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ARTICLE

In vitro activity of tigecycline against methicillin-resistant *Staphylococcus aureus*, including livestock-associated strains

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Abstract The in vitro activity of tigecycline was determined using a well-defined collection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates (n=202), including 33 livestock-associated strains. Susceptibility testing was performed using the Etest system. Among the 202 MRSA strains, three (1.5%) had a minimum inhibitory concentration (MIC) value for tigecycline greater than 0.5 mg/l, which are considered to be resistant. When these strains were tested using Iso-Sensitest medium, the MICs were substantially lower and no resistance was found. This discrepancy warrants further investigations into the preferred test conditions for tigecycline. In conclusion, tigecycline showed good activity against MRSA strains in vitro.

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Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have traditionally been a problem in healthcare settings [1]. According to a report from the National Nosocomial Infections Surveillance (NNIS) System, approximately 60% of all *S. aureus* isolated from patients in intensive care units in US hospitals were methicillin-resistant in 2003 [2]. For the last approximately 10 years, MRSA has expanded its territory to the community, causing severe infections in previously healthy persons all over the world [3, 4]. In 2003, a new clone of MRSA was observed in The Netherlands that is related to an extensive reservoir in pigs and cattle [5]. The livestock-associated clone is characterized by being non-typable by *Sma*I pulsed-field gel electrophoresis (PFGE). By the end of 2007, nearly 30% of all MRSA observed in The Netherlands were of this type [6]. There are important differences between livestock-associated MRSA (LA-MRSA), healthcare-associated MRSA (HA-MRSA), and community-associated MRSA (CA-MRSA) regarding the susceptibility against antimicrobial agents. HA-MRSA isolates are frequently multidrug-resistant, while CA-MRSA and LA-MRSA are relatively susceptible for most non-beta-lactam antibiotics, with the exception of tetracycline for LA-MRSA, for which they are almost always resistant. This is most likely due to the extensive use of this antimicrobial agent in animal husbandry. Because tigecycline is related to tetracycline, it is important to determine the activity of this new drug for LA-MRSA.

The treatment of serious MRSA infections has been based, for many years, upon the use of glycopeptides, i.e., vancomycin and teicoplanin. However, concerns over increasing rates of heteroresistance and tolerance to glycopeptides [7] has urged the development of newer agents. Tigecycline is the first commercially available

member of the glycylcyclines, a new class of antimicrobial agents. The glycylcyclines are derivatives of the tetracycline antibiotics, with structural modifications that result in activity against gram-positive, gram-negative, and anaerobic micro-organisms, including multidrug-resistant strains. It exhibits generally bacteriostatic action by reversibly binding to the 30S ribosomal subunit and inhibiting protein translation [8].

The purpose of the present study was to assess the in vitro activity of tigecycline against MRSA isolates collected in The Netherlands using a well-defined collection of strains that included a representative sample of LA-MRSA strains.

Materials and methods

A total of 202 MRSA isolates were tested in this study. All MRSA isolates are part of the MRSA strain collection of the National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands. The collection consisted of three subsets. The first set of isolates used in this study contained 76 MRSA isolates that were collected between 1990 and 1998 in The Netherlands (old MRSA). The second set was 93 MRSA isolates collected between 2003 and 2005 (recent MRSA). These MRSA strains all had a unique PFGE typing result. The third set of isolates tested consisted of 33 LA-MRSA strains and were collected between 2003 and 2005. They had been collected in a previous study and the strains in our evaluation are the index cases of the previous survey [9]. All 202 isolates have been confirmed as *S. aureus* and methicillin-resistant using a duplex polymerase chain reaction (PCR) for the *mec*A gene and coagulase gene as described previously [10, 11].

The minimum inhibitory concentration (MIC) for tigecycline was determined by using the Etest system (AB Biodisk, Solna, Sweden) with a concentration range of 0.016 to 256 μg/ml. Etest strips contained a concentration gradient of the antimicrobial agent with a standard amount of calcium throughout the strip. Etest strips were applied to the surface of 150–mm Mueller–Hinton agar plates. Plates were incubated at 35°C in ambient air for 24 h prior to reading the MIC results. In addition, the MICs of the following antimicrobial agents were determined simultaneously: oxacillin, gentamicin, cotrimoxazole, ciprofloxacin, erythromycin, clindamycin, rifampin, daptomycin, tetracycline, linezolid, vancomycin, and teicoplanin. All MICs were determined using the Etest system. For vancomycin and teicoplanin, the Etest strips were placed on brain heart infusion agar, using a high inoculum (2.0 McFarland) and an extended incubation time (48 h) to be able to detect hGISA isolates. Isolates were categorized as susceptible or resistant to an antimicrobial agent according to the breakpoints published by the Clinical and Laboratory Standards Institute (CLSI) [12]. The proposed breakpoint for tigecycline is greater than 0.5 mg/l for *S. aureus* (both methicillin-resistant and methicillin-susceptible strains). The 11 MRSA strains with the highest MIC for tigecycline on Mueller–Hinton agar plates were subsequently applied on 90-mm Iso-Sensitest agar plates (Oxoid Ltd.). The plates were then incubated at 35°C in ambient air for 24 h prior to reading the MIC results.

All results were entered into a database and further statistical analyses were performed using SPSS software. The MIC values for all tested antimicrobial agents of the different subsets of strains were compared using the Mann–Whitney *U*-test.

Results and discussion

The observed MIC range for tigecycline was 0.05 to 1.0 μg/ml, with MIC values at which 50 and 90% of the isolates tested are inhibited (MIC_{50} and MIC_{90}) of 0.19 and 0.38 μg/ml, respectively. The MIC_{50} and MIC_{90} of LA-MRSA, old MRSA, and recent MRSA isolates for tigecycline and other antibiotics are outlined in Table 1. No significant difference was found in the portion of tigecycline resistance between recent MRSA and old MRSA. None of the LA-MRSA isolates were resistant for tigecycline. Three (2%) of the 169 tested MRSA isolates were resistant for tigecycline. Of the 76 old MRSA isolates, two (3%) isolates had MICs for tigecycline greater than 0.5 μg/ml and are, therefore, considered to be resistant for tigecycline. In addition, one isolate (1%) of the 93 recent MRSA strains had an MIC value for tigecycline greater than 0.5 μg/ml. The 11 MRSA strains with the highest MIC for tigecycline on Mueller–Hinton agar plates were retested using Etest strips that were applied on Iso-Sensitest agar plates (Oxoid Ltd.) and simultaneously on Mueller–Hinton agar plates. The MIC values for tigecycline of the 11 MRSA strains applied on Mueller–Hinton agar plates were comparable with the previously obtained MIC values. On Iso-Sensitest medium, these 11 MRSA strains had significantly 2-fold lower MIC values for tigecycline using linear regression analysis ($p<0.001$). Figure 1 shows the shift towards lower MIC values for tigecycline when MRSA strains were applied on Iso-Sensitest medium. The MICs for tigecycline showed a significant correlation with those of tetracycline ($r=0.518$; $p<0.001$) and teicoplanin ($r=0.325$; $p<0.001$).

LA-MRSA was the most susceptible group of strains. They were only significantly more often resistant to tetracycline (Table 1). Old MRSA strains were more often resistant to most groups of antimicrobial agents, compared to recent MRSA. The only agent that was significantly ($p<0.001$) more resistant in recent strains in comparison with old strains was vancomycin.

Table 1 MIC_{50} and MIC_{90} values of antimicrobial agents against 76 MRSA collected between 1990 and 1998 (old MRSA), 93 MRSA collected between 2003 and 2005 (recent MRSA), and 33 livestock-associated MRSA (LA-MRSA) strains collected in The Netherlands between 2003 and 2005

Antibiotic	Old MRSA		Recent MRSA		LA-MRSA		*p*-value		
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	Old vs. recent	Old vs. LA	Recent vs. LA
Tigecycline	0.19	0.42	0.19	0.38	0.25	0.38	0.618	0.230	0.296
Oxacillin	256	256	32.0	256	12.0	48.0	<0.001[a]	<0.001[a]	<0.001[a]
Gentamicin	12	256	0.75	41.6	0.38	9.9	<0.001[a]	<0.001[a]	<0.001[a]
Cotrimoxazole	0.13	32	0.025	0.525	0.02	0.13	<0.001[a]	<0.001[a]	0.202
Ciprofloxacin	32	32	24.0	32.0	0.38	1.8	<0.001[a]	<0.001[a]	<0.001[a]
Erythromycin	256	256	0.38	256	0.25	256	<0.001[a]	<0.001[a]	0.036
Clindamycin	0.19	256	0.09	256	0.06	256	<0.001[a]	<0.001[a]	0.179
Rifampin	0.012	32	0.006	0.60	0.004	0.006	<0.001[a]	<0.001[a]	<0.001[a]
Daptomycin	0.38	0.75	0.38	0.75	0.13	0.19	0.243	<0.001[a]	<0.001[a]
Tetracycline	12	32	0.38	29.6	32	48	0.001[a]	<0.001[a]	<0.001[a]
Linezolid	1.0	1.5	1.0	1.5	0.75	1.0	0.032	<0.001[a]	<0.001[a]
Vancomycin[b]	3.0	4.0	4.0	8.0	4.0	4.0	<0.001[a]	0.176	0.077
Teicoplanin[b]	3.5	12	4.0	12.0	3.0	4.0	0.385	0.05	<0.001[a]

[a] A *p*-value of <0.01 is considered to be statistically significant

[b] The Etest system with a high inoculum and 48 h of incubation was used

In a well-defined collection of MRSA, we found that a minority (1.5%) was resistant for tigecycline. All strains were isolated before tigecycline had been used in patients. The results of this study slightly differed from other data on European and North American antibiotic-resistant clinical isolates that were phenotypically characterized [13–15]. In a recent study, the in vitro activity of tigecycline against 38 MRSA and the correlation of this activity with their resistance gene content were determined [16]. Tigecycline demonstrated good activity against MRSA, with MIC_{50} and MIC_{90} values of 0.12 and 0.25 μg/ml, respectively. Overall, tigecycline showed an MIC range of 0.06 to 0.25 μg/ml. The tigecycline MICs determined in our study were slightly higher.

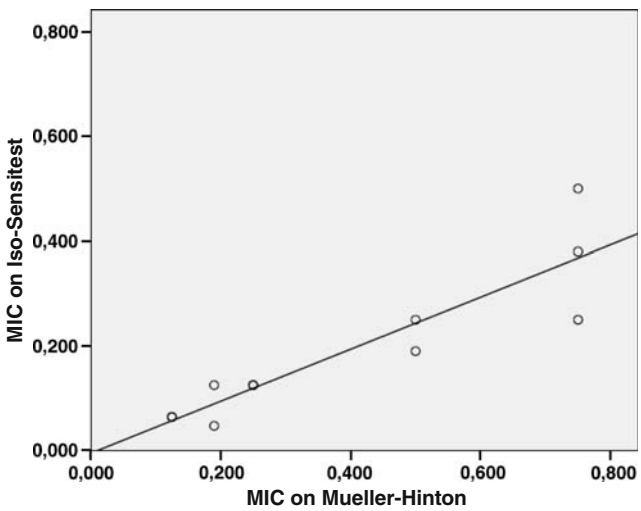

Fig. 1 Linear regression analysis of MIC values for tigecycline using Mueller–Hinton medium and Iso-Sensitest medium

In another study, Fluit et al. [17] found the MIC range for tigecycline to be 0.06 to 2.0 μg/ml. For the 106 *S. aureus* isolates tested, two (2%) isolates had MIC values for tigecycline greater than 0.5 μg/ml and are, therefore, considered to be resistant. These findings are identical to our results. In our study, the MICs for tigecycline showed a significant correlation with the MICs for tetracycline. Fluit et al. found no relation between the presence of tetracycline resistance determinants *tet*(K) or *tet*(M) and the MICs for tigecycline observed for *S. aureus*, although tetracycline-susceptible isolates were more often susceptible to tigecycline.

The possible correlation of the in vitro susceptibility of tigecycline and tetracycline prompted us to include LA-MRSA in the evaluation. These strains are known to have high levels of tetracycline resistance. Also, the current evaluation showed that 28 out of the 33 (85%) LA-MRSA strains were resistant against tetracycline. However, none of the LA-MRSA isolates tested was resistant against tigecycline. Conversely, we found tigecycline resistance in three of the HA-MRSA strains when incubated on Mueller–Hinton agar plates. Because of the recently reported influence of the test conditions on the in vitro susceptibility of tigecycline, we also tested a subset of the strains on Iso-Sensitest medium [18]. Eleven MRSA strains with the highest MICs for tigecycline were selected and retested on Mueller–Hinton agar and on Iso-Sensitest medium. On Mueller–Hinton agar, the results were identical to the initial

result, but on Iso-Sensitest medium, the MICs for tigecycline were much lower, and all strains were considered to be susceptible. The results for tigecycline are influenced by the concentration of manganese in the medium [18]. As Mueller–Hinton agar is a biological medium, the concentration of manganese may vary. Iso-Sensitest is a biochemical medium, which is well-defined. However, the CLSI standard recommends the use of Mueller–Hinton medium for the susceptibility testing of tigecycline using the Etest system [19]. This discrepancy requires further investigations into the underlying mechanisms.

An interesting aspect of this study is the remarkable difference in resistance against various classes of antibiotics between old and more recent strains of MRSA. The older strains were, in general, much more resistant than the more recent strains (Table 1). This may reflect the emergence of CA-MRSA in recent years, which are, in general, more susceptible [4]. The only antimicrobial agent with significantly higher MICs in recent MRSA was vancomycin. This has recently been reported by other groups and may reflect the increased use of this agent in hospitals all over the world [20, 21]. As vancomycin is considered to be the cornerstone of therapy for serious MRSA infections, the increasing MICs are a worrying finding. It stresses the need for alternative therapeutic agents. The LA-MRSA strains were also relatively susceptible to many classes of antibiotics, with the exception of tetracycline. MICs for tigecycline were comparable in all three groups of strains.

In conclusion, tigecycline exhibited broad in vitro activity against a collection of MRSA strains collected in The Netherlands, including livestock-associated strains. Using the recommended methodology, we found three strains to be resistant. However, these strains were considered to be susceptible when Iso-Sensitest medium was used. This discrepancy warrants further investigations into the preferred test conditions because the interpretation of the in vitro susceptibility of tigecycline is affected significantly.

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