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BRIEF REPORT

Susceptibility of clinical isolates of frequently encountered bacterial species to tigecycline one year after the introduction of this new class of antibiotics: results of the second multicentre surveillance trial in Germany (G-TEST II, 2007)

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Abstract Tigecycline, a broad-spectrum antibiotic for parenteral use, was introduced in Germany in May 2006. In the G-TEST-II trial, the susceptibility of isolates, recovered in 2007 from hospitalised patients in 15 centres, was assessed against tigecycline and comparators. Susceptibility tests were performed by the microdilution procedure. This study reports on the susceptibility of the isolates of 16 bacterial species and compares the results with those of a trial (G-TEST I) conducted prior to the introduction of tigecycline. Between 2005 and 2007, tigecycline retained activity against Gram-positive and Gram-negative organisms. By contrast, the rate of vancomycin-resistant strains among *Enterococcus faecium* isolates almost doubled. Moreover, an increase in resistance to broad-spectrum beta-lactams and fluoroquinolones was observed for members of the family *Enterobacteriaceae*. Against a background of a steadily rising number of pathogens that are resistant to various antibiotic classes, tigecycline represents an important treatment option.

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Tigecycline (Tygacil®), the first glycylcycline with in-vitro activity against many aerobic and anaerobic Gram-positive and Gram-negative organisms [1], was introduced in Germany in May 2006. The drug, which is administered intravenously, is indicated for complicated skin and skin structure infections and complicated intra-abdominal infections. The recommended standard dosage is 100 mg as a loading dose, followed by 50 mg every 12 h over a period of 5 to 14 days. In a second Tigecycline Evaluation Surveillance Trial conducted throughout Germany (G-TEST II), the susceptibility of over 2,400 bacterial isolates, collected one year after the introduction of the new compound, was tested against tigecycline and comparators. This study reports on the susceptibility of the isolates of 16 bacterial species and compares the results with those of a trial performed one year prior to the introduction of tigecycline (2005) (G-TEST I) [2].

The same 15 medical microbiology laboratories that had participated in G-TEST I cooperated in this trial. In accordance with the study protocol, in the period from May to September 2007, each laboratory was asked to include in the study a maximum of 200 isolates recovered from hospitalised patients with community-acquired or nosocomial infections. Data from the following bacterial species and groups were evaluated: *Enterococcus faecalis*, *E. faecium*, oxacillin (methicillin)-susceptible *Staphylococcus aureus* (MSSA), oxacillin (methicillin)-resistant *S. aureus* (MRSA), *S. haemolyticus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. pneumoniae*, *S. pyogenes*, *Acinetobacter baumannii* group, *Escherichia coli*, *Enterobacter cloacae*, *Haemophilus influenzae*, *Klebsiella oxytoca*, *K. pneumoniae*, *Serratia marcescens* and *Stenotrophomonas maltophilia*.

Only first isolates from the following sources were accepted for inclusion: peritoneal cavity, respiratory tract, blood, wounds and urine (<10% of the isolates). Coagulase-negative staphylococci were only included in the study if they were recovered from at least two consecutive blood samples.

Identification of the pathogens was performed using standard laboratory methods. The bacterial strains were then conserved at −70°C and sent to a central laboratory (Antiinfectives Intelligence) for susceptibility testing at the end of the collection period.

Minimal inhibitory concentrations (MICs) were determined using the microdilution broth method according to the standard ISO 20776-1:2006 [3]. The test medium was Mueller-Hinton II broth (Becton Dickinson GmbH, Heidelberg). Testing of the streptococci was performed using 3% lysed horse blood (Oxoid GmbH, Wesel). *Haemophilus* test medium was used to determine the susceptibility of *H. influenzae*. The following reference strains were included as quality controls: *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *H. influenzae* ATCC 49247, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300 and *S. pneumoniae* ATCC 49619.

Susceptibility to the following antibacterial agents was tested: Gram-positive bacteria – amoxicillin-clavulanic acid, cefuroxime, doxycycline, gentamicin, imipenem, linezolid, moxifloxacin, oxacillin, penicillin G, piperacillin-tazobactam, tigecycline and vancomycin; Gram-negative bacteria – amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ciprofloxacin, doxycycline, ertapenem, gentamicin, imipenem, moxifloxacin, piperacillin-tazobactam and tigecycline.

In order to categorise the bacteria as susceptible, intermediate or resistant, the species-related breakpoints approved by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used, if available [4, 5]. The MICs of doxycycline for *Enterobacteriaceae* and enterococci were interpreted by the cut-off values given by the German standard DIN 58940 [6].

Isolates of *E. coli* and *Klebsiella* spp. were tested for extended-spectrum beta-lactamase (ESBL) production according to the broth dilution procedure described by the Clinical Laboratory Standards Institute (CLSI) [7].

A total of 2,420 bacterial strains were included in the study. The majority of isolates were cultured from respiratory tract specimens (34%), wound swabs (29%), blood (21%) and intra-abdominal material (8%). Sixty-eight percent of the isolates came from patients on general wards and 32% from patients in intensive care units. More than 60% of the patients were male. The age of the patients ranged from <1 to 107 years (median: 63 years).

Each reference strain was included at least five times in the susceptibility testing. With one exception, MICs fell into tolerance ranges (as far as available). The MICs of imipenem for *E. coli* ATCC 25922 were, in part, above the upper breakpoint of the tolerance range.

Table 1 shows the resistance rates of 15 organisms for selected antibacterial agents, as well as changes compared to the 2005 trial (G-TEST I), while Tables 2 and 3 of the supplementary material present comprehensive results of both trials, including the MIC_{50} and MIC_{90} values and the susceptibility and resistance rates of all of the tested drugs for the 16 bacterial species.

Gram-positive bacteria

Compared with the situation prior to its introduction, tigecycline demonstrated unchanged high in-vitro activity against Gram-positive organisms. With the exception of three *S. haemolyticus* isolates, all Gram-positive bacteria tested were categorised as tigecycline-susceptible.

The MIC_{90} values of tigecycline for MSSA, MRSA, *S. epidermidis* and *S. haemolyticus* ranged between ≤0.125 μg/mL and 0.5 μg/mL. The MIC values of the *S. haemolyticus* isolates categorised as resistant were each one

Table 1 Proportion of resistant strains among Gram-positive and Gram-negative pathogens and percentage changes compared to the 2005 trial (G-TEST I)

Organism (*n*)	Antibacterial agent	% R	% Δ	Organism (*n*)	Antibacterial agent	% R	% Δ
E. faecalis (149)	Tigecycline	0	0	*A. baumannii* group (117)	Tigecycline	n.d.	–
	Doxycycline	59.7	−11.0		Imipenem	11.1	+10.4
	Amoxicillin-clavulanic acid	0.7	+0.7		Ciprofloxacin	27.4	−2.6
	Linezolid	0	0		Gentamicin	22.2	+8.6
	Vancomycin	0	0	*E. cloacae* (223)	Tigecycline	6.3	−0.6
	Gentamicin (high level)[a]	38.9	+0.9		Doxycycline	10.8	0
E. faecium (142)	Tigecycline	0	0		Piperacillin-tazobactam	22.0	+3.5
	Doxycycline	6.3	−5.4		Cefotaxime	42.6	+2.1
	Amoxicillin-clavulanic acid	93.0	+1.3		Ceftazidime	34.1	+4.8
	Linezolid	0	−0.7		Cefepime	2.2	−1.2
	Vancomycin	18.3	+8.6		Imipenem	0	0
	Gentamicin (high level)[a]	37.3	−6.1		Ciprofloxacin	7.2	+1.6
Oxacillin-susceptible *S. aureus* (153)	Tigecycline	0	0		Gentamicin	6.3	+1.6
	Doxycycline	2.6	−0.8	*E. coli* (292)	Tigecycline	0	0
	Moxifloxacin	11.8	−1.7		Doxycycline	40.4	−3.6
	Linezolid	0	0		Amoxicillin-clavulanic acid	28.8	+4.5
	Vancomycin	0	0		Piperacillin-tazobactam	6.2	+2.5
	Gentamicin	7.8	−0.4		Cefotaxime	12.3	+7.0
Oxacillin-resistant *S. aureus* (155)	Tigecycline	0	0		Ceftazidime	5.8	+3.8
	Doxycycline	3.9	−1.9		Cefepime	9.9	+6.9
	Moxifloxacin	94.8	+3.9		Imipenem	0	0
	Linezolid	0	0		Ciprofloxacin	28.4	+6.7
	Vancomycin	0	0		Gentamicin	10.3	+2.6
	Gentamicin	12.9	−9.8	*H. influenzae* (225)	Tigecycline	n.d.	–
S. epidermidis (142)	Oxacillin	83.1	−0.4		Doxycycline	0.9	+0.9
	Tigecycline	0	0		Amoxicillin-clavulanic acid	5.3	+2.1
	Doxycycline	9.2	−0.9		Ciprofloxacin	0	0
	Moxifloxacin	52.1	+9.8	*K. oxytoca* (109)	Tigecycline	1.8	−0.2
	Linezolid	0	0		Doxycycline	16.5	+12.5
	Vancomycin	0	0		Amoxicillin-clavulanic acid	25.7	+10.7
	Gentamicin	56.3	−9.2		Piperacillin-tazobactam	23.9	+9.9
S. haemolyticus (66)	Oxacillin	90.9	+0.5		Cefotaxime	15.6	+12.6
	Tigecycline	4.5	+3.1		Ceftazidime	0.9	+0.9
	Doxycycline	12.1	+6.6		Cefepime	2.8	+2.8
	Moxifloxacin	51.5	−6.0		Imipenem	0	0
	Linezolid	0	0		Ciprofloxacin	13.8	+7.8
	Vancomycin	0	0		Gentamicin	4.6	+3.6
	Gentamicin	84.8	+4.0	*K. pneumoniae* (185)	Tigecycline	7.0	−5.4
S. agalactiae (76)	Tigecycline	0	0		Doxycycline	26.5	−0.4
	Doxycycline	77.6	+6.9		Amoxicillin-clavulanic acid	19.5	+6.1
	Penicillin G	0	0		Piperacillin-tazobactam	10.3	+2.2
	Moxifloxacin	0	0		Cefotaxime	13.5	+8.1
	Linezolid	0	0		Ceftazidime	10.3	+6.0

Table 1 (continued)

Organism (*n*)	Antibacterial agent	% R	% Δ	Organism (*n*)	Antibacterial agent	% R	% Δ
	Vancomycin	0	0		Cefepime	7.6	+2.8
S. pneumoniae (70)	Tigecycline	n.d.	–		Imipenem	0	0
	Doxycycline	11.4	+6.2		Ciprofloxacin	16.8	+8.7
	Penicillin G	0	0		Gentamicin	5.4	+2.2
	Moxifloxacin	0	0	*S. marcescens* (124)	Tigecycline	2.4	−1.0
	Linezolid	0	0		Doxycycline	42.7	+1.2
	Vancomycin	0	0		Piperacillin-tazobactam	9.7	+7.2
S. pyogenes (63)	Tigecycline	0	0		Cefotaxime	17.7	+10.9
	Doxycycline	17.5	+4.5		Ceftazidime	1.6	−0.1
	Penicillin G	0	0		Cefepime	0	−2.5
	Moxifloxacin	0	0		Imipenem	0	0
	Linezolid	0	0		Ciprofloxacin	12.9	+7.0
	Vancomycin	0	0		Gentamicin	2.4	−0.1

% R = % resistant; % Δ = percentage changes compared to the 2005 trial (G-TEST I)

n.d. = not determined, as no species-related breakpoint has been approved by the EUCAST. All isolates of *S. pneumoniae*, *A. baumannii* group and *H. influenzae* were inhibited at <0.125, 4 and 0.5 mg tigecycline per mL, respectively

[a] MIC > 500 mg/l

level above the breakpoint of 0.5 μg/mL. The proportion of isolates with susceptibility to doxycycline was approximately 95% each for MSSA und MRSA and approximately 85% each for the two coagulase-negative species. Among MRSA isolates, the proportion of gentamicin-resistant strains, at 12.9%, was less in this study than in G-TEST I. A negative trend for gentamicin resistance was also observed for *S. epidermidis*, although at a markedly higher level. Staphylococci with reduced susceptibility to vancomycin or linezolid were not detected.

The proportion of vancomycin-resistant enterococci (VRE) among the *E. faecium* isolates was 18.3% in this study, almost twice as high as in G-TEST I (9.7%), whereas the isolates of *E. faecalis* were 100% susceptible to vancomycin. No linezolid-resistant enterococci were detected. Tigecycline showed unchanged high in-vitro activity against both enterococcal species (including VRE) with MIC_{90} values of ≤0.125 μg/mL. The proportion of doxycycline-resistant strains in this trial was less than in G-TEST I. The resistance rate in *E. faecalis* was, however, still approximately 60%.

Tigecycline again showed high in-vitro activity against the three streptococcal species investigated (MIC_{90} of ≤0.125 μg/mL in each case). By contrast, the proportion of doxycycline-resistant isolates was 11.4% for *S. pneumoniae*, 17.5% for *S. pyogenes* and 77.6% for *S. agalactiae*.

Gram-negative bacteria

Of the *E. coli* isolates, 99.3% were tigecycline-susceptible, while more than 40% were categorised as doxycycline-resistant. The MIC values of tigecycline for two doxycycline-resistant isolates were in the intermediate range (2 μg/mL). The proportion of *E. coli* isolates with resistance to ciprofloxacin rose between 2005 and 2007 from 21.7% to over 28.4%. The increase of resistance to cefotaxime correlated with a rise of ESBL-producing strains, from 5.3% to 12.3%.

The activity of tigecycline against *E. cloacae*, *K. pneumoniae*, *K. oxytoca* and *S. marcescens* was comparable in both studies. By contrast, the proportion of isolates classified as ESBL-producers increased from 9% to 17.4% for *K. oxytoca* and from 4.3% to 14.6% for *K. pneumoniae*. Concurrently, a marked increase in resistance to fluoroquinolones was detected in both species. In the case of *K. oxytoca*, there was also an increase in resistance to doxycycline and piperacillin-tazobactam, and for *S. marcescens*, an increase in resistance to fluoroquinolones, cefotaxime and piperacillin-tazobactam was observed.

As in G-TEST I, tigecycline again showed high activity against isolates of the *A. baumannii* group. The $MIC_{50/90}$ values (0.25/1 μg/mL) were comparable with the values obtained in G-TEST I (0.25/0.5 μg/mL). By contrast, the percentage of isolates with resistance to imipenem was 11.1% in this trial, compared with <1% in G-TEST I.

Based on the $MIC_{50/90}$ values, no changes in the antimicrobial susceptibility of *S. maltophilia* isolates were observed. In both trials, the lowest MIC_{90} values were determined for tigecycline (1–2 μg/mL) and moxifloxacin (2 μg/mL).

Comparison of the results of the present trial with those of G-TEST I shows no significant changes in the susceptibility to tigecycline of the species tested [2]. By contrast, the frequency of resistance of *E. coli* to fluoroquinolones increased considerably and was over 28% in this trial. This value corresponds well to the rate of ciprofloxacin resistance from a study recently performed by the Paul-Ehrlich-Society (PEG) in Germany [8]. The latest rate of ciprofloxacin resistance for German blood culture isolates published by the European Antimicrobial Resistance Surveillance System (EARSS) was 30% [9]. Of concern is the marked rise in cefotaxime resistance due to the spread of ESBL-producing isolates in *E. coli* and other *Enterobacteriaceae* species. Compared with the trial conducted in 2005, the increase in the prevalence of ESBL-producers observed for *E. coli* (from approximately 5% to over 12%) and *K. pneumoniae* (from 4.3% to almost 15%) also corresponds with the results of a resistance study performed by the PEG [8].

Overall, tigecycline remained universally active against *E. coli*, *E. cloacae*, *K. pneumoniae*, *S. maltophilia* and against isolates of the *A. baumannii* group.

No statement can be made about the prevalence of MRSA due to the specific study design. For tigecycline, there was no change in activity against staphylococci, enterococci and streptococci, including MRSA, VRE and doxycycline-resistant strains. However, tigecycline resistance has been described for *E. faecalis* and *S. aureus* [10, 11]. In two tigecycline-resistant laboratory mutants of *S. aureus* (MICs 4–16 μg/mL), obtained following serial passage in increasing concentrations of this drug, the overexpression of a novel MATE efflux pump (MepA) was shown to contribute to the reduced susceptibility [10], whereas the resistance mechanism in one tigecycline-resistant clinical isolate of *E. faecalis* (MIC 1 μg/mL) has not yet been elucidated [11].

In summary, one year after the introduction of tigecycline, it can be ascertained that no change in the resistance situation has yet been observed for any of the bacterial species tested. Against a background of a steadily rising number of pathogens that are resistant to various antibiotic classes, tigecycline represents an important treatment option.

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