ORIGINAL ARTICLE

Susceptibility of Tigecycline against Carbapenem Resistant *Enterobacteriaceae*

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ABSTRACT

Background: *Enterobacteriaceae*, a large family of Gram-negative bacteria, are one of the commonest etiological agents causing serious bacterial infections to humans. Carbapenems are the group of antibiotics with a broad spectrum of antimicrobial action. Infections caused due to Carbapenem resistant *Enterobacteriaceae* (CRE) are a huge challenge for existing medical practice. Therefore, this project aimed to find out the antimicrobial susceptibility pattern of Tigecycline against CRE.

Methods: This cross-sectional study with non-probability consecutive sampling was done at Ziauddin Hospital Microbiology Laboratory from 15th August 2017 to 15th April 2018. Accordingly, 151 isolates of CRE were collected from cultures of blood, respiratory tract, wound pus and other body fluids. The growth inhibition zones were measured following the Food and Drug Administration (FDA) disk diffusion breakpoint criteria. Frequencies and percentages were computed for gender, microorganism, and antimicrobial susceptibility. Chi-squared test was applied and $p \leq 0.05$ was considered as statistically significant.

Results: *Klebsiella species* were most commonly isolated pathogen, 67.5% (n=102) followed by *Escherichia coli* (*E. coli)* 23.2% (n=35), *Enterobacter* 7.3% (n=11) *and Serratia species* 2% (n=3). Tigecycline was 97% (34 /35*)* sensitive for *E. coli*, 86.3% (88/102) for *Klebsiella species)*, 91% (10/11) for *Enterobacter species*, and 100% for *Serratia species*. *Klebsiella species* showed the highest rate of resistance to tigecycline i.e., 13.7% of the total *Klebsiella* isolates.

Conclusion: Among the *Enterobacteriaceae* family, *Klebsiella species* have the greatest ability to acquire resistance. Tigecycline showed good activity against isolates of CRE recovered from infections of skin, soft tissue, intra-abdomen, lower respiratory tract and blood stream.

Keywords: Carbapenems; beta-Lactamases; Anti-Bacterial Agents; Tigecycline; *Klebsiella; Escherichia coli.*

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INTRODUCTION

Gram-negative bacteria belonging to the family *Enterobacteriaceae* are most important cause of healthcare-acquired and community-acquired infections[1]. The antimicrobial resistance in *Enterobacteriaceae* is increasing globally due to the production of extended spectrum beta-Lactamases (ESBLs) which hydrolyze penicillins, cephalosporins and monobactams[2].

Carbapenems are the class of beta-lactam antibiotics with the wide range of antimicrobial action against many Gram-positive cocci and rods, Gram-negative cocci and rods, and anaerobic organisms[3]. They include meropenem, imipenem, doripenem and ertapenem. They have been used successfully to treat infections due to multidrug resistant (MDR) including ESBLs [4]. With the excessive use of Carbapenems, *Enterobacteriaceae* producing carbapenemases have emerged which are of particular concern[5]. Carbapenemases are beta lactamase enzymes that hydrolyze and acquire resistance to a wide range of beta-lactam drugs

including carbapenems[6].

Carbapenemase enzymes fall into 3 of the Ambler classes of beta-Lactamases, class A, class B, and class D. Class A and Class D enzymes have a serine-based hydrolyzing mode of action, while class B are Metallo-β-lactamase enzymes that have zinc in their active site. The class A carbapenemases include members of the *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana extended spectrum (GES) family enzymes etc. The class D carbapenemases include Oxacillinase-48-carbapenemase (OXA-48). The class B Metallo-β-lactamases consists of New Delhi Metallo-β-lactamases (NDM), Imipenemase (IMP) and Verona Integron-encoded Metallo-β-lactamases (VIM). They have been found more frequently in *Pseudomonas aeruginosa*; however, they are increasing in number throughout the world and are to be found in the *Enterobacteriaceae* as well[7].

Carbapenem resistant *Enterobacteriaceae* (CRE) can cause a number of severe infections and the mortality rates are high ranging from 18% to 60% [2]. CRE are resistant to almost all available antibiotics, complicating therapy and limiting treatment options. Tigecycline is one of the anti-bacterial agents which is active against multi drug resistant *Enterobacteriaceae*[8]. One of the tetracycline is a glycylcycline that binds to the 30S subunit of bacterial ribosome and inhibits the synthesis of proteins[9]. It displays great action against several Gram-positive and a large number of Gram-negative bacteria, including multidrug-resistant strains[10]. In one study held in Pakistan on stool samples, 89% of the CRE were susceptible to Tigecycline[11].

Infections caused by CRE are a major challenge for current medical practice. It is crucial to find out alternative treatment options and undergo continuous surveillance to update the clinicians to choose appropriate antimicrobials. The purpose of this study was to explore the antimicrobial susceptibility of Tigecycline against the isolates of CRE. This study will offer additional and effective antimicrobial in the form of Tigecycline against CRE and it will help clinicians and general practitioners to treat difficult pathogens (CRE). This will also reduce selection pressure of antimicrobials and in due course reduce antimicrobial resistance.

METHODS

This study was held at the Microbiology Department, Ziauddin Medical University Hospital, Karachi from 15th August 2017 to 15th April 2018. To determine the antimicrobial susceptibility pattern of Tigecycline against CRE, sample size of patient was calculated with a 0.05 margin of error and 95% confidence interval. Prevalence of tigecycline sensitivity against CRE is 89%. After putting values in the formula $n= z^2_{1-\alpha/5}P(1-P)/d^2$=151, 151 samples were collected from inpatients and outpatients using consecutive sampling (non-probability) technique. All clinical samples of blood, pus, tissue, wound, sputum, tracheal aspirate and other body fluid cultures yielding growth of CRE were collected for the study and tigecycline sensitivity was checked. Clinical laboratory standard institute (CLSI) guidelines on Antimicrobial Susceptibility Testing were followed. Male and female patients of age 18-80 years were included. Exclusion criteria were strappingly fulfilled. All duplicate isolates, samples showing no growth or growth other than CRE and urine cultures were excluded. Written approval was taken from the institutional ethical committee (ERC Reference Code: 0150517 MFMB). Informed consents were taken from either the patient or any relative of the patient.

Age, gender, source of specimen and prior antibiotic use were collected and recorded. All the samples were received in sterile containers, inoculated on quality control (QC) checked media plates, Sheep blood agar (SBA), Chocolate agar and MacConkey agar. Streaking was performed with a sterile wire loop and culture plates were placed in incubator for 24-48 hours at 35°C-37°C following the standard protocols. Plates were noticed for any bacterial growth of colonies and gram stain was performed. To identify the suspected bacterial colonies of Gram-negative rods, few biochemical tests were performed like Oxidase test, Triple sugar iron (TSI), Sulphide indole motility (SIM), Citrate utilization and Urease tests. Final confirmation of bacterial identification was done by analytical profile index 20 Enterobacteriaceae (API 20E) [12].

The susceptibility testing of isolated organisms was done by using modified Kirby Bauer's disc diffusion test on Mueller Hinton agar (MHA) (Oxoid Limited, England). After 24-hours incubation, isolated colonies were suspended in normal saline to make a suspension of 0.5 McFarland turbidity which resulted in a confluent lawn by using sterile swabs over the MHA plates. In order to screen for carbapenem resistance in the bacteria identified, double disk diffusion technique was performed by using discs containing Meropenem (10µg), a zone diameter of ≤ 19 mm indicated that the organism is carbapenem resistant and were labeled as CRE[13].

All CRE positive isolates were now tested with a 15µg disc of Tigecycline (Oxoid, England) to find out the effectiveness of Tigecycline against CRE. The growth inhibition zones were measured following the Food and Drug Administration (FDA) disk diffusion breakpoint criteria for tigecycline (≥19 mm is sensitive and ≤14 mm is resistant) and recorded[14].

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Data entry was done and analyzed by using Statistical Package for Social Sciences (SPSS) version-20. Descriptive statistics were used to summarize the categorical variables such as gender, microorganism's identification, antimicrobial susceptibility (Tigecycline as sensitive or resistant) and were reported as frequencies and percentages. Continuous variables like those that age was presented as mean and standard deviation was calculated. Stratification was done with age and gender by outcome variables (Tigecycline: sensitive or resistant). Post stratification Chi-squared test was used and $p \leq 0.05$ was considered as statistically significant.

RESULTS

The mean age of the patients was 55.58±18.27 years. Most of the patients with infections caused by CRE were 71- 80 years of age, 27.8% (n=42). Out of 151 patients, 63 patients (41.7%) were falling in age group 18 to 50 years and 88 patients (58.3%) were in age group 51- 80 years. In Figure 1, the histogram shows age distribution in different age groups.

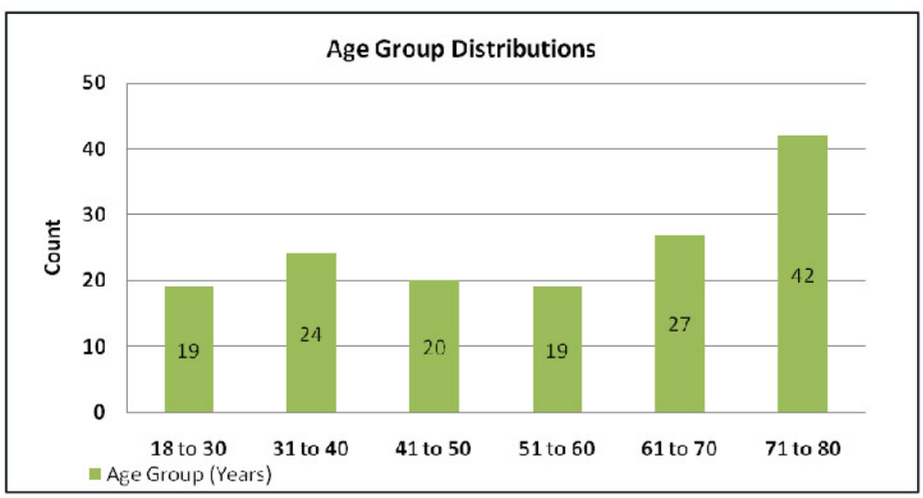


Figure 1: Age distribution of patients (n=151).

There were 94(62.25%) male and 57(37.75%) female patients. There were 113(74.83%) patients taking other antibiotics before the sample was collected. Frequency of CRE organisms with respect to the type of specimen and their rate of resistance to Tigecycline is shown in Table 1. Of the total samples collected, source of 21 samples were blood cultures, 68 were pus/tissue cultures, 53 were respiratory cultures and 09 were from other body fluids. *Klebsiella* species was the most common organism that was found in 102(67.5%) cases followed by *E. coli* 35(23.2%), *Enterobacter* 11(7.3%) and *Serratia* species was observed in only 3(2%) cases. Highest rate of resistance to Tigecycline was shown among respiratory cultures, 17% (n=9).

Table 1: Frequency of Carbapenem Resistant *Enterobacteriaceae* (CRE) with respect to type of specimen and their resistance to tigecycline.

Type of Specimen (Culture)	n	Microorganisms				Resistance to Tigecycline
		Escherichia coli	*Klebsiella species*	*Enterobacter species*	*Serratia species*	
Blood	21	2 (9.5%)	15 (71%)	4 (19%)	0 (0%)	3 (14.3%)
Pus / Tissue	68	23 (34%)	36 (53%)	6 (9%)	3 (4%)	4 (5.9%)
Respiratory (BAL, TA, Sputum)	53	8 (15%)	44 (83%)	1 (2%)	0 (0%)	9 (17%)
Other Body Fluids	09	2 (22%)	7 (78%)	0 (0%)	0 (0%)	0 (0%)
Total	151	35 (23.2%)	102 (67.5%)	11 (7.3%)	3 (2%)	16 (11%)

BAL = Bronchoalveolar lavage; TA= Tracheal Aspirate

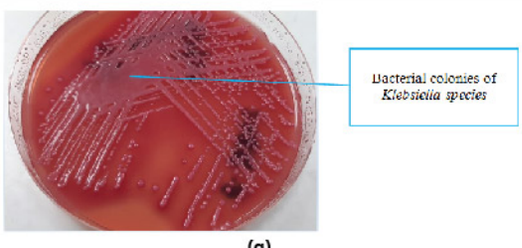
Susceptibility pattern of tigecycline against clinical isolates of carbapenem resistant *Enterobacteriaceae* with respect to age and gender is shown and stratification analysis was performed (Table 2). Tigecycline was more than 80% sensitive for *E. coli, Klebsiella species, Enterobacter species* and *Serratia species* in all age groups and in both males and females. Out of 151 isolates, only 16 (i.e., 11%) were resistant to Tigecycline in which 1isolate was *E. coli*, 14 isolates were *Klebsiella* species, 1 isolate was *Enterobacter species* and none of the *Serratia species* was resistant. *Klebsiella species* showed the

Table 2: Susceptibility Pattern of Carbapenem Resistant Enterobacteriaceae against tigecycline with respect to age groups and gender.

Microorganism	Age Group (Years)				Gender			
	18 – 50 years		51 -80 years		Male		Female	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Escherichia coli (n=35)	13 (37.14%)	1 (2.86%)	21 (60%)	0 (0%)	24 (68.57%)	0 (0%)	10 (28.57%)	1 (2.86%)
Klebsiella species (n=102)	35 (34.31%)	8 (7.84%)	53 (51.96%)	6 (5.88%)	55 (53.92%)	7 (6.86%)	33 (32.35%)	7 (6.86%)
Enterobacter Species (n=11)	4 (36.36%)	0 (0%)	6 (54.55%)	1 (9.09%)	6 (54.55%)	0 (0%)	4 (36.36%)	1 (9.09%)
Serratia species (n=3)	2 (66.67%)	0 (0%)	1 (33.33%)	0 (0%)	2 (66.67%)	0 (0%)	1 (33.33%)	0 (0%)
***p*-Value**	0.524		0.444		0.272		0.87	

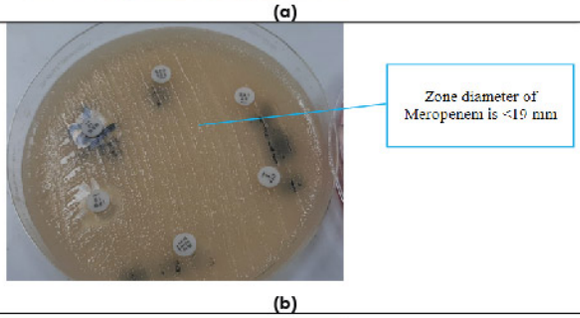
Chi-Squared test applied.

However, all bacteria showed no significant results among different age groups and gender. Figure 2 (a, b and c) shows a CRE isolated from a respiratory sample and its susceptibility testing. On the susceptibility plates, meropenem is resistant and tigecycline is sensitive.



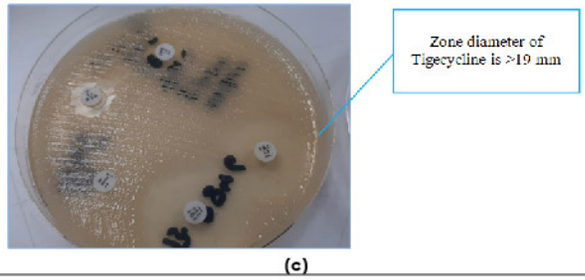
(a)

Figure 2(a): Bacterial colonies of *Klebsiella species* isolated from a respiratory specimen.



(b)

Figure 2(b): Antimicrobial susceptibility testing (AST) shows that this isolate is resistant to Meropenem (zone diameter of ≤19 mm) which indicates that it is Carbapenem Resistant *Enterobacteriaceae* (CRE) region.



(c)

Figure 2(c): Sensitive to tigecycline (zone diameter of ≥19 mm).

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DISCUSSION

Carbapenem resistant *Enterobacteriaceae* (CRE) has emerged as a great challenge to clinical microbiologists, physicians, surgeons and infection control consultants[15]. These organisms are among the fastest growing problems in the area of infectious diseases with worldwide distribution and their prevalence is increasing day by day. Detection of carbapenem producing *Enterobacteriaceae* is a key challenge for a clinical microbiology laboratory as its detection has important implications for clinical decision making[16]. Some health professionals prefer to choose a combination of antibiotics that have some ability to inhibit CRE bacteria. Antibiotics like aminoglycosides, fosfomycin, polymyxins, ceftazidime/avibactam, meropenem-vaborbactam, tigecycline and temocillin have been used to treat CRE infections[17]. The current study was held to ascertain the susceptibility pattern of tigecycline against CRE isolated from patients.

In the present study, *Klebsiella* species was the commonest CRE organism isolated, a result similar to another study that was carried out to show the epidemiology of healthcare-associated infections (HAI) caused by CRE in an Egyptian hospital that reports to the national HAI surveillance system. In this study, *Klebsiella* species was the commonest organism isolated (53.7%) [18]. In our study, Tigecycline was more than 80 percent sensitive for Carbapenem resistant *E. coli*, *Klebsiella species*, *Enterobacter species* and *Serratia species,* a result similar to a study that was conducted in a Mayıs Statement Hospital Ankara, Turkey where a total of 63 CRE were isolated from blood and tracheal aspirate. Out of 63 CRE isolates, the commonest isolate identified was *Klebsiella pneumonia* (60 isolates), 2 isolates were *E. coli* and 1 isolate was *Enterobacter cloacae*. In this study 45 out of 63 CRE isolates were sensitive to tigecycline (71.43%) and 18 isolates were resistant (28.57%)[19].

In another study, which evaluated 100 fecal samples of hospitalized patients that were colonized with CRE in the intensive care unit, all of the isolates were sensitive to colistin and tigecycline[20]. Another study conducted at the Department of Microbiology, Era's Lucknow Medical College and Hospital Lucknow, Uttar Pradesh, India. Out of 491 isolates which were tested, 186 were found to be CRE, Tigecycline was resistant in 12 isolates (8.3%) in which eight isolates were *K. pneumoniae* and four isolates were *E. coli* [21].
A semi-synthetic derivative of Minocycline, tigecycline is the pioneer glycylcycline for using in clinical practice[22]. In comparison to other tetracyclines, tigecycline has shown excellent sustained action against many Gram-positive and numerous Gram-negative bacteria, which include multi-drug-resistant organisms, except *P. aeruginosa* and *Proteus species*. The United States' Food and Drug Administration (FDA) as an organization have accepted Tigecycline for treating severe intra-abdominal infections, community-acquired lower respiratory tract infections, complicated skin, and soft tissue infections. Moreover, it escapes the mechanisms of ribosomal protection [tet (M)] and efflux [tet (A-E)] that makes other members of tetracycline family ineffective[23].

The common adverse effects of tigecycline were reported to be the same as those seen with the other tetracyclines including nausea (29.5%), vomiting (19.7%), and diarrhea (12.7%). Tigecycline has also shown to raise the hepatic enzymes and bilirubin levels in clinical trials. It may cause photosensitivity, pancreatitis and hyper-pigmentation similar to other tetracyclines; it is not prescribed in pregnancy (Pregnancy Category D) and not recommended in children less than 8 years of age. It is a Black Box Warning drug because there is a high risk of death among patients treated with Tigecycline[24].

Criteria for interpretation of tigecycline susceptibility are based currently on the breakpoints mentioned by the FDA or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [25]. However, the Clinical and Laboratory Standards Institute (CLSI) has not yet approved any guidelines regarding tigecycline. Differences in susceptibility results using MIC criteria of the two guidelines and the testing techniques have been published in various reports[26]. Furthermore, well-designed research studies on the clinical activity of tigecycline for infections caused by these pathogens, particularly for bacteremia and respiratory tract infections are required.

CONCLUSION

Tigecycline was found more than 80% sensitive for *Enterobacteriaceae* resistant to Carbapenems. *Klebsiella spp.* was the commonest bacteria to be identified among Carbapenem resistant *Enterobacteriaceae* and it showed the highest rate of resistance to Tigecycline followed by *E. coli* and *Enterobacter spp*. With tigecycline treatment, effective resolution of the infection has been achieved in many relevant reports. Since tigecycline may be one of the last resort agents against complicated and severe MDR infections.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

ETHICS APPROVAL

Ethics Review Committee of the Ziauddin University Hospital approved this study (ERC Reference Code: 0150517 MFMB).

PATIENT CONSENT

Verbal and written consents were obtained from the patients.

AUTHORS' CONTRIBUTION

MF conceived the idea, collected the data, and did manuscript writing and editing. HG performed statistics and reviewed the manuscript. FIA did the critical review and finalization of the manuscript.

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