ORIGINAL ARTICLE

Tigecycline Susceptibility of Carbapenem Resistant Enterobacteriaceae and *Acinetobacter spp.* isolates from Respiratory Tract: A Tertiary Care Centre Study

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Abstract:

Background: Tigecycline is used as a last line of defence against Multidrug Resistant (MDR) strains, and increasing rates of resistance are a growing concern globally. Tigecycline resistance has been reported in various pathogens including *Acinetobacter spp., Klebsiella spp., Enterobacter spp., E. faecalis, S. aureus, S. pneumoniae* and *Serratia marcescens*. *Aim and Objectives:* To study Tigecycline susceptibility pattern of isolates of Enterobacteriaceae and *Acinetobacter spp*. from Respiratory Tract Infections (RTI) in a tertiary care hospital. *Material and Methods:* A total of 7573 respiratory samples were received in Microbiology Department of Govind Ballabh Pant Institute of Postgraduate Medical Education and Research (GIPMER) from 1st January 2018 to 31st December 2018. The samples were processed as per standard techniques. Identification and antimicrobial susceptibility testing was done by VITEK-2 Compact automated system and Kirby – Bauer Disc Diffusion Method as per CLSI Guidelines. *Results:* Out of total 7573 respiratory samples received in laboratory, 1017 (13.42%) were culture positive for pathogens. *Klebsiella pneumoniae* 420(41.29%) was predominantly isolated microorganism followed by *Pseudomonas aeruginosa* 206(20.25%) and *Acinetobacter spp.* 193(18.97%). Most of Gram negative organisms were resistant to commonly used antibiotics. Carbapenem resistance was observed as 67.25%. *Conclusion:* Overall Tigecycline resistance among Carbapenem Resistant Enterobacteriaceae (CRE) and Carbapenem Resistant Acinetobacter (CRA) was found to be 15.50% and 10.69% respectively. Although Tigecycline is a promising antibiotic for the treatment of infections caused by drug resistant problematic pathogens, Tigecycline resistance is most frequently observed in *A. baumannii* and *Enterobacteriaceae*, especially in MDR strains. Hence, we advocate judicious use of Tigecycline in MDR infections and it should be kept as reserve.

Keywords: Tigecycline, Carbapenem Resistant Enterobacteriaceae, *Acinetobacter baumanii*, Respiratory Tract Infections

Introduction:

Intensive Care Unit (ICU) in tertiary care hospitals deal with immunocompromised condition of human life. Lower Respiratory Tract Infection (LRTI) is one of the most common infections among the patients in ICUs. Patients with chronic illnesses or untreated underlying pathology end up with admission in ICUs. Due to decreased immunity, multiple antimicrobials, decreased mobility and presence of life saving invasive devices, the risk of acquiring nosocomial infection is higher. Requirement of mechanical ventilation in patients with LRTIs lead to increase risk of microbial entry into the lower respiratory tract. Each hospital has its own local antibiogram pattern as per the patients it caters to. Increased bacterial

burden is observed in ICUs. Due to increased use of last resort antibiotics such as tigecycline and polymixins in ICUs, emergence of Multidrug Resistant (MDR) bugs is on the rise. *Methicillin Resistant Staphylococcus Aureus* (MRSA), *Vancomycin Resistant Enterococci* (VRE), *Extended Spectrum Beta Lactamase* (ESBL) *producing Gram negative bacteria (E. coli, Klebsiella species, Pseudomonas aeruginosa and Acinetobacter species)* are common pathogens reported from ICUs [1].

Carbapenems have been the therapeutic choice against multidrug resistant bacteria especially in ICU patients. Resistance to carbapenems among non-fermentative Gram negative bacteria is an emerging challenge. Emergence of carbapenemase has been reported across the world. Acinetobacter species are known for surviving in the hospital environment and rapid acquisition of drug resistance. The worldwide emergence of carbapenem resistant Acinetobacter isolates is a grave therapeutic challenge [2].

Tigecycline has a spectrum of activity unparalleled by any other broad spectrum agent and includes MRSA, VRE, *Penicillin resistant Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrhoeae,* ESBL producing Gram-negative bacteria, MDR *Acinetobacter spp.*, anaerobes and rapid growing Mycobacterial species [3].

In our institute, antimicrobial resistance is high in ICU patients, due to presence of history of exhaustive list of antibiotic intake before being referred to us. The antibiotic pressure thus arising leads to usage of higher generation or last resort of antibiotics to tackle the nuisance of MDR strains of Carbapenem Resistant Enterobacteriaceae (CRE) and Carbapenem Resistant Acinetobacter (CRA). This study was undertaken to evaluate Tigecycline susceptibility of Carbapenem Resistant isolates of Enterobacteriaceae and *Acinetobacter spp*. from Respiratory Tract Infections (RTI) in a tertiary care hospital. Findings of the study can be helpful in formulating antimicrobial stewardship program for ICUs and curtail the use of last resort antibiotics.

Material and Methods:

This cross-sectional study was carried out in ICUs of Govind Ballabh Pant Institute of Postgraduate Medical Education and Research (GIPMER), a tertiary care hospital in New Delhi catering to patients referred from various centres from India. The study was conducted from from January 2018 to December 2018 in various ICUs of the hospital. Specimens from lower respiratory tract (sputum and mucus/tracheal aspirate) received from various ICUs were evaluated. All the samples were subjected to Gram stain and culture. The samples were inoculated in 5% sheep blood agar, Chocolate agar and MacConkey Agar (HiMedia, Mumbai, India). All the plates were then incubated at 37ºC for 18-24 hrs. Identification and antimicrobial susceptibility testing was done by VITEK-2 Compact automated system and Kirby–Bauer Disc Diffusion Method as per CLSI Guidelines. Interpretation of Tigecycline for Entero-bacteriaceae was done as per 2018 EUCAST guidelines [4]. For *Acinetobacter spp*, Tigecycline interpretation was done as per Guidelines of British Society for Antimicrobial Chemotherapy [5].

Results:

Out of total 7573 respiratory samples received in laboratory, 1017 (13.42%) were culture positive for pathogens. Demographic details of the positive samples were as depicted in Table 1.

Most of Gram negative organisms were resistant to commonly used antibiotics. *Klebsiella pneumoniae* 420 (41.29%) was predominantly isolated microorganism followed by *Pseudomonas aeruginosa* 206 (20.25%) and *Acinetobacter spp.* 193(18.97%). About 67.25% resistance was observed against Carbapenems.

Overall Tigecycline resistance among CRE and CRA was found to be 15.40% (61/396) and 10.62% (17/160) respectively.

Table 1: Demographic Distribution of Culture Positive Samples

Age Groups (in years)	Number of samples (%)	Male (n)	Female (n)
0 to 15	118 (11.60%)	78	40
16 to 30	242 (23.79%)	94	148
31 to 45	190 (18.68%)	98	92
46 to 60	306 (30.08%)	155	151
Above 60	161 (15.83%)	95	66
Total	**1017 (100%)**	**520 (51.13%)**	**497 (48.87%)**

Table 2: Distribution of Various Bacteria Isolated from Culture Positive Samples

Organism isolated	Tracheal Aspirate (n=865)	Sputum (n=152)	Total n=1017 (100%)
Klebsiella pneumoniae	346	74	420 (41.29%)
Escherichia coli	68	25	93 (9.14%)
Enterobacter spp	22	0	22 (2.16%)
Proteus spp.	14	0	14 (1.37%)
Serratia marcescens	1	0	1 (0.09%)
Acinetobacter spp	178	15	193 (18.97%)
Pseudomonas aeruginosa	185	21	206 (20.25%)
Other Gram negative bacilli	5	0	5 (0.49%)
Staphylococcus aureus	46	17	63 (6.19%)

Table 3: Distribution of Bacterial Isolates from Various ICUs

Organism isolated	NES	NEUR	GIS	GAS	CTVS	CAR	GICU
Klebsiella pneumoniae (420)	111	133	32	23	51	4	66
Escherichia coli (93)	28	21	4	13	9	0	18
Enterobacter spp (22)	9	8	0	0	4	0	1
Proteus spp.(14)	4	10	0	0	0	0	0
Serratia marcescens (1)	0	1	0	0	0	0	0
Acinetobacter spp (193)	49	56	9	7	17	4	51
Pseudomonas aeruginosa (206)	56	79	15	11	8	1	36
Other gram negative bacilli (5)	2	3	0	0	0	0	0
Staphylococcus aureus (63)	24	8	5	4	12	0	10
Total (1017)	**283**	**319**	**65**	**58**	**101**	**9**	**182**

NES-Neurosurgery ICU, NEUR-Neurology ICU, GIS-Gastrointestinal surgery ICU, GAS-Gastroenterology ICU, CTVS-Cardiothoracic vascular surgery ICU, CAR-Cardiology ICU, GICU-General ICU

Table 4: Tigecycline Susceptibility Pattern among Carbapenem Sensitive and Carbapenem Resistant Bacterial Isolates

Organism isolated	Carbapenem Sensitive (n=270)	Carbapenem Sensitive +Tigecycline Sensitive (n=174)	Carbapenem Sensitive +Tigecycline Resistant (n=4)	Carbapenem Resistant (n=684)	Carbapenem Resistant + Tigecycline Sensitive (n=478)	Carbapenem Resistant + Tigecycline Resistant (n=78)
	***Enterobacteriaceae* (154)**			***Enterobacteriaceae* (396)**		
Klebsiella pneumoniae	96	93	3	324	269	55
Escherichia coli	38	38	0	55	52	3
Enterobacter spp	5	5	0	17	14	3
Serratia marcescens	1	1	0	0	0	0
Proteus spp	14	NT	NT	0	NT	NT
	Non-fermenting Gram Negative Bacilli (NFGNB) (116)			**Non-fermenting Gram Negative Bacilli (NFGNB) (288)**		
Pseudomonas aeruginosa	78	NT	NT	128	NT	NT
Acinetobacter spp	33	32	1	160	143	17
Other GNB	5	5	0	-	-	-

NT- Not Tested

Table 5: Overall Tigecycline Susceptibility Pattern in Respiratory Tract Isolates

Organism isolated	Tigecycline Resistant (n=82)	Tigecycline Sensitive (n=652)
***Enterobacteriaceae* (536)**		
Klebsiella pneumoniae	58	362
Escherichia coli	3	90
Enterobacter spp	3	19
Serratia marcescens	0	1
Proteus spp	NT	NT
Non-fermenting Gram Negative Bacilli (NFGNB) (198)		
Acinetobacter spp	18	175
Other Gram negative bacilli	0	5
Pseudomonas aeruginosa	NT	NT

NT- Not Tested

Discussion:

Tigecycline, a bacteriostatic agent, is broad-spectrum glycylcycline approved for the treatment of complicated skin and tissue infections, intra-abdominal infections and community-acquired pneumonia. It serves as a poor substrate for tetracycline-specific efflux pumps [6]. Tigecycline has potent in vitro activity against CRE (except *Proteus spp., Providencia spp. and Morganella morganii*) and *A. baumannii* [7].

In our study, CRE isolation rate was 67.25% which is quite more than reported susceptibility of 60-65% by Indian Council of Medical Research in their annual report of 2018 [8]. The reason for the same can be inclusion of only ICU isolates in our study and hence increased resistant isolates. Study by Tzouvelekis *et al.* reported that majority of CRE infections worldwide are caused by *K. Pneumoniae* [9]. This is similar to our study results (Table 2).

Carbapenem resistance in ICU isolates creates pressure to switch to tigecycline and colistin for treatment. Tigecycline attains suboptimal concentrations in serum, pulmonary epithelial lining fluid and bone, thus limits its clinical use selectively for multidrug resistant organisms [10-12]. Hence, due to its high clinical failure rate and higher mortality, tigecycline monotherapy is not recommended [13-14]. Thus, tigecycline in high-dose therapy or combination is recommended for treating severe multidrug resistant Gram negative infections. In our study, most of Gram negative organisms were resistant to commonly used antibiotics. Aminoglycosides, carbapenem and colistin are common combinations used with

tigecycline for treating CRE infections [15]. Various studies have investigated the clinical efficacy and safety of tigecycline in treating CRE infection and reported different findings. A systemic review and meta-analysis on the efficacy of tigecycline against CRE infection has reported that tigecycline-based combination therapy is more effective than monotherapy [16]. Further, tigecycline combination therapy was reported with lower mortality than monotherapy [17].

Tigecycline is derived from minocycline by the addition of a 9-tert-butyl-glycylamido side chain to the D ring at the ninth position. This side chain aids in overcoming the ribosomal protection proteins and efflux pumps which confer resistance to other tetracyclines [18-19]. Both the antibiotics act by inhibiting protein synthesis. With increased usage, resistance to tigecycline has increased [20]. In our study, overall Tigecycline resistance among CRE and CRA was found to be 15.40% (61/396) and 10.62% (17/160) respectively.

Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter species, E.coli and *S. aureus* were the top five isolates in our study. These pathogens described by Infectious Diseases Society of America as a part of ESKAPE group, are likely to escape the effect of many commonly used antibiotics [21]. Other studies done in ICUs have also reported similar organisms [22-23]. Infections caused by ESKAPE group organisms especially among ICU patients are difficult to treat and have high morbidity and mortality [24]. Patil *et al.* also advocated judicious use of Tigecycline in treatment of MDR *Acinetobacter spp* causing ventilator associated pneumonia. [25].

Inappropriate use of tigecycline in ICUs for treating carbapenem resistant Gram negative organisms is leading to development of resistant against this last resort antibiotic. To reduce the development of resistance for the same, its usage should be limited only for culture proven multidrug resistant isolates. Knowledge of local antibiogram of the hospital comes in handy in these cases.

Conclusion:

Overall Tigecycline resistance among CRE and CRA was found to be 15.50% and 10.69% respectively. Although Tigecycline is a promising antibiotic for the treatment of infections caused by drug resistant problematic pathogens, Tigecycline resistance is most frequently observed in *A. baumannii* and *Enterobacteriaceae*, especially in MDR strains. Hence, we advocate judicious use of Tigecycline in MDR infections.

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