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Experimental Research

In vitro activity of tigecycline against multidrug-resistant *Enterobacteriaceae* isolates from skin and soft tissue infections

Srujana Mohanty *, Ashoka Mahapatra

Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar, 751019, Odisha, India

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ABSTRACT

Background: Tigecycline, a new agent against multidrug-resistant (MDR) bacteria, is especially licensed for use in complicated skin and soft tissue and intra-abdominal infections. We aimed to study the recent *in vitro* activity of tigecycline against MDR *Enterobacteriaceae* skin and soft tissue isolates.

Methods: Consecutive isolates (56 *Escherichia coli*, 48 *Klebsiella pneumoniae*) were subjected to tigecycline susceptibility testing by Ezy MIC test and interpreted as per European Committee on Antimicrobial Susceptibility Testing.

Results: The minimum inhibitory concentrations (MICs) of tigecycline ranged from 0.016 to 48 μg/mL, with MIC_{50} 0.19 μg/mL and MIC_{90} 1.0 μg/mL respectively. Seven (6.7%) isolates were resistant to tigecycline, all *K. pneumoniae*.

Conclusion: Tigecycline remains a viable therapeutic option against MDR isolates, with excellent *in vitro* activity against *E. coli* and promising activity against *K. pneumoniae*. However, the limited availability of alternate therapeutic armamentarium necessitates its use with extreme judiciousness along with continuous monitoring for the emergence and spread of resistance.

1. Introduction

Skin and soft tissue infections (SSTIs) are a frequent cause of visits to health-care providers, including emergency departments [1,2]. *Staphylococcus aureus,* a gram-positive cocci, is the predominant pathogen isolated in approximately 38.05%–51.6% of the culture-positive SSTIs cases [3,4]. *Enterobacteriacaeae*, such as *Escherichia coli* and *Klebsiella pneumoniae*, are the common gram-negative pathogens isolated in 6.8%–17.39% and 3.3.0%–8.1% cases respectively [3,4]. A recent study showed gram-negative bacteria as emerging pathogens affecting mortality in SSTIs, in which *E. coli* and *K. pneumoniae* accounted for 28.4% and 17.6% of the culture positive isolates respectively [5]. A cause of concern has been the increasing prevalence and widespread dissemination of antimicrobial resistance, especially, the emergence of multidrug-resistant (MDR) and carbapenem-resistant *Enterobacteriaceae* (CRE), which has been a severe impediment to the successful therapy of SSTIs caused by these gram-negative pathogens [6,7]. Infections caused by MDR pathogens are also associated with increased costs, length of hospital stay, and morbidity and mortality rates [6,7]. In this context, tigecycline represents a new therapeutic alternative having potent *in vitro* activity against most of the MDR Gram-positive and Gram-negative bacteria, except for *Pseudomonas aeruginosa* and *Proteae* and is especially licensed for use in complicated skin and soft tissue infections and intra-abdominal infections [8,9]. Its unique mechanism of action involves binding with high affinity to the bacterial 30S ribosomal subunit (almost five times that of tetracycline) as well as overcoming the effect of ribosomal protection proteins and efflux pumps, thus remaining unaffected by the typical mechanisms that render bacteria resistant to the tetracycline class [8–10]. Tigecycline is also not associated with cross-resistance to other antibiotics, conferring another advantage in its activity against several ESBL- and carbapenemase-producing *Enterobacteriaceae* [8–10]. Herein, we aimed to study the recent *in vitro* activity of tigecycline against multidrug-resistant *Enterobacteriaceae* isolates from skin and soft tissue infections.

2. Methods

The study, exempted from review by the Institutional Ethical Committee, included a total of 104 consecutive, non-repeat, discrete MDR *Enterobacteriaceae* skin and soft tissue isolates (56 *E. coli*, 48

* Corresponding author.
E-mail address: srujana_micro@yahoo.co.in (S. Mohanty).

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Table 1
Distribution of tigecycline minimum inhibitory concentrations in multidrug-resistant *Enterobacteriaceae* isolates.

Organism	No. of isolates with MIC (μg/mL)														Total	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
	.016	.047	.064	.094	.125	.19	.25	.38	.5	.75	1	3	6	48			
E. coli	–	1	1	24	13	5	5	3	2	2	–	–	–	–	56	.125	.38
K. pneumoniae	1	–	1	1	6	5	7	2	5	9	4	1	4	2	48	.5	6
Total (%)	1 (0.9)	1 (0.9)	2 (1.9)	25 (24.0)	19 (18.3)	10 (9.6)	12 (11.5)	5 (4.8)	7 (6.7)	11 (10.6)	4 (3.8)	1 (0.9)	4 (3.8)	2 (1.9)	104	.19	1

K. pneumoniae) from January to June 2019, displaying extended-spectrum beta-lactamase (ESBL)-production, carbapenem-resistance and/or multi-drug resistance to three or more antibiotic classes. The isolates were subjected to tigecycline susceptibility testing by Ezy MIC test (HiMedia, Mumbai, Maharashtra, India) and results were interpreted as per the European Committee on Antimicrobial Susceptibility Testing (sensitive $\leq$ 1 μg/mL, resistant $>$ 2 μg/mL) [11] guidelines in view of the lack of tigecycline breakpoints for *Enterobacteriaceae* by the Clinical and Laboratory Standards Institute (CLSI). Other antibiotics were tested by disc-diffusion (HiMedia, Mumbai, Maharashtra, India) and interpreted as per CLSI guidelines [12]. Standard strains of *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 (ESBL- positive) were used as controls.

3. Results

The minimum inhibitory concentrations (MICs) of tigecycline ranged from 0.016 μg/mL to 48 μg/mL, with MIC_{50} 0.19 μg/mL and MIC_{90} 1.0 μg/mL respectively (Table 1). Maximum isolates (25, 24.0%) demonstrated MIC of 0.094 μg/mL followed by 0.125 μg/mL (19, 18.3%) and 0.25 μg/mL (12, 11.5% each). Seven (6.7%) isolates were observed to be resistant to tigecycline, all *K. pneumoniae*. The proportion of *K. pneumoniae* isolates displaying tigecycline resistance was 14.5% (7/48). The MIC_{50} and MIC_{90} of *K. pneumoniae* isolates were 4 and 16 times higher compared to that of *E. coli* (0.5/0.125 μg/mL and 6/0.38 μg/mL respectively) suggesting a lower activity of tigecycline against *K. pneumoniae* isolates (Table 1).

4. Discussion

The current study showed that tigecycline had excellent (100%) *in vitro* activity against MDR skin and soft tissue isolates of *E. coli*, with a comparatively lower activity against MDR *K. pneumoniae* isolates. A similar finding was noted when the potency and spectrum of tigecycline was tested against an international collection of bacterial pathogens associated with skin and soft tissue infections [13]. It was observed that 99% of *E. coli* were inhibited by tigecycline at $\leq$ 1 μg/mL (MIC_{50} and MIC_{90} values, 0.12 and 0.5 μg/mL), while only 90% of *Klebsiella* spp. were inhibited at $\leq$ 1 μg/mL (MIC_{50} and MIC_{90} values, 0.5 and 1 μg/mL) [13]. In Africa-Middle East countries, *K. pneumoniae* and *E. coli* displayed *in vitro* susceptibility rates of 96.8% and 100%, respectively to tigecycline [14]. Other studies from India and outside have reported an increasing prevalence of tigecycline resistance in carbapenem-resistant/MDR *K. pneumoniae* clinical isolates [15–17]. A study on isolates from burn wound infections in an Indian tertiary care hospital, reported tigecyline resistance in 9.09% and 11.76% of *E. coli* and *K. pneumoniae*, respectively [16]. An increasing prevalence of tigecycline- and carbapenem-resistant *K. pneumoniae* isolates has been reported from China [15]. In Vietnam, tigecycline susceptibility was observed in only 82% of 57 carbapenem-resistant clinical isolates of *K. pneumoniae*, belonging to strain ST15 [17]. The various mechanisms of tigecycline resistance in *Enterobacteriaceae* include the *tetA* and *OqxAB* genes that encode efflux pumps; mutations in the tigecycline target site of ribosomal protein S10 encoded by the *rpsJ* gene and mutations in the *ramR* gene, which results in the overexpression of the AcrAB multi-drug pump [15,18,19]. Of these, mutations in the *ramR* and *tetA* efflux genes have been found to constitute the major resistance mechanisms in recent studies [15,18].

Of concern, was the finding that, there was low susceptibility of the MDR pathogens to other available antibiotics: amikacin (60/104, 57.7%), gentamicin (55/104, 52.8%), piperacillin/tazobactam (37/104, 35.6%), trimethoprim/sulfamethoxazole (31/104, 29.8%), and ciprofloxacin (16/104, 15.4%) highlighting the extremely limited alternate therapeutic options. Such low susceptibility has also been observed in carbapenem-resistant *K. pneumoniae* ST258 strains from Northeast Ohio, in which trimethoprim/sulfamethoxazole, gentamicin, and amikacin susceptibility rates were 31%, 39% and 76% respectively [20] as well as in carbapenem-resistant ST15 *K. pneumoniae* isolates from Vietnam with trimethoprim/sulfamethoxazole, amikacin and ciprofloxacin susceptibility rates of 70%, 0% and 0% respectively [17].

To conclude, tigecycline remains a viable therapeutic option for MDR and carbapenem-resistant *Enterobacteriaceae* skin and soft tissue isolates, with excellent *in vitro* activity against MDR *E. coli* and promising activity against MDR *K. pneumoniae*. However, its use should be guided by the observation that tigecycline combination therapy and high-dose regimens have been found to be more effective than monotherapy and standard-dose regimens, respectively, in treating CRE infections [9,10]. The limited availability of suitable alternate therapeutic armamentarium necessitates the use of tigecycline with extreme judiciousness along with a critical and urgent need to continuously monitor the emergence and spread of resistance.

Ethical approval

Ethical approval has been obtained from the Institutional Ethics Committee of our Institute i.e., the Institutional Ethics Committee of All India Institute of Medical Sciences, Bhubaneswar, Odisha, India.

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Author contribution

Srujana Mohanty: Corresponding author, concept and design, writing the paper and giving final approval. Ashoka Mahapatra: literature search, writing and correction of the paper.

Registration of research studies

1. Name of the registry.
2. Unique Identifying number or registration ID.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked).

Guarantor

Srujana Mohanty.

Consent

Not applicable. The study is on bacterial isolates only.

Declaration of competing interest

None to declare.

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