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Original article

Colistin and tigecycline susceptibility among carbapenemase producing *Enterobacteriaceae* at a tertiary care hospital of South India

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ABSTRACT

Background: Carbapenem resistance among *Enterobacteriaceae* is a serious clinical problem and the global spread of such resistant strains has hampered the treatment effort leaving with few choices of antibiotics like tigecycline and colistin. **Methods:** Therefore, we looked for the susceptibility pattern of tigecycline and colistin among carbapenem-resistant *Enterobacteriaceae* (CRE) by Epsilometer (E) test at Sri Ramachandra University Hospital, Porur, Chennai, India. This study used the combined disc test with phenylboronic acid and EDTA; and modified Hodge test (MHT) to differentiate the carbapenemases. The minimum inhibitory concentration of tigecycline and colistin susceptibility was determined for CRE isolates by using the E-test strips ranging from 0.016-256 µg /ml. **Results:** A total of 238 extended spectrum beta lactamase producers from Family *Enterobacteriaceae* were included in the study. Among those, 37 isolates were MHT positive. On combined disc test, 14 were metallo beta lactamase positive, 4 were *Klebseillae pneumoniae* carbapenemase positive and only one isolate was found to be positive for both. Out of 51 CRE isolates, the number of tigecycline and colistin resistant were found to be in 30 and 3 respectively according to EUCAST criteria. **Conclusion:** Tigecycline may be effective but it needs to be monitored routinely. Colistin remains a reliable option for CRE infections. The increasing resistance of CRE to the available antibiotics like tigecycline and colistin is a threat to the therapeutic management of such patients.

Introduction

Carbapenems are potent and effective broad-spectrum β-lactam antibiotics commonly used for the treatment of multidrug-resistant *Enterobacteriaceae* infections. Carbapenemase-producing *Enterobacteriaceae* (CPE) causes cystitis, pneumonia, meningitis, bacteremia, septicemia, and wound infections with prolonged hospital stays and increased mortality rates in debilitated and immunocompromised patients. [1] Carbapenem-resistant *Enterobacteriaceae* (CRE) is not uncommon, mostly because of the acquisition of carbapenemase gene and a decrease in bacterial outer membrane permeability. Widespread of carbapenemase producers in particular *Klebsiella pneumoniae (K. pneumoniae)* and *Escherichia coli*, is a serious clinical issue because of clonal spread and plasmid-mediated transmission that confer resistance to most β-lactams. Also, carbapenemase

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producers are usually associated with many other non–β-lactam resistance determinants, which give rise to multidrug- and pan drug-resistant isolates [2]. Various carbapenemases have been reported in *Enterobacteriaceae*. These enzymes include the class A carbapenemases (*Klebsiella pneumoniae* carbapenemase KPC types), the class B or metallo β-lactamases (MBLs), and the class D oxacillinases (e.g., OXA-48-like enzymes) [3].

Carbapenemases production is not the only mechanism of acquired resistance to carbapenems but is the most important one for infection control concern. A confirmation of CPEs is needed in most cases to infection prevention and control teams. Delayed recognition of this mechanism of resistance will lead to inappropriate treatment and the spread of such strains to the community and environment. Therefore, the identification of carbapenemase producers for these reasons is important [4,5].

The increasing prevalence of CRE at a rapid pace in India and worldwide poses a challenge in the treatment with fewer antibiotic options [6,7]. In this scenario, tigecycline and colistin remains the choice of treatment for such infections. and needs to be explored further [5,8,9]. Although colistin retained activity against CRE, more recent data suggest that resistance to colistin is emerging, and outbreaks of colistin-resistant strains have been reported [10].

In such circumstances of the increasing number of reports of the variable susceptibility of tigecycline and colistin against CRE from different regions of the world and the fewer therapeutic options, this study investigated carbapenemase resistance in *Enterobacteriaceae* and determined in vitro activity of tigecycline and colistin against resistant isolates at Sri Ramachandra Medical Center, Chennai, India.

Material and Methods

This study was carried out at Sri Ramachandra Medical Center, Porur, Chennai, India between November 2014 and April 2015. The hospital is 1600 bedded tertiary care center and serves a wide range of patients across the country. The hospital laboratory is accredited with the National Accreditation Board of Laboratories (NABL). Ethical approval for this study was obtained from Institutional Review Board, Sri Ramachandra University (CSP/14/OCT/37/209). All non-duplicate *Enterobacteriaceae* other than *Salmonella*, *Shigella*, *Proteus* and *Morganella* isolated from patient specimens except stool that were resistant to any of the third-generation cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) and/or had reduced susceptibility to imipenem, or meropenem on disc diffusion test were included in the study. Epidemiological data was retrieved from a registration form containing information on sex, age, in- or outpatient status, hospital department, and specimen type.

Laboratory procedures

Primary isolation, identification and routine susceptibility testing

The primary isolation was carried according to the laboratory standard protocol depending on the type and site of the sample on Cysteine lactose electrolyte deficient medium (CLED), blood agar, Mac Conkey agar, and chocolate agar. Clinically significant colonies were identified by using a set of in house biochemical tests as per standard protocols [11] and Vitek 2 GN ID (BioMerieux, France) for isolates from ICU sample with appropriate quality control [12].

Antimicrobial susceptibility testing was performed using the Clinical and Laboratory Standards Institute (CLSI) disc diffusion method with Mueller-Hinton agar (MHA) (Hi-Media, India). *Escherichia coli* strain ATCC 25922 were tested as internal control each time performed the susceptibility test [13].

Carbapenem susceptibility test

All the isolates that were resistant to routine susceptibility were tested for susceptibility to meropenem (10µg), imipenem (10µg), and ertapenem (10µg) (Hi media) by disc diffusion method according to CLSI criteria. Resistance of *Enterobacteriaceae* strains to carbapenem were reported if zone diameter to ertapenem is ≤ 18 and/or ≤ 19 for meropenem [13]. Isolates with reduced susceptibility to meropenem with or without ertapenem were confirmed by Modified Hodge Test, KPC, and MBL for carbapenemase production.

Modified Hodge test

All CRE isolates detected by disc diffusion were further tested by the Modified Hodge test as per CLSI guidelines [13]. Briefly, standard suspension of *E. coli* ATCC 25922 was inoculated on Mueller Hinton agar as for the routine disc-diffusion procedure. Meropenem disc (10µg) was placed on the center of the plate. Using a loop, test organism grown was inoculated in a straight line out from the edge of the disc. Following incubation, the plate was

examined for the presence of enhanced growth around the test streak at the intersection of the streak and the zone of inhibition which was considered as positive for carbapenemase production [13].

Differentiation of KPC and MBL

The use of inhibitor phenylboronic acid (PBA), EDTA, or both along with meropenem disc (10µg) were used for the detection of KPC and MBL, respectively [14].

The stock solution of PBA in the concentration of 20 mg/ml was prepared by dissolving PBA (Hi media) in dimethyl sulfoxide. Twenty microliters (400 µg of PBA) from this solution was dispensed onto meropenem discs. The stock solution of EDTA was prepared by dissolving anhydrous EDTA (Hi media) in distilled water at a concentration of 0.1 M. Ten microliters (292 µg of EDTA) from this solution was dispensed onto meropenem discs. The meropenem discs with an inhibitor added were dried in an incubator at 37°C and used within 60 min [14].

On Mueller Hinton agar plate inoculated with test strain, four discs of meropenem were used. One disc of meropenem without any inhibitor, one disc of PBA (400 µg) only, one disc of EDTA (292 µg) only, and the fourth disc of meropenem having both PBA plus EDTA were used. The agar plates were incubated at 37°C overnight and the diameter of the growth inhibitory zone around these meropenem discs with an inhibitor added was compared with that around the plain meropenem disc [14].

Interpretation

The isolate was considered KPC-producing when the growth- inhibitory zone diameter around the meropenem disc with PBA and the meropenem disc with both PBA and EDTA was increased by ≥5 mm compared with the growth-inhibitory zone diameter around the disc containing meropenem alone.

The isolate was considered MBL producing when the growth- inhibitory zone diameter around the meropenem disc with EDTA and the meropenem disc with both PBA and EDTA was increased by ≥5 mm compared with the growth-inhibitory zone diameter around the disc containing meropenem alone.

The isolate was considered producing both KPC and MBL enzymes when the growth-inhibitory zone diameter around the meropenem disc with both PBA and EDTA were increased by ≥5 mm compared with the growth-inhibitory zone diameter around the disc containing meropenem alone while the growth-inhibitory zone diameters around the meropenem disc with PBA and the meropenem disc with EDTA was increased by <5 mm compared with the growth-inhibitory zone diameter around the disc containing meropenem alone. The isolate was considered negative for MBL and KPC production, when none of the three combined-disc tests was positive [14].

Controls

a. The concentration of PBA and EDTA employed for this study was tested for any detectable effect on bacterial growth.
b. Positive control for KPC – *K. pneumoniae* ATCC BAA-1705.

Tigecycline and colistin susceptibility testing

The minimum inhibitory concentration (MIC) of tigecycline and colistin susceptibility was determined for CRE isolates by using the E-test strips ranging from 0.016-256 µg /ml (Hi-Media, India) according to the manufacturer's instructions. Bacteria were cultured on a blood agar plate for 18h at 37°C and colonies resuspended in sterile saline to 0.5 McFarland standards. Each suspension was inoculated on a 90-mm diameter Mueller Hinton agar plate and E test strips were applied as recommended by the manufacturer. Results were recorded after 16-20h of incubation. Quality of media, and E test strips were checked with *E. coli* ATCC 25922.

The interpretation of tigecycline and colistin MIC was done by using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline for *Enterobacteriaceae*. The tigecycline MIC breakpoints were used as ≤1 and ≥2 mcg/ml for the susceptible and the resistant strains, respectively. For colistin, MIC ≤ 2 mcg/L was regarded as susceptible while ≥ 2 mcg/ml as resistant [15].

Results

A total of 238 samples were collected from the clinical microbiology department from different specimens in Sri Ramachandra Laboratory services. Of the 238 third-generation cephalosporin-resistant isolates, 180 were found to be resistant to meropenem according to the CLSI criteria. Out of 180, the isolated organisms were *E. coli* (n=114), *K. pneumoniae* (n=59), *C. freundii* (n= 2), *Enterobacter* spp. (n=3) and *K. oxytoca* (n=2). The source of these isolates were urine (n=114), pus (n=44), blood (n=17), tracheal aspirate (n=2), ascitic fluid (n=1), bronchial wash (n=1) and eye swab (n=1). The organisms isolated from the patients mostly belong to the general medicine, general

surgery, orthopedics, and pediatrics. Out of 180; 25 were from ICU.

Antibiotic susceptibility pattern

Maximum susceptibility was noticed with colistin (99%) followed by tigecycline (90 %). Nitrofurantoin was susceptible to 77% of the urinary isolates. Amikacin 77%, tazobactum -piperacillin 70%, cefaperazone-sulbactum 64%, and amoxicillin-clavulanic acid 58% were the other antibiotics that were susceptible **(Figure 1)**.

Detection of carbapenem resistance by disc diffusion

Of 180 meropenem resistant isolates, 69 were resistant to imipenem. Maximum resistance (100%) to carbapenems was noted in *Enterobacter* species and *C. freundii* followed by *K. pneumoniae* (78%). A total of 112 isolated strains either resistant to meropenem only or together with imipenem were further subjected to modified Hodge test (MHT). 33% (37/112) of the carbapenem-resistant isolates were MHT positive **(Table 1, Figure 2)**.

Detection of carbapenemases by combined disc test

A combined disc test detected both metallo beta-lactamases and *K. pneumoniae* carbapenemases simultaneously. Among the 112 carbapenem-resistant isolates by disc diffusion, 14 (12.5%) were positive for metallo beta-lactamases by screening with EDTA, and 4 (3.57%) were *K. pneumoniae* carbapenemases (KPC) by screening with PBA. Only one isolate was found to be positive for both MBL and KPC **(Table 2)**.

Distribution of various types of carbapenemases

The MHT/-52 was positive in 25 (41.66%) of sixty imipenem resistant isolates which were also resistant with meropenem. Fourteen (23.33%) of these imipenem and meropenem resistant isolates gave positive results for MBL and 4 for KPC. Only one isolate was found to be both KPC and MBL positive by the inhibitor-based method **(Table 2)**.

Distribution of carbapenemases in family *Enterobacteriaceae*

Among 61 *E. coli*, 13 were positive for MHT, and 4 were positive for MBL. None of the strains were found to be KPC producers. In *K. pneumoniae* (n=45), 20 were MHT positive, 10 were MBL positive, 1 was KPC positive and 1 was positive for both MBL and KPC. Strains of *C. freundii*, *K. oxytoca*, and *Enterobacter* species were positive only for MHT and were negative for either MBL or KPC **(Table 3)**.

In-vitro susceptibility of tigecycline in carbapenemase producers

All the 51 carbapenem-resistant isolates (meropenem & imipenem or both resistant) were subjected to MIC for tigecycline by E-strip. Minimum inhibitory concentration ranged from 0.125µg /ml to 64 µg /ml as shown in **table (4)**. Out of 51, only 16 were found to be sensitive, 5 were intermediate and 30 were found to be resistant according to EUCAST criteria as depicted in **table (5)**.

Susceptibility pattern of carbapenem-resistant isolates to colistin

Colistin MIC was detected by E-strip and ranged from 0.125µg/ml to 4 µg/ml **(Table 6)**. As seen in **table (7)**, out of 51, only 3 were found to be resistant and rest were sensitive according to EUCAST criteria.

Susceptibility pattern of tigecycline and colistin in family *enterobacteriaceae*

Higher resistance to tigecycline was observed among *K. pneumoniae* (n=22) followed by *E. coli* (5), *Enterobacter* spp. (2) and *C. freundii* (1) **(Table 8)**.

As seen in **table (9)**, higher resistance to colistin was observed among *K. pneumoniae* (n=2) and *Enterobacter* spp. (1). None of the *E. coli* was resistant to colistin.

Comparison of MIC and disc diffusion test for tigecycline and colistin

Among a total of 51 comparable isolates tested by both disc diffusion and E-test, 10 isolates were identified as resistant, 14 were identified as sensitive and 3 were identified as intermediate by both methods. A total of 14 isolates were identified as resistant by E-test which were sensitive by disc diffusion whereas 1 isolate identified as resistant by disc diffusion was found to be sensitive by E-test **(Table 10)**.

For colistin, a 100% match existed by both methods. A total of 48 as sensitive and 3 as resistant were identified by both method of drug susceptibility method **(Table 11)**.

Figure 1. Antibiotic susceptibility pattern.

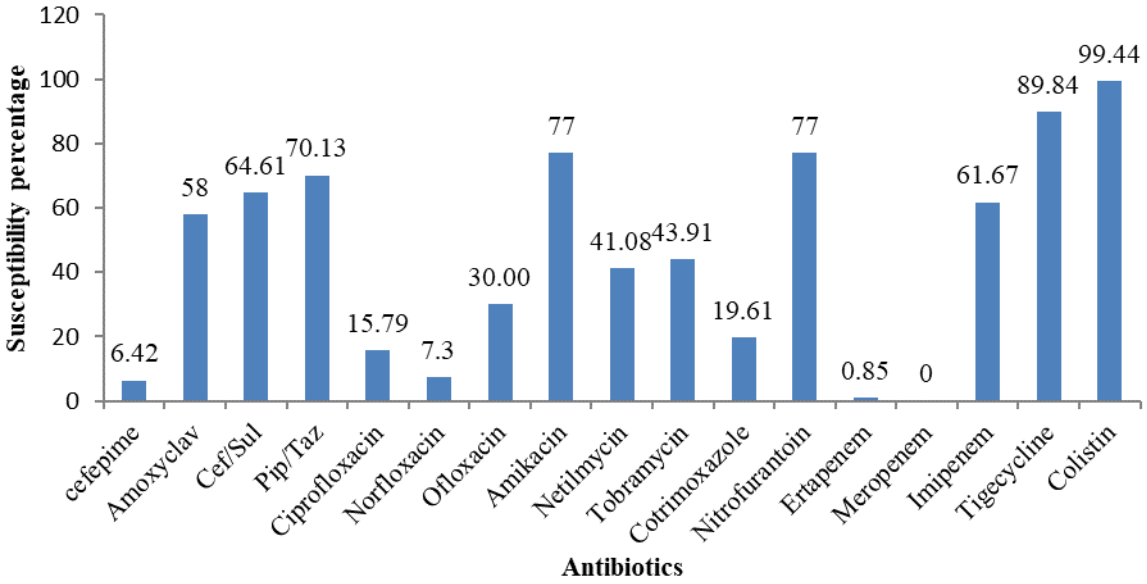

Figure 2. Modified Hodge test.

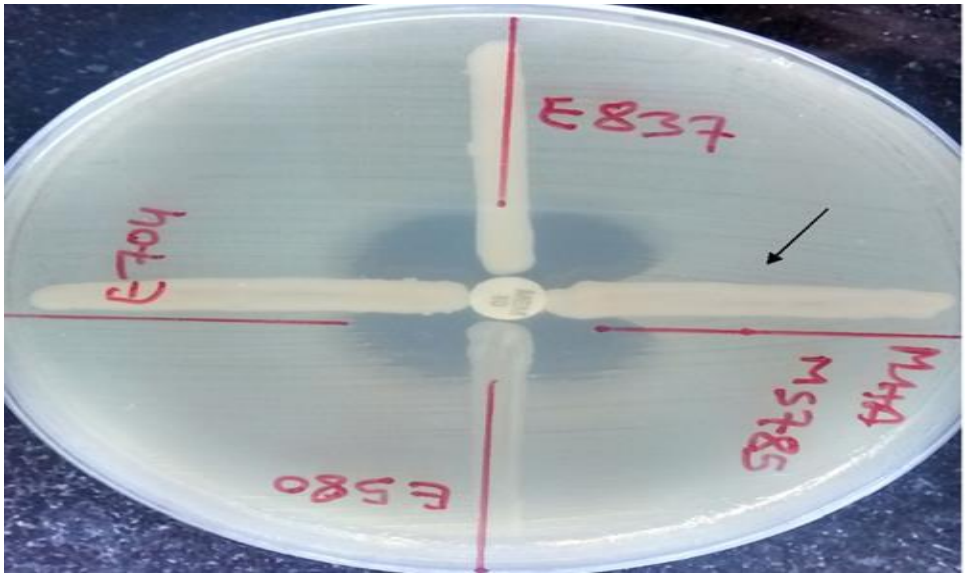

Table 1. Bacterial isolates showing resistance to carbapenems.

Organism (No. of carbapenem resistant isolates)	**Resistance (%)**		
	Meropenem	**Imipenem**	**Both (Meropenem+Imipenem)**
E. coli (114)	114 (100%)	18 (15.78%)	18 (15.78%)
K. pneumoniae (59)	59 (100%)	46 (77.96%)	46 (77.96%)
Enterobacter spp. (3)	3 (100%)	3 (100%)	3 (100%)
K. oxytoca (2)	2 (100%)	0 (0%)	0 (0%)
C. freundii (2)	2 (100%)	2 (100%)	2 (100%)
Total (180)	180 (100%)	(38.33%)	69 (38.33%)

Table 2. Distributions of carbapenemases.

Carbapenemases		Carbapenem resistance (No. of isolates)		
		Meropenem (112)	**Imipenem (60)**	**Both Meropenem & Imipenem (60)**
MHT (%)	Positive	37 (33%)	25 (41.66%)	25 (41.66%)
	Negative	75 (67%)	35 (58.34%)	35 (58.34%)
MBL (%)	Positive	14 (12.5%)	14 (23.33%)	14 (23.33%)
	Negative	98 (87.5%)	46 (76.67)	46 (76.67)
KPC (%)	Positive	4 (3.57%)	4 (6.66%)	4 (6.66%)
	Negative	108 (96.43%)	56 (93.34)	56 (93.34)
MBL+KPC (%)	Positive Negative	1 (0.89%) 111 (99.11%)	1 (1.66%) 59 (98.34%)	1 (1.66%) 59 (98.34%)

Table 3. Carbapenemases among *Enterobacteriaceae*.

Organisms	**Type of Carbapenemases**				**Total Carbapenemases**
	MHT (%)	**MBL (%)**	**KPC (%)**	**MBL+KPC (%)**	
***E. coli* (61)**	13 (21.31%)	4 (6.55%)	0	0	14
***K. pneumoniae* (45)**	20 (44.44%)	10 (22.22%)	4 (8.88%)	1 (2.22%)	30
***Enterobacter* spp. (3)**	2 (66.66%)	0	0	0	2
***C. freundii* (2)**	1 (50%)	0	0	0	1
***K. oxytoca* (1)**	1 (100%)	0	0	0	1
Total (112)	37 (33%)	14 (12.5%)	4 (3.57%)	1 (0.89%)	48

Table 4. Tigecycline MIC among carbapenemase producers.

Carbapenemase		**No. of isolates with MIC Tigecycline (µg/ml)**									**Total**
		1	2	4	8	16	32	64	128	256	
MHT	Positive	6	1	2	0	0	10	5	0	0	24
	Negative	10	6	3	1	0	4	3	0	0	27
MBL	Positive	4	2	2	0	0	2	0	0	0	10
	Negative	12	5	3	1	0	12	8	0	0	41
KPC	Positive	2	0	0	0	0	0	0	0	0	2
	Negative	14	7	5	1	0	14	8	0	0	49

Table 5. Tigecycline sensitivity pattern according to EUCAST guidelines.

Tigecycline MIC	Frequency	Percentage (%)
≤1 (S)	16	31.4
1.1-2 (I)	5	9.8
>2 (R)	30	58.8
Total	51	100.0

Table 3. Colistin MIC among carbapenemase producers**.**

Type of carbapenemase		MIC of Colistin in (µg/ml)				Total
		1	2	4	>4	
MHT	Positive	2	21	1	0	24
	Negative	1	24	2	0	27
MBL	Positive	0	10	0	0	10
	Negative	3	35	3	0	41
KPC	Positive	0	2	0	0	2
	Negative	3	43	3	0	49

Table 4. Colistin sensitivity pattern according to EUCAST guidelines.

Colistin MIC (CLSI)	Frequency	Percent
<2 (S)	48	94.1
≥2 (R)	3	5.9
Total	51	100

Table 5. Tigecycline sensitivity in *Enterobacteriaceae.*

Organism	Tigecycline			Total
	≤1(S)	1.1-2 (I)	>2 (R)	
E. coli	6	1	5	12
K. pneumoniae	9	4	22	35
Enterobacter spp.	0	0	2	2
C. freundii	1	0	1	2
Total	16	5	30	51

Table 6. Colistin activities in *Enterobacteriaceae.*

Organism isolated	Colistin MIC (mcg/ml)		Total
	<2 (S)	≥2 (R)	
E. coli	12	0	12
K. pneumoniae	33	2	35
Enterobacter spp.	2	1	3
C. *freundii*	2	0	2
Total	48	3	51

Table 10. Comparison of disc diffusion and MIC for tigecycline.

Tigecycline MIC				
Disc diffusion	Sensitive	Intermediate	Resistant	Total
Sensitive	14	2	14	30
Intermediate	1	3	4	8
Resistant	1	2	10	13
Total	16	7	28	51

Table 11. Comparison of disc diffusion and MIC for colistin.

Colistin MIC			
Disc diffusion	Sensitive	Resistant	Total
Sensitive	48	0	48
Resistant	0	3	3
Total	48	3	51

Discussion

Enterobacteriaceae isolates are important nosocomial pathogens responsible for various infections. Carbapenem resistant *Enterobacteriaceae* related infections are related to high mortality. The increasing burden of carbapenem resistance has obscured its clinical efficacy in preventing and treating life-threatening nosocomial infections. Therefore, it is necessary to evaluate the resistance level.

The present study included 238 third-generation resistant *Enterobacteriaceae* isolates collected for six months. 75.6% (N=180) of them were found to exhibit carbapenem resistance by the disc diffusion method. This was in concordance with a study done in this same institute by **Sekar et al.** in 2010 [16]. Numerous studies from India have discovered varying rates of carbapenem resistance. **Manoharan et al.**, **Priyadutta et al.**, **Wattal C et al.**, **and Gupta et al.** found 17%, 7.87%, 13-57%, and 17-22% carbapenem resistance, respectively [17-20].

A major challenge in determining an appropriate antibiotic regimen to treat infections with CRE occurred because of increasing resistance to most other antibiotic classes, leaving very few antimicrobial options available. These options include polymyxins, newer aminoglycosides, tigecycline, and ceftazidime-avibactam. Carbapenem-resistant *Enterobacteriaceae* are often resistant to all β-lactam drugs and frequently carry mechanisms conferring resistance to other antimicrobial classes, due to the frequent occurrence

of other resistance genes on the same mobile genetic elements. In this study, all the carbapenem-resistant isolates showed cross-resistance to β-lactams and β-lactamase inhibitor combinations **(Figure 1)**. Carbapenem resistance was seen mainly among *E. coli* (n=114) followed by *K. pneumoniae* (n=59). The majority of these isolates were from urine, pus, and blood. Similar results were also reported by **Nagaraj et al.** and **Shanmugam et al.** [21,22].

Based upon the updated CLSI criteria (CLSI-2014 guidelines), 38.33% of the meropenem resistant isolates (N=180) were also resistant to imipenem in this study whereas **Rai et al.** reported 61.7% imipenem resistance in meropenem resistant isolates (n=102) [23]. A study by **Mohamudha et al.** also reported 43.68% of imipenem resistance in meropenem resistant isolates among 103 *K. pneumoniae* isolates while **Parveen et al**. reported 73.33% of imipenem resistance in meropenem resistant isolates [24]. The higher resistance to meropenem than imipenem might be due to the greater use of meropenem over imipenem as a result of its higher tolerance and greater efficacy against gram-negative pathogens [25]. *Klebsiella pneumoniae* showed a higher resistance rate of 77.96% (n=46) when compared to *E. coli which* showed 15.78% (n=18) in this study.

The prevalence of carbapenem-resistant *Enterobacteriaceae* varies with geographical region. For example, KPC is the most common carbapenemase in Israel and the United States, VIM is endemic in Greece, and IMP is endemic in Japan. The NDM and OXA-48-like carbapenemases originated in India and Turkey, respectively, where they are endemic, but have successfully disseminated worldwide. In this study, MBL (12.5%) was the most common carbapenemase followed by KPC (3.57%). In contrast, a study done by **Mohamudha et al.** reported 13.33% to be KPC producers in 45 meropenem resistant *K. pneumoniae* and none were MBL producers [24]. Similarly, **Bansal et al**. reported KPC (55.9%) to be the main carbapenemase followed by MBL (29.39%). Another study done in 26 CRE isolates by **Datta et al.** showed 73.07% MBL while none were KPC producers [18]. The reason for this varied percentage may be due to sample size, selection criteria, geographical region, and various screening methods were employed.

The incidence of CPE in this study was 37.5% (n=48/112). The rate of CPE in other studies done in various parts of India was found to be ranging from as low as 7.87% to 51% at the maximum [18,23,26]. In the present study, *K. pneumonia* (75.5%) was found to be the predominant CPE producer followed by *Enterobacter* spp. (66.66%) and *E. coli* (27.6%). This was in concordance with a previous study done in this center by **Sekar et al.** in 2010. Whereas a study done by **Gupta et al.** found that *Enterobacter* (43.52%) to be the major carbapenemase producer followed by *E. coli* (20.75%) [27].

Only 37.33% were carbapenemase producers and the rest (N=64) 67% were negative for any type of carbapenamase despite being resistant to the carbapenems by disc diffusion method in this study. The probable cause could be due to overproduction of ESBL or AmpC, with porin loss, or combination of these [28]. Moreover, the presence of OXA mediated resistance and NDM-MBL producers could not be easily detected by phenotypic methods [29].

The number of isolates positive by both MHT and identified as MBL was 6, 3 of *K. pneumoniae,* and *E. coli* each emphasizing that these strains could be producing other types of carbapenemases. Those identified as KPC's along with positive MHT were 1 strain of *K. pneumoniae* strengthening the fact that KPC production was identified also by MHT. Those strains that were only positive by MHT alone were 30. These CPE strains could be due to the production of other types of carbapenemases which need confirmation with the molecular study.

The main drawback of the study is the inability to compare the phenotypic results with that of the molecular studies available for the detection of carbapenemase production. Though there is a lacuna in detecting all types of carbapenamase by using both MHT and combined disc test, still this technique can be used as an effective tool in distinguishing the types of CPE and thereby improve patient outcome by aiding infection control and in curbing the rise in CPE.

Emerging resistance to carbapenems has left only fewer options for treatment. In this situation, tigecycline and colistin use have been widely advocated and used. However, reports of colistin and tigecycline resistant isolates have emerged and these isolates are commonly referred to as pan-resistant. This study therefore, investigated the tigecycline and colistin susceptibility by disc diffusion for all of the 180 meropenem resistant

isolates and detected the MIC by E-test for 51 carbapenemase-producing isolates.

Tigecycline MIC ranged between 0.125 to 64 µg/ml. A total of 14 isolates that appeared to be sensitive on disc diffusion were resistant to E-test. Discrepancies were also observed between disc diffusion and E-test for tigecycline which may be due to testing with different methodologies and interpretive criteria. The effect of test media and breakpoint criteria may have contributed to some extent to the reported a marginally higher tigecycline resistance in this study [30].

The higher percentage of tigecycline resistance was worrisome. **Livermore et al.** reported a similar susceptibility to tigecycline - 47%, of 81 isolates tested by agar dilution method among carbapenemase-producing *Enterobacteriaceae* and lower rates of tigecycline susceptibility in non-*E. coli* species in the UK. The present study found that all the 238 ESBL producing strains were 90% susceptible to tigecycline. Similar reports were furnished by **Shanthi et al.** in 2010 and 2011 in 2 different studies at our center. Different studies done by **Rouchelle et al.**, **Gupta et al.** and **Behera et al.** also reported tigecycline susceptibility against all ESBL producing isolates [27,31,32].

According to EUCAST breakpoint criteria for colistin, only three isolates resistant to colistin were encountered which were also resistant by disc diffusion criteria thus producing the 100% concordance between both methods. The MIC for colistin ranged from 0.125µg/ml to 4 µg/ml. Carbapenemase producing *Enterobacteriacea* producers were relatively susceptible to colistin 94.1% when compared to tigecycline (41%). Moreover, 99% of the ESBL producers were also susceptible to colistin. *K. pneumoniae* was comparatively more resistant to tigecycline 63% and colistin 5.7% whereas *E. coli* exhibited 41.6% and 100% respectively.

The rising trend of increasing MIC of *Enterobacteriaceae* towards both colistin and tigecycline has raised concerns for an early detection and containment of CPE and not the treatment alone.

Conclusion

The increasing resistance of CPE to the available antibiotics like tigecycline and colistin is a threat to the clinical management thereby effective measures for early identification and control should be done to prevent the potential continuous dissemination of these carbapenem-resistant pathogens. Combined disc test and MHT can differentiate the carbapenemases to understand the drug resistance mechanism. Tigecycline may be effective with regular monitoring to track the development and dissemination of resistance. Colistin remains a reliable option for CRE infections.

Conflicts of interest: None.

Financial diclosure: None.

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