JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

In-vitro activity of tigecycline and comparator agents against common pathogens: Indian experience

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Abstract

Introduction: Tigecycline Evaluation and Surveillance Trail (TEST) study is an on-going global surveillance. The study was performed to determine the susceptibility of common pathogens to tigecycline and comparator antibiotics by broth microdilution (BMD) at two tertiary care centres in India from 2015 to 2017.

Methodology: Total of 989 isolates collected from various clinical specimens between January 2015 and September 2017 from two centres in India were included. BMD was performed to determine the minimum inhibitory concentration (MIC) for tigecycline and comparator antibiotics.

Results: Among Gram-negative bacteria, susceptibility to tigecycline was lowest among *Klebsiella* spp. being 84% while others such as *E. coli*, *Enterobacter* spp., *Serratia* spp. and *H. influenzae* showed susceptibility of 98%, 95%, 98% and 100% respectively. Overall, 99 isolates among *Enterobacteriaceae* (*E. coli, Klebsiella spp*. and *Enterobacter* spp.) were ESBL producers, susceptible to tigecycline. Among the 101 meropenem resistant *Enterobacteriaceae*, 85 were susceptible to tigecycline (84%). Among the Gram-positive bacteria, *S. aureus* and *Enterococcus* spp. were 99% and 98% susceptible to tigecycline respectively. Among 68 MRSA isolates in the study, 66 (97%) were susceptible to tigecycline. Seven vancomycin resistant *E. faecalis* were isolated and all were susceptible to tigecycline.

Conclusion: Tigecycline has retained activity over both Gram-positive and Gram-negative organisms with MIC values comparable to global reports. About 98% of the MDR Gram-positive and Gram-negative bacteria in the study are susceptible to tigecycline. With increased incidence of extensively drug resistant organisms, tigecycline is a potential reserve drug.

Key words: Tigecycline; India; surveillance; *Enterobacteriaceae*; *S. aureus*.

J Infect Dev Ctries 2019; 13(3):245-250. doi:10.3855/jidc.10376

(Received 20 March 2018 – Accepted 28 January 2019)

Introduction

Tigecycline is a glycylcycline which is a derivative of minocycline and is structurally better in overcoming the ribosomal protection proteins and efflux pumps which confer resistance to other tetracyclines [1,2]. It is often used in the treatment of multidrug resistant organisms as a last resort apart from colistin. It is active against both Gram-positive and Gram-negative bacteria. Studies focusing on the usage and prevalence of tigecycline resistance are crucial in judicious use of the reserved drug in order to prevent resistance. Though tigecycline has been licensed only for skin and soft tissue infection (SSTI) and intra-abdominal infections (IAI) [3], it is used for the treatment of other infections such as bacteraemia secondary to SSTI and IAI. Tigecycline Evaluation and Surveillance Trail (TEST) study was performed to determine the susceptibility of common pathogens to tigecycline and comparator antibiotics. The present study details the observation made at two tertiary care centres in India as a part of TEST study from 2015 to 2017. This study determines the susceptibility to tigecycline by broth-micro dilution and MIC_{50} and MIC_{90} have been calculated to determine tigecycline susceptibility.

Methodology

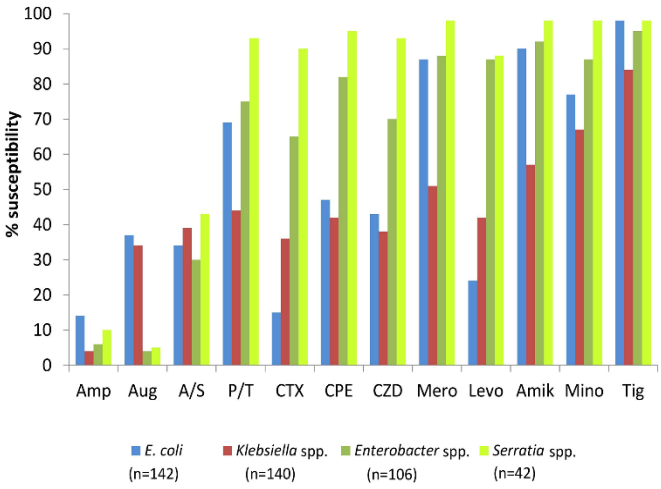
Total of 989 isolates collected from various clinical specimens between January 2015 and September 2017 from two centres in India namely Christian Medical College, Vellore, and Breach Candy Hospital Trust, Mumbai, were included in the study. Only the first isolate from each patient was included in the study. Identification was performed by standard biochemical methods [4]. Antimicrobial susceptibility was

performed by Kirby Bauer disc diffusion method for preliminary screening as per CLSI guidelines [5]. The number of isolates for each organism included in the study from the study centres is mentioned in Table 1. Minimum inhibitory concentration (MIC) of antibiotics was determined by broth micro dilution and the results were interpreted according to CLSI guidelines for all antibiotics except tigecycline for which the US Food and Drug Administration prescribed breakpoints were used [6,7]. The panel of antibiotics for Gram-negative bacteria includes ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, ceftriaxone, cefepime, ceftazidime, meropenem, levofloxacin, amikacin, minocycline and tigecycline. Gram-positive panel includes ampicillin, penicillin, amoxicillin/clavulanic acid, piperacillin/ tazobactam, ceftriaxone, meropenem, vancomycin, linezolid, minocycline and tigecycline. E-test (Liofilchem, Roseto Degli Abruzzi, Italy) was performed to determine MIC for ampicillin/sulbactam and cefoperazone/ sulbactam for all the study isolates. β-lactamase production in *H. influenzae* was performed using nitrocefin disc. Currently, there are no breakpoints described for interpretation of cefoperazone/ sulbactam susceptibility and hence the MIC range was determined. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as the controls for susceptibility testing.

Results

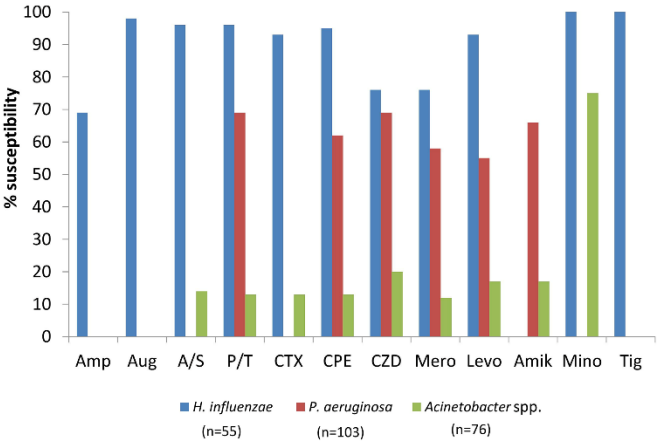
Table 2 mentions the MIC_{50} and MIC_{90} of all the antibiotics tested for *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *P. aeruginosa*. Figure 1 and Figure 2 are the susceptibility profiles of *Enterobactericeae* and other Gram-negative bacteria such as *P. aeruginosa*, *Acinetobacter* spp. and *H. influenza*e respectively. Overall, 99 isolates among *Enterobacteriaceae* [*E. coli* (n = 62), *Klebsiella spp*. (n = 18) and *Enterobacter* spp. (n = 19)] were ESBL producers (meropenem susceptible, ceftazidime resistant) and were susceptible to tigecycline. Among the 101 meropenem resistant *Enterobacteriaceae* [*E. coli* (n = 18), *Klebsiella spp*. (n = 69) *Enterobacter* spp.

Figure 1. Susceptibility of *Enterobacteriaceae* to antimicrobials.



Amp: Ampicillin; Aug: Amoxicillin/clavulanic acid; A/S: Ampicillin/sulbactam; P/T: Piperacillin/tazobactam; CTX: Ceftriaxone; CPE: Cefepime; Czd: Ceftazidime; Mero: Meropenem: Levo: levofloxacin; Amik: Amikacin; Mino: Minocycline; Tig: Tigecycline.

Figure 2. Susceptibility of other Gram-negative bacteria to antimicrobials.



Amp: Ampicillin; Aug: Amoxicillin/clavulanic acid; A/S: Ampicillin/sulbactam; P/T: Piperacillin/tazobactam; CTX: Ceftriaxone; CPE: Cefepime; Czd: Ceftazidime; Mero: Meropenem: Levo: levofloxacin; Amik: Amikacin; Mino: Minocycline: Tig: Tigecycline.

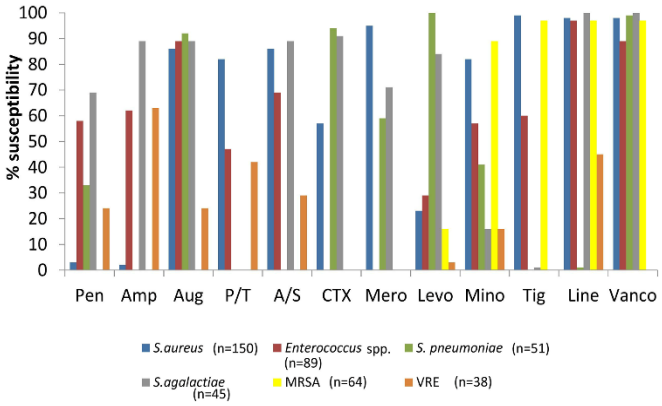
Table 1. Number of isolates for each organism included in the study.

Organism	*E. coli*	*Klebsiella spp.*	*Enterobacter spp.*	*Serratia spp.*	*P. aeruginosa*	*Acinetobacter spp.*	*H. influenzae*	*S. aureus*	*S. pneumoniae*	*S. agalactiae*	*Enterococcus spp.*	Total
No. of isolates from CMC	75	75	75	30	60	45	37	75	45	25	45	587
From BCH	67	65	31	12	43	31	18	75	6	20	44	412
Total	142	140	106	42	103	76	55	150	51	45	89	989

(n = 13) and *Serratia* spp. (n = 1)] isolates, 85 were susceptible to tigecycline (84%). Tigecycline is found to be effective against multi-drug and extensively drug resistant isolates. Figure 3 describes the susceptibility profile of Gram-positive bacteria such as *S. aureus*, *S. pneumoniae*, *Enterococci* and *S. agalactiae*. MIC_{50} and MIC_{90} of tigecycline for *E. coli, Klebsiella* spp. and *Enterobacter* spp. are within the susceptible range indicating the effectiveness of tigecycline for these commonly encountered organisms. For meropenem, MIC_{50} (0.5 µg/mL) is within the susceptible range (≤ 1 µg/mL) while MIC_{90} (> 16 µg/mL) falls in the resistance range (> 1 µg/mL). Though there are large number of MDR organisms, tigecycline retains its activity over these bacteria.

MIC_{50} and MIC_{90} for all the antimicrobials included in the study for *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa* and *Acinetobacter* spp. are mentioned in Table 2. Among Gram-negative bacteria, susceptibility to tigecycline was lowest among *Klebsiella* spp. being 84% while others such as *E. coli*, *Enterobacter* spp., *Serratia* spp. and *H. influenzae* showed susceptibility of 98%, 95%, 98% and 100% respectively. Currently, there is no tigecycline susceptibility breakpoint for *Acinetobacter* spp. *P. aeruginosa* is intrinsically resistant to tigecycline. MIC_{50} and MIC_{90} for *Acinetobacter* spp. for tigecycline were found to be 1 µg/mL and 2 µg/mL respectively. The MIC for *Acinetobacter* spp. ranged from 0.06 to 2 µg/mL for tigecycline. Meropenem, commonly used in

Figure 3. Susceptibility of Gram-positive bacteria to antimicrobials.



Pen: Penicillin; Amp: Ampicillin; Aug: Amoxicillin/clavulanic acid; A/S: Ampicillin/sulbactam; P/T: Piperacillin/tazobactam; CTX: Ceftriaxone; Mero: Meropenem; Levo: levofloxacin; Mino: Minocycline: Tig: Tigecycline; Line: Linezolid; Vanco: Vancomycin.

Table 2. MIC_{50} and MIC_{90} of the tested antibiotics for *E. coli, Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa* and *Acinetobacter* spp.

Antimicrobial		*E. coli*	*Klebsiella* spp.	*Enterobacter* spp.	*P. aeruginosa*	*Acinetobacter* spp.
Ampicillin	MIC_{50}	> 32	> 32	> 32	> 32	> 32
	MIC_{90}	> 32	> 32	> 32	> 32	> 32
Amoxicillin/ clavulanic acid	MIC_{50}	16/8	> 32/16	> 32/16	> 32/16	> 32/16
	MIC_{90}	> 32/16	> 32/16	> 32/16	> 32/16	> 32/16
Piperacillin/ tazobactam	MIC_{50}	4/4	128/4	2	8	> 128/4
	MIC_{90}	> 128/4	> 128/4	> 128/4	> 128/4	> 128/4
Ampicillin/ sulbactam	MIC_{50}	24	256	48	> 256	> 256
	MIC_{90}	> 256	> 256	> 256	> 256	> 256
Cefoperazone/ sulbactam	MIC_{50}	6	12	0.75	256	>256
	MIC_{90}	> 256	> 256	> 256	> 256	> 256
Ceftazidime	MIC_{50}	8	> 16	≤1	4	> 16
	MIC_{90}	> 16	> 16	> 16	> 16	> 16
Ceftriaxone	MIC_{50}	> 32	> 32	0.25	> 32	> 32
	MIC_{90}	> 32	> 32	> 32	> 32	> 32
Cefepime	MIC_{50}	16	> 32	< 0.5	4	>32
	MIC_{90}	> 32	> 32	> 32	> 32	> 32
Meropenem	MIC_{50}	< 0.06	0.5	< 0.06	2	>16
	MIC_{90}	16	> 16	8	> 16	> 16
Levofloxacin	MIC_{50}	> 8	> 8	0.06	2	8
	MIC_{90}	> 8	> 8	> 8	> 8	> 8
Amikacin	MIC_{50}	2	4	2	4	>64
	MIC_{90}	16	>64	16	>64	>64
Minocycline	MIC_{50}	2	4	2	>16	2
	MIC_{90}	16	>16	8	>16	8
Tigecycline	MIC_{50}	0.12	1	0.5	8	1
	MIC_{90}	0.5	2	1	>8	2

A/C: Amoxicillin/clavulanic acid; P/T: Piperacillin/tazobactam; A/S: Ampicillin/sulbactam; C/S: Cefoperazone/sulbactam.

the Indian setting, shows significantly decreased susceptibility to *Klebsiella* spp., *P. aeruginosa* and *Acinetobacter* spp. with 51%, 58% and 12% respectively. Among the non-fermenters, *P. aeruginosa* retains 60% susceptibility to most antibiotics while *Acinetobacter* spp. shows 20% susceptibility to the antimicrobials tested.

MIC_{50} and MIC_{90} for all the antimicrobials for *S. aureus* in mentioned in Table 3. Among the Gram-positive bacteria, *S. aureus* and *Enterococcus* spp. were 99% and 60% susceptible respectively to tigecycline. Among 68 MRSA isolates in the study, 66 (97%) were susceptible to tigecycline. MIC_{50} and MIC_{90} of vancomycin for *S. aureus* were 0.5 µg/mL and 1 µg/mL respectively. Seven vancomycin resistant *E. faecalis* were isolated and all were susceptible to tigecycline. A total of 15 vancomycin resistant *Enterococcus* spp. were seen which were susceptible to linezolid. Among MDR Gram-positive bacteria, tigecycline is a preferable therapeutic agent. However, only one isolate of *S. agalactiae* was susceptible to tigecycline while none of the *S. pneumoniae* included in the study was susceptible to tigecycline. Vancomycin resistant *Enterococci* (VRE) was found to be 11% (n = 15) among the study isolates. For *Enterococcus* spp., MIC_{50} and MIC_{90} of vancomycin were 1 µg/mL and 4 µg/mL respectively.

Table3. MIC_{50} and MIC_{90} of tested antibiotics for *S. aureus.*

Antimicrobial		MIC value
Ampicillin	MIC_{50}	8
	MIC_{90}	>16
Penicillin	MIC_{50}	8
	MIC_{90}	> 8
Amoxicillin/ clavulanic acid	MIC_{50}	2/1
	MIC_{90}	8/4
Piperacillin/ tazobactam	MIC_{50}	1/ 4
	MIC_{90}	16/4
Ampicillin/ sulbactam	MIC_{50}	1
	MIC_{90}	6
Cefoperazone/ sulbactam	MIC_{50}	3
	MIC_{90}	12
Ceftriaxone	MIC_{50}	4
	MIC_{90}	32
Meropenem	MIC_{50}	0.25
	MIC_{90}	2
Levofloxacin	MIC_{50}	4
	MIC_{90}	8
Minocycline	MIC_{50}	≤ 0.25
	MIC_{90}	≤ 0.25
Tigecycline	MIC_{50}	0.12
	MIC_{90}	0.25
Linezolid	MIC_{50}	2
	MIC_{90}	4
Vancomycin	MIC_{50}	0.5
	MIC_{90}	1

A/C: Amoxicillin/clavulanic acid; P/T: Piperacillin/tazobactam; A/S: Ampicillin/sulbactam; C/S: Cefoperazone/sulbactam.

Discussion

Tigecycline is a broad spectrum antibiotic with activity against Gram-positive, Gram-negative, anaerobic, atypical and multidrug-resistant organisms [8]. In particular, it is active against methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococci* (VRE) and carbapenem-resistant *Enterobacteriaceae* [9,10]. It has reduced activity against *Pseudomonas spp.*, *Proteus spp.*, *Providencia spp.* and *Morganella spp.* Its primary role for multidrug resistant Gram-negative infections is for *Klebsiella spp.*, *E coli* and *Acinetobacter spp*.

In the present study, tigecycline retains activity against 90% of the *Enterobacteriaceae* and 100% of *H. influenzae*, 98% of *S. aureus* and *E. faecalis*. The current FDA breakpoints do not describe the criteria for *E. faecium*. Among the organisms tested, *Klebsiella* spp. has susceptibility of 84% to tigecycline with good activity compared to carbapenems which is 51% susceptible. The main mechanism of resistance to tigecycline is by mutations in the regulators of efflux pumps such as *acrAB* and *oqxAB,* which also contribute to resistance to other antimicrobials in *Klebsiella* spp. [11]. Tigecycline has retained good activity against *H. influenzae* over the years with 98 to 100% susceptibility as reported in various studies [2,12].

Similarly, TEST studies conducted in various countries, determined MIC_{90} for tigecycline as 2 µg/mL which was also observed in the present study [12-14]. For *Acinetobacter baumannii,* none of the organisation (EUCAST, USCAST, FDA) has suggested tigecycline breakpoints. In this scenario, most of the clinical laboratories are extrapolating tigecycline FDA breakpoints of *Enterobacteriaceae* for *A. baumannii* as well (MIC, ≥ 8 µg/mL as non-susceptible).

Among the Gram-positive bacteria, *S. aureus* and *Enterococci* show 98% susceptibility tigecycline unlike *S. pneumoniae* and *S. agalactiae* which are resistant to tigecycline. In contrast to the present study, Garrison *et al*., reported 99.7% susceptibility to tigecycline among *S. agalactiae* [12]. This vast difference in susceptibility rates can be attributed to the time and geographical variation. Earlier study [12] included isolates from 2004 to 2010 collected globally while the present study isolates are from 2015 to 2017 from Indian setting. Also, the usage of antibiotics differs significantly among various countries. Susceptibility of *S. pneumoniae* was not interpreted in the study by

Garrison and colleagues due to lack of breakpoints. Hoban *et al*., reported 99.7% susceptibility of *E. faecalis* to tigecycline similar to the present study wherein 53 out of 54 *E. faecalis* were susceptible to tigecycline [13].

For *S. aureus*, MIC_{50} and MIC_{90} of vancomycin were 0.5 µg/mL and 1 µg/mL respectively in the present study while earlier studies report MIC_{50} and MIC_{90} to be 1 µg/mL. Globally, there has not been an increase in MIC of vancomycin for *S. aureus* [12,13]. 88% of the *Enterococci* are susceptible to vancomycin while other Gram-positive bacteria in the present study retain 100% susceptibility. For *Enterococcus* spp., MIC_{50} and MIC_{90} of vancomycin were 1 µg/mL and 4 µg/mL respectively in the present study while earlier reports show lower values such as 1-2 µg/mL for MIC_{50} and MIC_{90} [13]. The susceptibility to tigecycline among MRSA and VRE was found to be 97% (66/68) and 100% (7/7) respectively. Among MDR Gram-positive bacteria, tigecycline is a potential therapeutic agent.

A recent publication on results of TEST 2016 from regions including North America, Europe, Latin America and Asia Pacific show that tigecycline has retained its activity against both Gram-positive and Gram-negative bacteria worldwide [15]. MIC_{50} and MIC_{90} of tigecycline for carbapenem resistant *Enterobacteriaceae* is reported as 0.5 µg/mL and 2 µg/mL respectively which is in the susceptible range. Among Gram-positive bacteria such as *S. aureus*, MIC_{50} and MIC_{90} of tigecycline was as low as 0.06 µg/mL and 0.12 µg/mL respectively. Similar to the study by Pfaller *et al*., our present study also finds MDR organisms such as carbapenem resistant *Enterobacteriaceae*, MRSA and VRE to retain > 95% susceptibility to tigecycline.

Cefoperazone/sulbactam has good activity against some ESBL producers. Its activity varies among *Enterobacteriaceae* species which include some AmpC β-lactamase producers and is also active against some important non-fermentative Gram-negative bacteria species that are resistant to cefoperazone [16]. But currently to determine its efficacy against MDR isolates, no breakpoints are defined for this combination. Determining the MIC range and MIC_{50} can aid in establishing breakpoints. This study showed lowest MIC_{50} and MIC_{90} among *H. influenzae* and the highest among *Acinetobacter* spp. Among *Enterobacteriaceae, Enterobacter* spp. had lowest MIC_{50} of 0.75 µg/mL. Gram-positive bacteria had lower MIC_{50} and MIC_{90} values when compared to Gram-negative bacteria. Jean *et al*., [16] reported lower MIC_{50} and MIC_{90} values of ≤8 µg/mL as determined by Vitek2 for *Enterobacteriaceae* including *E. coli*, *K. pneumoniae*, *E. cloacae*, *C. freundii*, *Salmonella* spp. and *S. marcescens*. However, for *Acinetobacter* spp. MIC_{50} and MIC_{90} were ≤8 µg/mL and 32 µg/mL respectively in contrast to the present study where MIC_{50} and MIC_{90} was found to be > 256 µg/mL (Table 2). For *P. aeruginosa* Jean *et al*., report MIC_{50} and MIC_{90} ≤ 8 µg/mL and > 64 µg/mL respectively while in the present study MIC_{50} and MIC_{90} were observed to be > 256 µg/mL.

Surveillance of susceptibility to commonly used antimicrobials against various pathogens helps in understanding the antibiogram and optimises the standard therapeutic practices. This in turn helps the use of reserve drugs such as tigecycline, colistin, linezolid and vancomycin with discernment. In India, determining the MIC of antimicrobials across the country in various centres along with monitoring the usage of tigecycline will enable in establishing the susceptibility trend over the years.

Conclusion

Tigecycline has retained activity over both Gram-positive and Gram-negative organisms with MIC values comparable to global reports. About 98% of the MDR Gram-positive and Gram-negative bacteria in the study are susceptible to tigecycline. With increased incidence of extensively drug resistant organisms, tigecycline is a potential reserve drug. However, there is need to establish uniformity of testing methodology for tigecycline and also define breakpoints for *Acinetobacter* spp. for which tigecycline is a potential therapeutic option.

Acknowledgements

We acknowledge International Health Management Association (IHMA) Inc. for their coordination of T.E.S.T which is funded by Pfizer.

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Conflict of interests: No conflict of interests is declared.