–7.
32. Verde PE, Curcio D. Imbalanced mortality evidence for tigecycline: 2011, the year of the meta-analysis. Clin Infect Dis. 2012;55(3):471–472.
33. Elizabeth BH, Vincent HT. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother. 2010;65(6):1119–25.
34. Cohen SJ, Leverstein-Van HMA. Guideline for phenotypic screening and confirmation of carbapenemases in *Enterobacteriaceae*. Int J Antimicrob Agents. 2010;36(3):205–10.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=525&id=8&aid=4792