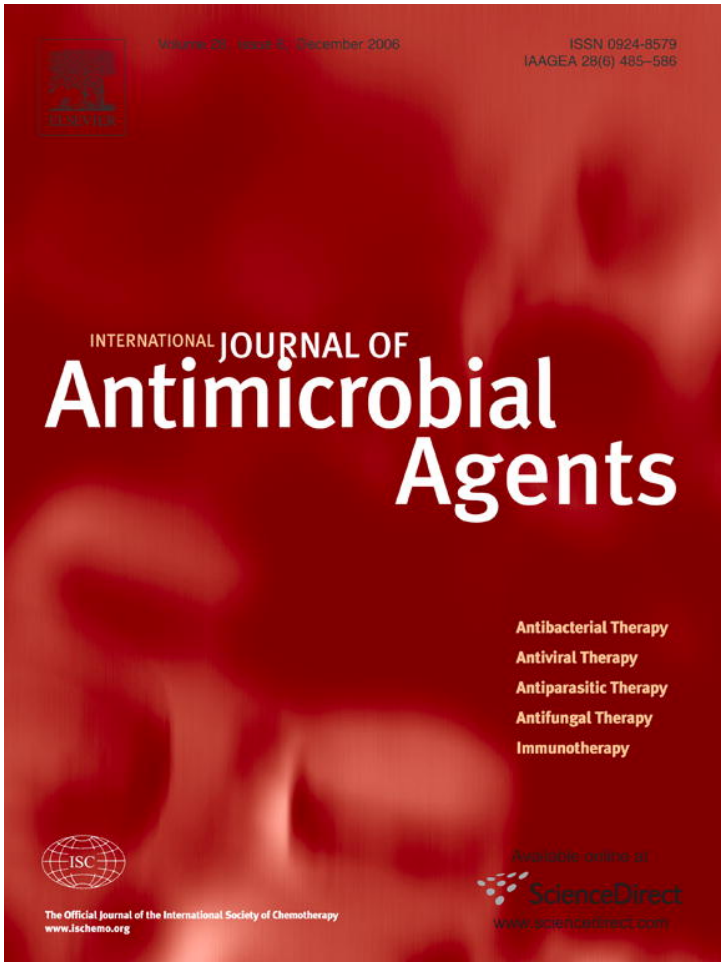

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Activity of tigecycline against clinical isolates of *Staphylococcus aureus* and extended-spectrum β-lactamase-producing *Escherichia coli* in Granada, Spain

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Abstract

We evaluated the in vitro activity of tigecycline using the Etest and disk diffusion method according to Clinical and Laboratory Standards Institute guidelines against clinical isolates of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) as well as for CTX-M-9 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and SHV ESBL-producing *E. coli*. All isolates were susceptible to tigecycline according to US Food and Drug Administration cut-off points. There were no differences in the activity of tigecycline between MSSA and MRSA isolates or between the presence of either type of ESBL. For each type of microorganism studied, we established the equation relating the minimum inhibitory concentration to the diameter of the zone of inhibition.

Keywords: Tigecycline; *Staphylococcus aureus*; *Escherichia coli*; ESBL

1. Introduction

Tigecycline is a semi-synthetic tetracycline (glycylcycline) derived from minocycline [1]. It is active against Gram-positive cocci and Gram-negative rods, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp., macrolide- or penicillin-resistant *Streptococcus pneumoniae*, extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae and carbapenem-resistant *Acinetobacter* spp. It is also active against anaerobic bacteria (*Bacteroides* spp., *Clostridium perfringens* and *Peptostreptococcus* spp.), intracellular microorganisms and non-tuberculous mycobacteria [2]. Furthermore, tigecycline is active against tetracycline- and minocycline-resistant microorganisms and does not present cross-resistance with other antibiotics such as β-lactams or fluoroquinolones [3]. Nevertheless, in vitro studies show that it is not active against *Pseudomonas aeruginosa*, *Proteus mirabilis* or *Providencia* spp. [4].

Tigecycline crosses the external membrane of bacteria through porins via passive diffusion and reaches the cytoplasm by an energy-dependent mechanism. It binds to the ribosome thereby inhibiting protein synthesis. This effect is produced by blocking binding of the tRNA aminoacyl site to the 30 S ribosomal subunit. The association is reversible, which explains its bacteriostatic effect [5]. The absence of anti-eukaryotic activity means that it has selective antibacterial properties.

Several cases of reduced sensitivity to this antibiotic have been reported in Enterobacteriaceae owing to induction of the efflux pump gene *acrAB* [6].

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Tigecycline is administered parenterally as a 1 h infusion twice daily and is only available as an injectable formulation. It crosses the placental barrier and is generally eliminated in high concentrations in breast milk. It is metabolised in the liver and the main routes of elimination are via the biliary tract and kidney [3].

To date, the indications approved by the US Food and Drug Administration (FDA) are treatment of complicated skin and soft tissue infections and complicated intra-abdominal infections.

In recent years in Spain we have observed a marked increase in the number of infections produced by multiresistant microorganisms as well as in the morbidity and mortality of infections caused by MRSA and ESBL-producing Enterobacteriaceae.

In Spain, the increased incidence of MRSA (from 1.5% in 1986 to 31.2% in 2002) was accompanied by a marked increase in resistance to other antibiotics such as macrolides, lincosamides, aminoglycosides or fluoroquinolones [7]. Although this is not currently a significant problem in Europe, there have been reports of infections caused by *S. aureus* with reduced susceptibility to glycopeptides (glycopeptide-intermediate *S. aureus* (GISA)) [8]. This situation is particularly problematic given the lack of available therapeutic alternatives.

ESBLs are plasmid-borne enzymes produced by Gram-negative rods that confer resistance to all the penicillins, cephalosporins (with the exception of cephamycins) and monobactams. The plasmids encoding these enzymes can also carry genes for resistance to other antibiotics such as cotrimoxazole, aminoglycosides and tetracyclines and cross-resistance is very common [9]. ESBL-producing microorganisms are also resistant to fluoroquinolones more frequently than other non-ESBL-producing isolates [10]. Therefore, sometimes the only possibility of treatment is using carbapenems [11]. However, these should be used in moderation as they have been associated with an increase in infections by carbapenem-resistant *Acinetobacter baumannii* or *P. aeruginosa* [12], with the result that treatment of these infections is remarkably difficult.

Tigecycline may therefore be an alternative in the treatment of skin and soft tissue infections caused by *S. aureus* and intra-abdominal infections caused by Enterobacteriaceae (especially ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*) in hospitalised patients. This study used different methods to describe the activity of tigecycline against clinical isolates of methicillin-sensitive *S. aureus* (MSSA), MRSA and ESBL-producing *E. coli*.

2. Material and methods

2.1. Bacterial isolates

We evaluated the in vitro activity of tigecycline against 220 clinical isolates identified using the WIDER system (Francisco Soria Melguizo S.A., Madrid, Spain) [13] at the clinical microbiology laboratory of Hospital 'San Cecilio', Granada, Spain.

One hundred and five isolates were identified as *S. aureus*. Resistance to methicillin was confirmed using the Mueller–Hinton agar diffusion procedure (bioMérieux, Marcy l'Etoile, France) with 1 μg oxacillin disks, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [14]. Fifty-four MSSA and 51 MRSA isolates were obtained.

The remaining 115 isolates were ESBL-producing *E. coli* in which the presence of the enzyme in each isolate was confirmed by the diffusion method with disks containing cefotaxime (30 μg), cefotaxime/clavulanic acid (30/10 μg), ceftazidime (30 μg) and ceftazidime/clavulanic acid (30/10 μg), as recommended by the CLSI [14]. Following phenotypic confirmation, determination of the existing β-lactamase and clonality was carried out by means of biochemical (determination of the isoelectric point) and molecular (polymerase chain reaction) studies following the procedures previously described by our group [10,15]. Sixty-seven isolates produced the CTX-M-9 enzyme and 48 isolates produced the SHV enzyme.

Isolates were stored at −40 °C until the susceptibility study.

2.2. Susceptibility study

After checking the purity of the isolates, a 0.5 McFarland suspension was prepared and inoculated onto Mueller–Hinton agar plates (bioMérieux). An agar plate was used for each isolate and the Etest and disk diffusion procedures were carried out in parallel.

Tigecycline Etest strips (AB Biodisk, Solna, Sweden) were used to determine the minimum inhibitory concentration (MIC) of tigecycline. To determine the diameter of the zone of inhibition, the agar diffusion method was used with 15 μg tigecycline disks (BBL, Becton Dickinson, Franklin Lakes, NJ).

The control strains used in all procedures were *K. pneumoniae* ATCC 700603, *E. coli* ATCC 25922 and *S. aureus* ATCC 29213.

3. Results

3.1. Staphylococcus aureus

Using the cut-off established by the FDA in 2005 for *S. aureus* (MIC ≤ 0.5 μg/mL), 100% of the *S. aureus* isolates were susceptible to tigecycline. They were all inhibited by a concentration of ≤0.19 μg/mL and presented a zone of inhibition around the disk ≥18 mm. For *S. aureus* ATCC 29213, the values were 0.125 μg/mL and 20 mm, respectively.

The MIC range and the MIC for 50% and 90% of the organisms (MIC_{50} and MIC_{90}, respectively) obtained by the

Table 1
MIC range and MIC for 50% and 90% of the organisms (MIC_{50} and MIC_{90}, respectively) obtained by the Etest, and range, mean and S.D. of the diameter of the zone of inhibition obtained by the disk diffusion method for *Staphylococcus aureus* isolates

Organism	MIC (μg/mL)			Inhibition zone (mm)		
	Range	MIC_{50}	MIC_{90}	Range	Mean	S.D.
S. aureus ($n = 105$)	0.047–0.19	0.094	0.125	18–27	22.2	1.7
MRSA ($n = 51$)	0.047–0.19	0.094	0.125	20–27	21.8	1.5
MSSA ($n = 54$)	0.047–0.19	0.094	0.125	18–27	22.6	1.7

MIC, minimum inhibitory concentration; S.D., standard deviation; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

Table 2
Percentage of clinical isolates at each minimum inhibitory concentration (MIC) of tigecycline obtained by the Etest

	MIC of tigecycline (μg/mL)								
	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75
ESBL-producing *Escherichia coli*	0.9	9.6	24.3	19.2	18.2	8.7	8.7	4.3	6.1
CTX-M-9-producing *E. coli*	1.5	10.4	23.9	23.9	16.4	10.4	6	1.5	6
SHV-producing *E. coli*	0	8.3	24.9	12.5	20.9	6.3	12.5	8.3	6.3
Staphylococcus aureus	7.6	23.9	35.2	27.6	5.7	0	0	0	0
MSSA	12.9	35.2	27.8	18.6	5.5	0	0	0	0
MRSA	2.1	11.7	43.1	37.2	5.9	0	0	0	0

ESBL, extended-spectrum β-lactamase; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

Table 3
MIC range and MIC for 50% and 90% of the organisms (MIC_{50} and MIC_{90}, respectively) obtained by the Etest, and range, mean and S.D. of the diameter of the zone of inhibition obtained by the disk diffusion method for isolates of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*

Organism	MIC (μg/mL)			Inhibition zone (mm)		
	Range	MIC_{50}	MIC_{90}	Range	Mean	S.D.
ESBL-producing ($n = 115$)	0.047–0.75	0.125	0.38	19–29	24.5	2.2
CTX-M-9-producing ($n = 67$)	0.047–0.75	0.125	0.38	20–29	24.8	2
SHV-producing ($n = 48$)	0.064–0.75	0.19	0.5	19–29	24.2	2.4

MIC, minimum inhibitory concentration; S.D., standard deviation.

Etest, and the range, mean and standard deviation (S.D.) of the diameter of the zone of inhibition obtained by the disk diffusion method for the 105 *S. aureus* isolates are shown in Table 1. The MIC values obtained by Etest show the excellent activity of tigecycline against these clinical isolates (range 0.047–0.19 μg/mL, $MIC_{50} = 0.094$ μg/mL, $MIC_{90} = 0.125$ μg/mL). These values were observed both in the MSSA and MRSA isolates.

Table 2 shows the percentage of *S. aureus* isolates at each tigecycline MIC determined by the Etest.

Finally, we studied the relationship between the MIC values and the zone of inhibition around the 15 μg disks for *S. aureus*. The equation relating MIC (y) and the diameter of the zone of inhibition (x) was $y = 0.4566–0.0162x$ and the correlation coefficient was $r = -0.808$, which demonstrates a significant relationship between both variables (Fig. 1).

3.2. ESBL-producing E. coli

Using the cut-off established by the FDA in 2005 for Enterobacteriaceae (MIC ≤ 2 μg/mL), 100% of the ESBL-producing *E. coli* isolates were susceptible to tigecycline. All the isolates were inhibited by a concentration ≤0.75 μg/mL and presented a zone of inhibition around the disk ≥19 mm. For *K. pneumoniae* ATCC 700603, the values were 0.5 μg/mL and 23 mm, and for *E. coli* ATCC 25922 they were 0.38 μg/mL and 22 mm, respectively.

The range, MIC_{50} and MIC_{90} obtained by the Etest, and the range, mean and S.D. of the diameter of the zone of inhibition obtained by disk diffusion for the 115 ESBL-producing *E. coli* isolates are shown in Table 3. The MIC values

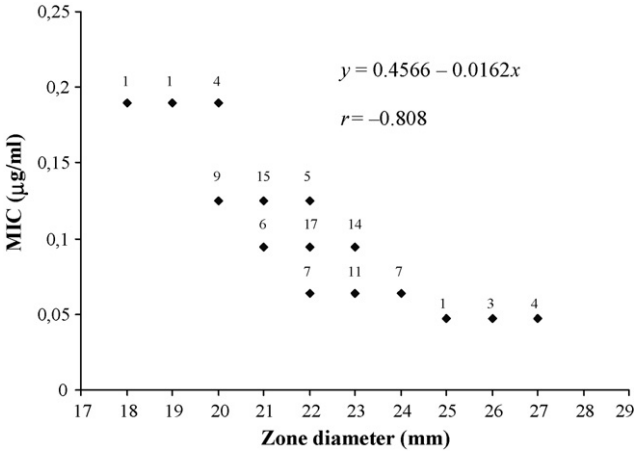

Fig. 1. Scattergram comparing zones of inhibition around 15 μg tigecycline disks (x) with the tigecycline minimum inhibitory concentration (MIC) (y) determined by the Etest method for all the isolates of *Staphylococcus aureus*.

Table 4
Activity of tigecycline against species of *Staphylococcus aureus* (MRSA and MSSA) and *Escherichia coli* (ESBL-producing and non-ESBL-producing) in different studies

Reference	Microorganism	Minimum inhibitory concentration (μg/mL)		
		Range	MIC_{50}	MIC_{90}
Gales and Jones [4]	MRSA	≤0.06–0.5	0.25	0.25
	MSSA	0.12–0.25	0.25	0.25
	E. coli (non-ESBL)	0.12–0.25	0.25	0.25
Biedenbach et al. [17]	*S. aureus*	≤0.015–1	0.06	0.25
	E. coli (ESBL)	0.06–0.5	0.12	0.5
Betriu et al. [18]	MRSA	≤0.06–0.5	0.125	0.5
	E. coli (non-ESBL)	0.5–2	1	1
Milatovic et al. [19]	MRSA	0.12–1	0.25	0.25
	MSSA	0.06–0.5	0.12	0.25
	E. coli (non-ESBL)	0.06–1	0.25	0.5
Zhang et al. [20]	MRSA	≤0.06–0.5	0.25	0.25
	MSSA	≤0.06–0.25	0.125	0.125
	E. coli (non-ESBL)	0.125–1	0.25	0.5
Reynolds et al. [21]	MRSA	0.125–1	0.25	0.5
	MSSA	0.125–0.5	0.25	0.25
	E. coli (ESBL and non-ESBL)	0.125–2	0.5	1
Fritsche et al. [22]	MRSA	–	0.25	0.5
	MSSA	–	0.25	0.5
Fritsche et al. [23]	All *S. aureus*	0.03–1	0.12	0.5
	MRSA	0.03–1	0.12	0.5
	MSSA	0.03–1	0.12	0.5
	E. coli (non-ESBL)	0.06–2	0.12	0.5
Sader et al. [24]	*S. aureus*	≤0.016–1	0.12	0.5
	E. coli (non-ESBL)	0.03–1	0.25	0.25
Fritsche et al. [25]	*E. coli* (ESBL)	0.06–2	0.25	0.5

MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; ESBL, extended-spectrum β-lactamase.

obtained by the Etest show the excellent activity of tigecycline against these clinical isolates (range 0.047–0.75 μg/mL, MIC_{50} = 0.125 μg/mL, MIC_{90} = 0.38 μg/mL). Similar values were obtained for isolates producing CTX-M-9 or SHV enzymes.

Table 2 shows the percentage of isolates of ESBL-producing *E. coli* for each tigecycline MIC determined by the Etest.

The equation relating the MIC value (y) and the diameter of the zone of inhibition around the 15 μg disks (x) was $y = 1.8656–0.0674x$ and the correlation coefficient was $r = -0.845$, which, once again, shows a significant relationship between both variables (Fig. 2).

4. Discussion

Staphylococcus aureus and ESBL-producing *E. coli* are two important causes of nosocomial and community-acquired infections. Carbapenems are sometimes the only therapeutic alternative against infections caused by ESBL-producing pathogens owing to the resistance associated with other groups of antibiotics [11]. Glycopeptides such as vancomycin or teicoplanin are generally the antibiotics of choice for the treatment of MRSA infections. The emergence of GISA [8] suggests that the use of glycopeptides may be limited and it may be necessary to look for alternatives. Several studies show that tigecycline is as active as imipenem in the treatment of intra-abdominal infections (where it is necessary to cover the presence of Gram-negative pathogens such as Enterobacteriaceae) and as active as the combination of vancomycin and aztreonam in skin and soft tissue infections (where it is necessary to cover the presence of MRSA and Gram-negative pathogens) [16].

Table 4 shows the results of susceptibility to tigecycline among *S. aureus* isolates (MRSA and MSSA) and

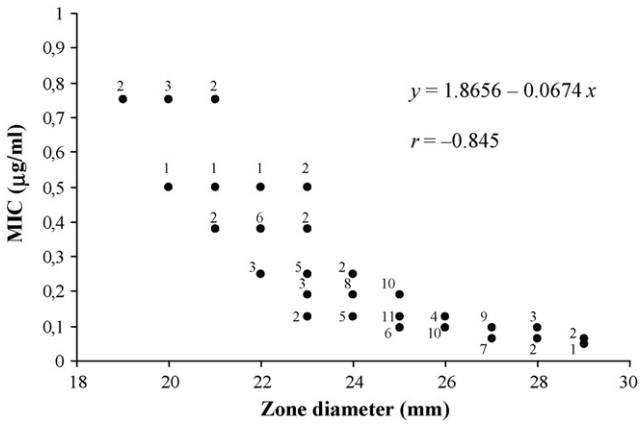

Fig. 2. Scattergram comparing zones of inhibition around 15 μg tigecycline disks (x) with the tigecycline minimum inhibitory concentration (MIC) (y) determined by the Etest method for all the isolates of extended-spectrum β-lactamase-producing *Escherichia coli*.

ESBL-producing *E. coli* in different studies [4,17–25]. The MIC_{50} and MIC_{90} values for MRSA isolates range from 0.12 μg/mL to 0.25 μg/mL and from 0.25 μg/mL to 0.5 μg/mL, respectively (compared with 0.094 μg/mL and 0.125 μg/mL in the present study). For MSSA isolates, the MIC_{50} and MIC_{90} values range from 0.12 μg/mL to 0.25 μg/mL and from 0.125 μg/mL to 0.5 μg/mL, respectively (compared with 0.094 μg/mL and 0.125 μg/mL in the present study). For *E. coli* isolates (ESBL-producing and non-ESBL-producing), the MIC_{50} and MIC_{90} values in the different studies range from 0.12 μg/mL to 1 μg/mL and from 0.25 μg/mL to 1 μg/mL, respectively (compared with 0.125 μg/mL and 0.38 μg/mL in the present study). Our results are therefore similar to those of other studies.

Given the importance of these microorganisms in intra-abdominal infections and infections of the skin and soft tissues as well as the initial indications for therapy with tigecycline, our results show that tigecycline has excellent activity. Furthermore, in our series this activity is maintained regardless of the presence of methicillin resistance or type of ESBL produced.

To conclude, tigecycline is a therapeutic alternative against infections caused by *S. aureus* (including MRSA isolates) and ESBL-producing *E. coli*.

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