# *In vitro* activity of tigecycline against 313 Gram-positive and Gram-negative clinical isolates

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**Key words:** tigecycline, broad-spectrum activity, antibiotic-resistance

**Attività *in vitro* di tigeciclina su 313 ceppi Gram-positivi e Gram-negativi di isolamento clinico**

**SUMMARY**

**Objectives.** In this study the *in vitro* activity of tigecycline, member of a new class of antimicrobial agents, the glycylcyclines, was evaluated against clinical isolates collected in Italy.

**Study Design.** A total of 313 clinical pathogens were collected and identified in our Institution during 2007-2008. Minimum inhibitory concentrations (MICs) of the antimicrobial agents were determined by the CLSI (2007) recommended broth microdilution method.

**Results.** Globally 205 Gram-negative and 108 Gram-positive pathogens were evaluated. Tigecycline demonstrated excellent inhibitory activity against *Acinetobacter* spp., *H. influenzae*, *E. coli, Enterococcus* spp., *S. aureus*, *S. agalactiae* and *S. pneumoniae* with $MIC_{90}$ ≤1mg/l.

**Conclusion.** Tigecycline exhibited potent *in vitro* antibacterial activity (comparable to or greater than most commonly employed antimicrobials) against both Gram-positive and negative clinical pathogens. These data suggest that tigecycline, with an expanded broad-spectrum antimicrobial activity, may be an effective empiric therapeutic option for the treatment of serious infections caused by clinically relevant pathogens.

Tigecycline (formerly GAR-936), a synthetic analogue of tetracycline, is the first member of a new class of antimicrobial agents, the glycylcyclines, that inhibits bacterial protein biosynthesis blocking the attachment of amino-acyl tRNA to the A site of the ribosome and preventing the elongation of peptide chains (7). The drug manteins its activity even in presence of mechanisms that otherwise confer tetracycline resistance (2, 6). In previous studies this new drug had demonstrated significant antibacterial properties with an excellent in vitro activity against clinical and laboratory Gram-positive and - negative bacteria, anaerobic and atypical pathogens.

Moreover the broad spectrum of tygecycline includes difficult to treat methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant E*nterococcus* spp. (VRE), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* (3, 6-7).

Tigecycline resistance is very infrequent and is also difficult to induce in laboratory, with a selection frequency lower than $10^{-9}$ (3, 6).

This study was undertaken to document the *in vitro* activity of tigecycline against clinical isolates collected in Italy from in-patient and out-patient populations.

A total of 313 clinical isolates, 205 Gram-negative (50 *Escherichia coli,* 37 *Klebsiella pneumoniae,* 36 *Pseudomonas aeruginosa,* 21 *Enterobacter cloacae,* 21 *Acinetobacter. baumannii*, 14 *Serratia marcescens,* 13 *Klebsiella oxytoca,* 13 *Haemophilus influenzae*) and 108 Gram-positive (48 *Staphylococcus aureus,* 25 *Streptococcus pneumoniae*, 20 *Streptococcus agalactiae,* 15 *Enterococcus faecalis*), were collected and identified to the species level in Sezione di Microbiologia of University of Genova during 2007-2008. Minimum inhibitory concentrations (MICs) of the antimicrobial agents were determined by the CLSI recommended broth microdilution testing method (1). Quality controls (QC) were performed using the following strains: *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. Tigecycline was supplied by Wyeth Pharmaceuticals (Milano).

On Gram-negative pathogens (Table 1), Tigecycline demonstrated excellent inhibitory activity.

Tigecycline activity particulary on *E. coli* isolates, with a $MIC_{90}$ of 1 mg/l, was comparable to more active agents (meropenem, levofloxacin and amikacin) and lower than beta-lactams, beta-lactam/beta-lactamase inhibitor combinations.

Against other *Enterobacteriaceae* tigecycline's $MIC_{90}$ was minor or equal to 4 mg/l. This new agent, as the other tetracyclines, showed limited *in vitro* activity against *P. aeruginosa*, with $MIC_{90}$ values more than 16 mg/l.

Against *H. influenzae* ($MIC_{50}$ 0.25 mg/l and $MIC_{90}$ 0.5 mg/l), included beta-lactamase-producer strains, the new agent demonstratedan excellent inhibitory activity.

*In vitro* tigecycline's activity on *Acinetobacter* spp. ($MIC_{50}$ 0.5 mg/l and $MIC_{90}$ 1 mg/l) was superior to beta-lactams, beta-lactam/beta-lactamase inhibitor combinations, cephalosporins, aminoglycosides and levofloxacin.

Tigecycline exhibited a potent *in vitro* activity even in Gram-positive pathogens (Table 2).

The drug presented $MIC_{90}$ of 0.25 mg/l against *Enterococcus* spp., regardless of susceptibility to vancomycin. This value was the lowest of all comparative agents, in particular in vancomycin-resistant *E. faecium* and *E. faecalis* was several folds lower than linezolid, minocycline and levofloxacin (Table 2).

The drug has shown a potent inhibitory activity against *S. aureus* regardless of methicillin-resistant phenotype. Tigecycline, with $MIC_{50}$ and $MIC_{90}$ values of 0.25 and 0.5 mg/l, respectively, demonstrated *in vitro* activity comparable to linezolid and vancomycin and greater than levofloxacin against MRSA. In MSSA $MIC_{50}$ and $MIC_{90}$ had the same value of 0.25 mg/l (Table 2).

Tigecycline demonstrated potent inhibitory activity against *S. agalactiae* and *S. pneumoniae*, even in penicillin-intermediate or resistant strains (Table 2), with $MIC_{90}$ values ≤ 1 mg/l.

Resistance to currently available antibiotics and incidence of infection due to multidrug-resistant bacteria has dramatically increased worldwide during the last twenty years.

In presence of a serius infection, appropriate empirical antibiotic therapy can be life-saving and the choice of an antimicrobial to which the pathogens are susceptible may be critical. For these reasons, the development of new antimicrobial agents with expanded antibacterial spectrum (increased activity against Gram-negative as well as Gram-positive microrganisms) is more urgent than ever. (4).

Tigecycline's *in vitro* activity was comparable to or greater than most commonly prescribed antimicrobial against important clinical pathogens. Tigecycline, for example, demonstrated on methicillin-resistant *S. aureus* (MRSA) *in vitro* activity comparable to linezolid and vancomycin, the antimicrobial agents currently used for the treatment of serious staphylococ-

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cal infections and exhibits greater activity to linezolid against vancomycin-resistant *E. faecalis* and *E. faecium* (VRE).
This promising compound may be usefull even in the treatment of serious infections caused by resistant Gram-negative strains with limited therapeutic options. Tigecycline has shown to be a highly effective against *Acinetobacter* spp., particulary *A. baumannii* that are commonly associated with serious nosocomial infections. Resistance of *Acinetobacter* to cephalosporins, aminoglycosides and quinolones is widespread with an increasing of multi-drug resistance.
Tigecycline is a potent antimicrobial agent even against the infections due to this important pathogen. The drug demonstrated a limited activity, similar to other tetracyclines, only against *P. aeruginosa*. The *in vitro* activity of tigecycline observed in this study suggests that this drug is a suitable antimicrobial agent for empiric treatment of serious infections sustained by some of the commonly encountered pathogens (5).
Tigecycline has showed and demonstrated a significant antibacterial activity against Gram-negative and Gram-positive, with MIC50 ≤2 mg/l and MIC90 ≤4 mg/l for every species, except *P. aeruginosa*.
The *in vitro* activity demonstrated by tigecycline, suggest that this drug can be considered a promising alternative for the treatment of serious infections caused by commonly clinically pathogens encountered.

**Table 1.** *In vitro activity of tigecycline and other comparative agents against Gram-negative pathogens.*

| Microrganisms (number of strains) | Antibiotics | MIC-range (mg/L) | MIC50 | MIC90 |
|---|---|---|---|---|
| *E. coli (50)* | tigecycline | 0.12-4 | 0.25 | 1 |
| | minocycline | ≤0.5->16 | 2 | >16 |
| | ampicillin | 2->32 | >32 | >32 |
| | amoxicillin/clavulanate | 2->32 | 8 | >32 |
| | piperacillina/tazobactam | 1->128 | 2 | 128 |
| | ceftriaxone | ≤0.06->64 | 0.12 | 64 |
| | ceftazidime | | | ?8 |
| | cefepime | ≤0.5->32 | ?0.5 | 8 |
| | meropenem | 0.25-1 | 0.25 | 0.5 |
| | levofloxacin | 0.03-2 | 0.06 | 2 |
| | amikacin | 1-4 | 1 | 2 |
| *Klebsiella* spp. (50) (1) | tigecycline | 0.25-4 | 1 | 4 |
| | minocycline | ≤0.5->16 | 4 | 16 |
| | ampicillin | 32->32 | >32 | >32 |
| | amoxicillin/clavulanate | 2->32 | 4 | >32 |
| | piperacillina/tazobactam | 1->128 | 4 | 32 |
| | ceftriaxone | ≤0.06->64 | 0.25 | >64 |
| | ceftazidime | ≤8->32 | ?8 | 16 |
| | cefepime | ≤0.5-32 | ?0.5 | 1 |
| | meropenem | | | ?0.06 |
| | levofloxacin | 0.03-1 | 0.06 | 0.12 |
| | amikacin | 2-16 | 2 | 4 |
| *Enterobacter* spp. (21) (2) | tigecycline | 0.25-16 | 1 | 2 |
| | minocycline | 2->16 | 4 | 16 |
| | ampicillin | | | >32 |
| | amoxicillin/clavulanate | | | >32 |
| | piperacillina/tazobactam | 2->128 | 8 | >128 |
| | ceftriaxone | 0.25->64 | 8 | >64 |
| | ceftazidime | ≤8->32 | ≤8 | >32 |
| | cefepime | ≤0.5-4 | ≤0.5 | 2 |
| | meropenem | ≤0.06-1 | ≤0.06 | 0.12 |
| | levofloxacin | 0.06->8 | 0,25 | >8 |
| | amikacin | 1-16 | 2 | 8 |
| *S. marcescens* (14) | tigecycline | 1-4 | 2 | 4 |
| | minocycline | 4-16 | 8 | 16 |
| | ampicillin | | | >32 |
| | amoxicillin/clavulanate | | | >32 |
| | piperacillina/tazobactam | 2-8 | 2 | 4 |
| | ceftriaxone | 0.25-2 | 0.5 | 2 |
| | ceftazidime | | | ?8 |
| | cefepime | ≤0.5-1 | ≤0.5 | 1 |
| | meropenem | 0.06-1 | 0.5 | 1 |
| | levofloxacin | 0.06-1 | 0.25 | 0.5 |
| | amikacin | 1-8 | 1 | 8 |
| *P. aeruginosa* (36) | tigecycline | 2->16 | 16 | >16 |
| | minocycline | 4->16 | >16 | >16 |
| | ampicillin | >32 | | |
| | amoxicillin/clavulanate | >32 | | |
| | piperacillina/tazobactam | 4->128 | 8 | 128 |
| | ceftriaxone | >64 | | |
| | ceftazidime | ≤8->32 | ≤8 | 32 |
| | cefepime | 2->32 | 4 | 16 |
| | meropenem | 0.5-8 | 0.5 | 2 |
| | levofloxacin | 0.5->8 | 1 | >8 |
| | amikacin | 1-32 | 1 | 4 |

| Microrganisms (number of strains) | Antibiotics | MIC-range | MIC50 (mg/L) | MIC90 |
|---|---|---|---|---|
| *A. baumanii* (21)[3] | tigecycline | 0.06-1 | 0.5 | 1 |
| | minocycline | ≤0.5->16 | 8 | 16 |
| | ampicillin | >32 | | |
| | amoxicillin/clavulanate | >32 | | |
| | piperacillina/tazobactam | >128 | | |
| | ceftriaxone | >64 | | |
| | ceftazidime | >32 | | |
| | cefepime | 32->32 | 32 | >32 |
| | meropenem | 4->16 | >16 | >16 |
| | levofloxacin | >8 | | |
| | amikacin | 1->64 | 2 | 64 |
| *H. influenzae* (13) | tigecycline | 0.12-0.5 | 0.5 | 0.25 |
| | minocycline | 1-4 | 1 | 1 |
| | ampicillin | ≤0.5-32 | ≤0.5 | 1 |
| | amoxicillin/clavulanate | 0.25-1 | 0.5 | 1 |
| | piperacillina/tazobactam | ≤0.06-0.12 | ≤0.06 | 0.12 |
| | ceftriaxone | ≤0.06 | | |
| | ceftazidime | ≤8 | | |
| | cefepime | ≤0.5 | | |
| | meropenem | <0.006 | | |
| | levofloxacin | ≤0.008-0.5 | 0.03 | 0.25 |
| | amikacin | ≤0.5-16 | 8 | 16 |

1. *K. pneumoniae 17, K. oxytoca 8.*
2. *E. cloacae 20, E. aerogenes 5.*
3. *A. baumannii 21.*

**Table 2.** *In vitro activity of tigecycline and other comparative agents against Gram-positive pathogens.*

| Microrganisms (number of strains) | Antibiotics | MIC50 | MIC90 (mg/L) | MIC-range |
|---|---|---|---|---|
| *Enterococcus spp.* (15) (1) | ttigecycline | 0.25 | 0.25 | 0.06-0.5 |
| | minocycline | >8 | >8 | ≤0.25->8 |
| | penicillin | 4 | >8 | 2->8 |
| | ampicillin | 2 | >16 | 1->16 |
| | amoxicillin/clavulanate | 1 | >8 | 0.5->8 |
| | piperacillina/tazobactam | 8 | >16 | 2->16 |
| | ceftriaxone | >64 | >64 | 0.25->64 |
| | meropenem | 8 | >16 | 2->16 |
| | vancomycin | 1 | >32 | 0.25->32 |
| | levofloxacin | 16 | >32 | 1->32 |
| | linezolid | 2 | 4 | 1-4 |
| *S.aureus* MRSA (48) | tigecycline | 0.25 | 8 | 0.06->8 |
| | minocycline | 1 | 1 | ≤0.25-8 |
| | penicillin | >8 | >8 | 0.06->8 |
| | ampicillin | >8 | >16 | 0.25->16 |
| | amoxicillin/clavulanate | 1 | >8 | 0.25->8 |
| | piperacillina/tazobactam | 2 | >16 | 0.5->16 |
| | ceftriaxone | 4 | >64 | 4->64 |
| | meropenem | 0.12 | 8 | 0.12->8 |
| | vancomycin | 1 | 1 | 0.5-1 |
| | levofloxacin | 1 | 32 | 1-32 |
| | linezolid | 2 | 4 | 2-4 |
| *S. pneumoniae* (25) | tigecycline | 0.5 | 1 | 0.06-1 |
| | minocycline | 0.5 | 4 | ≤0.25-8 |
| | penicillin | 0.06 | 2 | 0.06-2 |
| | ampicillin | 0.25 | 0.5 | ≤0.06-2 |
| | amoxicillin/clavulanate | 0.12 | 0.25 | ≤0.03-2 |
| | piperacillina/tazobactam | ?0.25 | 1 | ≤0.25-2 |
| | ceftriaxone | 0.25 | 1 | 0.06-4 |
| | meropenem | 0.12 | 1 | ≤0.12-1 |
| | vancomycin | 0.5 | 1 | ≤0.12-1 |
| | levofloxacin | 1 | 2 | 1-2 |
| | linezolid | 1 | 2 | ≤0.5-2 |
| *S. agalactiae* (20) | tigecycline | 0.03 | 0.12 | 0.03-0.25 |
| | minocycline | 8 | >8 | 8 - >8 |
| | penicillin | | | ≤0.06 |
| | ampicillin | ≤0.06 | 0.12 | ≤0.06-0.12 |
| | amoxicillin/clavulanate | | | 0.12 |
| | piperacillina/tazobactam | ≤0.25 | ≤0.25 | ≤0.25 |
| | ceftriaxone | 0.06 | 0.12 | 0.03-0.12 |
| | meropenem | ≤0.12 | 0.25 | ≤0.12-0.25 |
| | vancomycin | | | 0.5 |
| | levofloxacin | 0.5 | 1 | 0.5-1 |
| | linezolid | 1 | 1 | ≤0.5-1 |

*E. faecalis 8, E. faecium 5, E. avium 1, E. durans 1.*

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