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In vitro Activity of Tigecycline Against Clinical Isolates of Gram-Positive and Gram-Negative Bacteria Displaying Different Resistance Phenotypes

Aktywność *in vitro* tigecykliny wobec izolatów klinicznych bakterii Gram-dodatnich i Gram-ujemnych o różnych fenotypach oporności

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Abstract

Objectives. The aim of the study was to evaluate the *in vitro* activity of tigecycline against clinically relevant isolates of Gram-positive and Gram-negative bacteria resistant to different antimicrobial drugs.
Material and Methods. A total of 495 clinical isolates, including methicillin-resistant *Staphylococcus aureus* (MRSA) (n = 19), methicillin-resistant coagulase-negative *Staphylococcus* spp. (MRCNS) (n = 56), methicillin-susceptible *Staphylococcus aureus* resistant to tetracycline (MSSA T^R) (n = 113), high-level aminoglycoside-resistant (HLAR) *Enterococcus* spp. (n = 181), and extended-spectrum β-lactamase (ESBL)-producing bacilli (n = 126), were used in this study. The clinical isolates were obtained from patients hospitalized at the Medical University Hospital in Wrocław, Poland, during a three-year period (2005–2007). The minimal inhibitory concentrations (MICs) of tigecycline for the strains were determined by the agar dilution technique on Mueller-Hinton agar.
Results. Tigecycline exhibited excellent activity against all Gram-positive cocci tested, with MIC_{90} values ranging from 0.03 to 0.06 mg/L. In addition, this antimicrobial was also very potent against all ESBL-producing strains tested with an MIC_{90} of 0.25 mg/L. The activity of tigecycline against ESBL-producers was comparable to that of imipenem.
Conclusions. Tigecycline could be considered an alternative antibacterial agent for the treatment of serious infections caused by drug-resistant bacterial strains (**Adv Clin Exp Med 2008, 17, 5, 545–551**).

Key words: tigecycline, MRS, HLAR, ESBL.

Streszczenie

Cel pracy. Określenie aktywności *in vitro* tigecykliny wobec ważnych z klinicznego punktu widzenia szczepów bakterii Gram-dodatnich i Gram-ujemnych opornych na różne leki przeciwbakteryjne.
Materiał i metody. W badaniach zastosowano 495 izolatów klinicznych obejmujących: metycylinooporne szczepy *Staphylococcus aureus* (MRSA) (n = 19), koagulazoujemne szczepy *Staphylococcus* spp. oporne na metycylinę (MRCNS) (n = 56), metycylinowrażliwe szczepy *Staphylococcus aureus* oporne na tetracyklinę (MSSA T^R) (n = 113), szczepy *Enterococcus* spp. oporne na wysokie stężenia aminoglikozydów (HLAR) (n = 181) oraz pałeczki wytwarzające β-laktamazy o rozszerzonym spektrum substratowym (ESBL) (n = 126). Szczepy kliniczne wyizolowano od pacjentów hospitalizowanych w Akademickim Szpitalu Klinicznym we Wrocławiu w ciągu 3 lat (2005–2007). Minimalne stężenia hamujące tigecykliny (MIC) dla badanych szczepów oznaczono metodą seryjnych rozcieńczeń w podłożu agarowym Mueller-Hintona.
Wyniki. Tigecyklina wykazywała doskonałą aktywność wobec wszystkich badanych ziarenkowców Gram-dodatnich, dla których wartości MIC_{90} zawierały się w przedziale 0,03–0,06 mg/l. Antybiotyk ten okazał się także bardzo skuteczny wobec wszystkich szczepów wytwarzających ESBL; wartość MIC_{90} dla tych izolatów wynosiła 0,25 mg/l. Aktywność tigecykliny wobec ESBL-dodatnich szczepów była porównywalna z aktywnością imipenemu.
Wnioski. Tigecyklina może być alternatywnym lekiem przeciwbakteryjnym w leczeniu poważnych zakażeń powodowanych przez lekooporne szczepy bakteryjne (**Adv Clin Exp Med 2008, 17, 5, 545–551**).

Słowa kluczowe: tigecyklina, MRS, HLAR, ESBL.

Tetracyclines represent a valuable group of therapeutic agents demonstrating activity against a broad range of microorganisms, including Gram-positive, Gram-negative, and "atypical" bacteria, such as *Mycoplasma* spp. and *Chlamydia* spp. Unfortunately, the clinical use of these antibiotics is often limited because of tetracycline resistance due to both ribosomal protection and active efflux [1]. Therefore there is an increasing need for new antimicrobials which overcome these resistance mechanisms. Tigecycline, a derivative of minocycline formerly known as GAR-936, is a member of a new group of semisynthetic antibacterial drugs called glycylcyclines that overcome the mechanisms of tetracycline resistance [2–4]. Similar to tetracyclines, tigecycline acts as an inhibitor of bacterial translation. It binds to the 30S ribosomal subunit and blocks the entry of amino-acyl tRNA molecules into the A site of the ribosome. Compared with tetracycline, tigecycline binds five times more efficiently to the ribosomal receptor [5]. Tigecycline demonstrates *in vitro* bacteriostatic activity against a variety of Gram-positive and Gram-negative bacteria, including those that display different resistance phenotypes, such as extended-spectrum β-lactamase (ESBL)-producing enteric bacilli [6–8], methicillin-resistant staphylococci (MRS) [8, 9], vancomycin-resistant enterococci (VRE), and glycopeptide-intermediate staphylococci [9, 10]. In addition, tigecycline also exhibits activity against the Gram-negative nonfermentative rods *Acinetobacter* spp. and *Stenotrophomonas malthophilia*, but not *Pseudomonas aeruginosa* [11, 12].

The aim of the present study was to determine the *in vitro* activity of tigecycline against clinical strains of MRS, high-level aminoglycoside-resistant (HLAR) enterococci, and ESBL-producing bacilli isolated from patients hospitalized at the Medical University Hospital in Wrocław, Poland. To the present authors' knowledge, this study is the first survey in Lower Silesia (Poland) focusing on tigecycline activity against alert-pathogens displaying different resistance patterns.

Material and Methods

Bacterial Strains

A total of 495 clinical isolates, including *Staphylococcus* spp. (n = 188), *Enterococcus* spp. (n = 181), and ESBL-producing species of the *Enterobacteriaceae* family (n = 126), were selected for the study. Among the 188 *Staphylococcus* spp. strains, 19 were methicillin-resistant *S. aureus* (MRSA), 56 were methicillin-resistant coagulase-negative *Staphylococcus* spp. (MRCNS), and 113 were methicillin-susceptible but tetracycline-resistant *S. aureus* (MSSA T^R). All the enterococcal strains, including *Enterococcus faecium* (n = 118) and *E. faecalis* (n = 63), exhibited the HLAR phenotype. ESBL-producing strains were represented by the species *Escherichia coli* (n = 44), *Enterobacter cloacae* (n = 39), *Klebsiella pneumoniae* (n = 26), *Serratia marcescens* (n = 10), and *Citrobacter freundii* (n = 7). The isolates were recovered from different specimens, mostly from urine, stool, pus, respiratory tract specimens (sputum, throat swabs, bronchial lavage), and blood samples from patients hospitalized at the Medical University Hospital in Wroclaw (Poland) during a three-year period (2005–2007). Only one isolate per patient was included in this study to avoid duplication.

Species Identification and Detection of Resistance Mechanisms

Identification of the *Enterobacteriaceae* strains was performed by the ATB automated identification system (bioMérieux, France) using the ID 32 E tests according to the manufacturer's instructions. ESBL production was detected by the double-disk synergy test (DDST) on Mueller-Hinton agar (Oxoid) according to Jarlier et al. [13]. This test was performed by placing disks of cefotaxime, ceftazidime, and aztreonam (30 μg each) at a distance of 20 mm (center to center) from a disk containing amoxicillin + clavulanic acid (20 and 10 μg, respectively). Strains showing synergy between oxyimino-lactams and clavulanic acid were considered to produce ESBL enzymes.

The staphylococcal isolates were identified by the ATB automated identification system (bio-Mérieux, France) using the ID 32 Staph tests. Resistance to methicillin and tetracycline was determined by the Mueller-Hinton diffusion procedure using disks containing cefoxitin and tetracycline (30 μg each), respectively, according to the Clinical and Laboratory Standards Institute guidelines [14].

The enterococcal isolates were identified to the species level using the API 20 Strep tests (bioMérieux, France). HLAR phenotype was confirmed by the Mueller-Hinton diffusion procedure using disks containing gentamicin (120 μg) and streptomycin (300 μg) according to the Clinical and Laboratory Standards Institute guidelines [14].

Antibiotic Susceptibility Testing

The minimal inhibitory concentration (MIC) of tigecycline was determined by the agar dilution technique on Mueller-Hinton agar (Oxoid) according to the Clinical and Laboratory Standards Institute guidelines [15]. The inoculum of bacterial strains was 10^4 colony forming units (cfu) per spot deposited on the Mueller-Hinton agar. MIC values were read after 18 h of incubation at 35°C. *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 29212 were used as quality reference strains. The *in vitro* susceptibility breakpoints of tigecycline for *Staphylococcus* spp., *Enterococcus* spp., and enterobacteria were ≤ 0.5, ≤ 0.25, and ≤ 2 mg/l, respectively [16]. Additionally, susceptibility to imipenem was also assessed for the ESBL-producing Gram-negative isolates tested. Standard powders of the antimicrobials tested were obtained from Wyeth (tigecycline) and Merck Sharp & Dohme Research (imipenem).

Results

Staphylococcus spp.

The results of the *in vitro* susceptibility testing of tigecycline for 188 clinical isolates of *Staphylococcus* spp. are shown in Table 1 and presented in terms of MIC_{50} (the MIC at which 50% of the strains tested were inhibited), MIC_{90} (the MIC at which 90% of the strains tested were inhibited), and the range of the MICs. Tigecycline exhibited excellent activity against all the staphylococcal isolates, with a concentration of ≤ 0.125 mg/l inhibiting methicillin-resistant staphylococci (MRSA and MRCNS), whereas methicillin-susceptible *S. aureus* strains resistant to tetracycline (MSSA T^R) were inhibited by a concentration of ≤ 0.06 mg/l. MRSA and MRCNS isolates displayed similar susceptibility to tigecycline, with MIC_{90} = 0.06 mg/l for both and MIC_{50} differing by only one dilution step (0.06 and 0.03 mg/l, respectively). In the case of the MSSA T^R isolates, the MIC_{50} and MIC_{90} values were found to be identical (0.03 mg/l). For the *S. aureus* ATTC 29213 reference strain, MIC was 0.125 mg/l.

Enterococcus spp.

Tigecycline was equally active against both *E. faecium* and *E. faecalis* isolates tested exhibiting HLAR phenotype. All the isolates were inhibited by a concentration of ≤ 0.03 mg/l. The MIC_{50} and MIC_{90} values were 0.015 and 0.03 mg/l, respectively for both enterococcal species (Table 2). For the *E. faecalis* ATCC 29212 reference strain, MIC was 0.06 mg/l.

Table 1. *In vitro* activity of tigecycline against *Staphylococcus* spp. strains tested (n = 188)

Tabela 1. Aktywność *in vitro* tigecykliny wobec badanych szczepów *Staphylococcus* spp. (n = 188)

Strains tested (Badane szczepy)	No. of isolates with indicated MIC values – mg/L (Liczba izolatów wykazujących wartości MIC – mg/l)					MIC – mg/L		
	≤ 0.0075	0.015	0.03	0.06	0.125	Range	MIC_{50}	MIC_{90}
Staphylococcus spp. (n = 188)	2	25	138	21	2	≤ 0.0075–0.125	0.03	0.06
MRSA (n = 19)	–	3	6	9	1	0.015–0.125	0.06	0.06
MRCNS (n = 56)	2	14	30	9	1	≤ 0.0075–0.125	0.03	0.06
MSSA T^R (n = 113)	–	8	102	3	–	0.015–0.06	0.03	0.03

MIC – minimal inhibitory concentration.
MIC – minimalne stężenie hamujące.

MIC_{50} – MIC at which 50% of the isolates tested were inhibited.
MIC_{50} – wartość MIC, przy której zostaje zahamowanych 50% badanych szczepów.

MIC_{90} – MIC at which 90% of the isolates tested were inhibited.
MIC_{90} – wartość MIC, przy której zostaje zahamowanych 90% badanych szczepów.

MRSA – methicillin-resistant *Staphylococcus aureus*.
MRSA – szczepy *Staphylococcus aureus* oporne na metycylinę.

MRCNS – methicillin-resistant coagulase-negative *Staphylococcus* spp.
MRCNS – koagulazoujemne szczepy *Staphylococcus* spp. oporne na metycylinę.

MSSA T^R – methicillin-susceptible *Staphylococcus aureus* resistant to tetracycline.
MSSA T^R – metycylinowrażliwe szczepy *Staphylococcus aureus* oporne na tetracyklinę.

Table 2. *In vitro* activity of tigecycline against high-level aminoglycoside-resistant (HLAR) isolates of *Enterococcus* spp. tested (n = 181)

Tabela 2. Aktywność *in vitro* tigecykliny wobec badanych szczepów *Enterococcus* spp. (n = 188) opornych na wysokie stężenia aminoglikozydów (HLAR)

Strains tested (Badane szczepy)	No. of isolates with indicated MIC values – mg/L (Liczba izolatów wykazujących wartości MIC – mg/l)			MIC – mg/L		
	≤ 0.0075	0.015	0.03	Range	MIC_{50}	MIC_{90}
Enterococcus spp. (n = 181)	79	60	42	≤ 0.0075–0.03	0.015	0.03
E. faecium (n = 118)	56	42	20	≤ 0.0075–0.03	0.015	0.03
E. faecalis (n = 63)	23	18	22	≤ 0.0075–0.03	0.015	0.03

ESBL-producing *Enterobacteriaceae*

Table 3 summarizes the *in vitro* susceptibilities to tigecycline and imipenem for the 126 ESBL--producing isolates of the family *Enterobacteriaceae*. Tigecycline was shown to have potent activity, inhibiting all the isolates at concentrations between 0.015 and 1.0 mg/L. Among the ESBL producers tested, tigecycline demonstrated the highest activity against the *E. coli* strains, with MIC_{50} and MIC_{90} values of 0.03 and 0.06 mg/L, respectively. The MIC_{50} value for the remaining strains tested was 0.125 mg/L, while the MIC_{90} values ranged from 0.25 to 0.5 mg/L. For the *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 reference strains the MIC values were 0.5 mg/L and 0.25 mg/L, respectively.

In this study, the *in vitro* activity of tigecycline was compared with that of imipenem. All the ESBL-producing strains were successfully inhibited by imipenem at concentrations between 0.015 and 0.5 mg/L. This carbapenem displayed especially high activity against the *E. coli* and *Klebsiella pneumoniae* isolates, with an MIC_{50} of 0.06 mg/L for both species and MIC_{90} values of 0.06 and 0.125mg/L, respectively. The MIC_{50} values for the *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* strains tested ranged from 0.125 to 0.25 mg/L, while the MIC_{90} values were identical (0.25 mg/L).

Discussion

In the last two decades the incidence of infections due to drug-resistant Gram-positive cocci has been on the increase in many countries throughout the world [17–19]. Enterococci displaying high-level aminoglycoside resistance (HLAR) and methicillin-resistant staphylococci (MRS) are undoubtedly the most prevalent Gram-positive cocci responsible for hospital-acquired infections. Since antibiotic therapy based on aminoglycoside in combination with a β-lactam drug is ineffective against enterococci exhibiting the HLAR phenotype, there is a need for alternative antimicrobials. Glycopeptides (vancomycin and teicoplanin) and linezolid (oxazolidinone) are useful alternatives for the treatment of infections caused by both HLAR and MRS. Unfortunately, resistance to these antibiotics among Gram-positive cocci has been reported in many countries worldwide [18, 20–22].

The results of the present study confirm the high activity of tigecycline against Gram-positive cocci previously reported by other authors [8, 11, 12, 23]. Among the enterococci studied, tigecycline was equally active against *E. faecium* and *E. faecalis* strains. These results are in agreement with data reported by Betriu et al. [11]. Moreover, the MIC_{50} and MIC_{90} values obtained in this study were eightfold lower than those previously reported by Boucher et al. [23]. Furthermore, tigecycline exhibited excellent activity against the staphylococcal strains studied. There were no significant differences in activity of tigecycline between methicillin-resistant and methicillin-susceptible staphylococci. The results confirm similar observations reported by Sorlozano et al. [8]. Moreover, tigecycline proved to be very active against all the tetracycline-resistant *Staphylococcus aureus* isolates tested, indicating that tigecycline remains stable and unaffected by tetracycline-resistance mechanisms [3, 4]. The MIC_{50} values were identical for both methicillin-resistant and methicillin--susceptible strains. These results are similar to those reported by Milatovic et al. [12].

β-lactam resistance, due to plasmid-mediated ESBLs, is one of the most important mechanisms of resistance in members of the family of *Enterobacteriaceae*. These enzymes effectively hydrolyze the majority of β-lactam antibiotics, including penicillins, cephalosporins, and monobactams, but are not active against cephamycins and carbapenems [24, 25]. ESBL producers often

Table 3. *In vitro* activities of tigecycline and imipenem against ESBL-producing *Enterobacteriaceae* isolates tested (n = 126)

Tabela 3. Aktywność *in vitro* tigecykliny i imipenemu wobec badanych szczepów *Enterobacteriaceae* (n = 126) wytwarzających ESBL

Strains tested (Badane szczepy)	Antibiotics (Antybiotyki)	No. of isolates with indicated MIC values – mg/L (Liczba izolatów wykazujących wartości MIC – mg/l)								MIC – mg/L		
		≤ 0.0075	0.015	0.03	0.06	0.125	0.25	0.5	1.0	range	MIC_{50}	MIC_{90}
Escherichia coli (n = 44)	Tigecycline	–	2	22	16	3	–	–	1	0.015–1.0	0.03	0.06
	Imipenem	–	1	17	24	2	–	–	–	0.015–0.125	0.06	0.06
Enterobacter cloacae (n = 39)	Tigecycline	–	–	5	9	12	4	8	1	0.03–1.0	0.125	0.5
	Imipenem	–	–	3	13	16	7	–	–	0.03–0.25	0.125	0.25
Klebsiella pneumoniae (n = 26)	Tigecycline	–	–	2	8	13	2	1	–	0.03–0.5	0.125	0.25
	Imipenem	–	–	2	12	10	1	1	–	0.03–0.5	0.06	0.125
Serratia marcescens (n = 10)	Tigecycline	–	–	–	–	6	2	2		0.125–0.5	0.125	0.5
	Imipenem	–	–	–	–	4	6	–	–	0.125–0.25	0.25	0.25
Citrobacter freundii (n = 7)	Tigecycline	–	–	3	–	3	–	1	–	0.03–0.5	0.125	0.5
	Imipenem	–	–	–	–	5	2	–	–	0.125–0.25	0.125	0.25

display high-level resistance to other classes of antibacterial agents, such as aminoglycosides, tetracycline, and co-trimoxazole [26]. This results from the fact that the determinants encoding ESBL enzymes and those conferring resistance to non-β-lactam antibiotics may reside within the same transferable plasmids that can easily be exchanged between bacterial species of Gram-negative rods via conjugation [27]. Thus the rapid spread of multiresistant ESBL-producing microorganisms poses a serious clinical problem worldwide, limiting therapeutic options. In the present study all the ESBL-producing isolates were uniformly susceptible to imipenem (MIC_{90} range: 0.06–0.25 mg/L), supporting the previous observations that carbapenems are the major treatment option for infections caused by ESBL-producing bacilli [28, 29]. Moreover, the efficacy of carbapenems might be considerably weakened because of the emergence of carbapenem-resistant Gram-negative strains, such as *Klebsiella* spp. and *Enterobacter* spp. [30, 31]. This resistance, more often resulting from carbapenemase production in association with impermeability and/or efflux, is still rare among *Enterobacteriaceae*, but it may be of the great clinical importance in future. The data obtained by Bratu et al. [30] showed that tigecycline may be considered the first therapeutic option for the treatment of infections involving such carbapenem-resistant strains.

The emergence of ESBL-producing *Enterobacteriaceae* isolates with diminished susceptibility to tigecycline was previously reported by Morosini et al. [7], Milatovic et al. [12], and Souli et al. [32]. However, in the present study all the ESBL-positive strains tested, representing five species of the family of *Enterobacteriaceae*, were fully susceptible to tigecycline. This glycylcycline inhibited all of the isolates at concentrations between 0.015 and 1.0 mg/L. These results are in line with data of previous studies; however, the MIC_{90} values in the present study were slightly lower than those reported by Biedenbach et al. [6], Morosini et al. [7], and Sorlozano et al. [8]. Based on the MIC_{90} values, the potency of tigecycline against *E. coli* isolates was found to be equivalent to that of imipenem. Regarding the other enterobacteria tested, the MIC_{90} values of tigecycline and those of imipenem differed by only one dilution step. The MIC_{90} for *E. coli* isolates (0.06 mg/L) was lower than those determined for the other enterobacteria tested (0.25–0.5 mg/L), indicating that tigecycline was four- to eightfold more active against the *E. coli* strains than against the remaining enteric bacilli. This is in agreement with the findings of Souli et al. [32].

In conclusion, the excellent activity of tigecycline against all the clinically relevant strains studied suggests that this compound could be considered an encouraging antimicrobial for the treatment of infections caused by pathogens displaying various resistance patterns.

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