ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2006, p. 505–510 Vol. 50, No. 2
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.2.505–510.2006

Diagnostic PCR Analysis of the Occurrence of Methicillin and Tetracycline Resistance Genes among *Staphylococcus aureus* Isolates from Phase 3 Clinical Trials of Tigecycline for Complicated Skin and Skin Structure Infections

C. Hal Jones,* Margareta Tuckman, Anita Y. M. Howe, Mark Orlowski, Stanley Mullen, Karen Chan, and Patricia A. Bradford

Infectious Diseases Discovery Research, Wyeth Research, Pearl River, New York

Received 3 October 2005/Returned for modification 27 October 2005/Accepted 3 November 2005

Diagnostic PCR assays were developed to track common genetic determinants of oxacillin resistance as well as resistance to classical tetracyclines in *Staphylococcus aureus* isolates from the recently completed worldwide phase 3 clinical trials of tigecycline. A total of 503 unique *S. aureus* strains isolated from complicated skin and skin structure infections were analyzed. The *mecA* gene was amplified from 120 strains (23.9%) determined to be resistant to oxacillin (MICs ≥ 4 μg/ml). The prevalence of the *mecA* gene was found to vary regionally from 6.5% to 50.9% among isolates originating in Eastern Europe and North America, respectively. The presence of a tetracycline resistance determinant, *tet*(M) or *tet*(K), among methicillin-resistant *S. aureus* (MRSA) isolates also varied regionally, with a range of 11.9% to 46.2% among isolates tested from North America and Eastern Europe, respectively. The occurrence of a tetracycline resistance marker in methicillin-susceptible *S. aureus* (MSSA) strains varied from 2.5 to 16.1% among the isolates tested across the regions of study. The presence of *tet*(M) or *tet*(K) had no discernible effect on the tigecycline MICs for either MRSA or MSSA strains, which is consistent with the ability of the glycylcyclines to retain activity in the presence of both the ribosomal protection and efflux mechanisms of resistance to the tetracyclines.

Staphylococcus aureus is a remarkable pathogenic organism that has acquired resistance to all classes of antimicrobials and thus is a continuing threat in both the hospital and community health care settings (1, 21). The acquisition of the *mecA* gene, which confers resistance to methicillin, spawning so-called methicillin-resistant *S. aureus* (MRSA), has resulted in a highly resilient pathogen that has reached epidemic levels in many parts of the world (5, 12, 16, 43). As MRSA strains are often resistant to other antibiotic classes (22, 23, 46, 50), surveillance for this important pathogen is a priority. Moreover, with the recent reports of vancomycin-resistant strains of *S. aureus* in the United States and Japan, treatment options are clearly limited and support ongoing efforts to identify novel agents for infection control as well as the reengineering of successful agents from the past (23, 53).

Numerous studies have shown that the incidence of MRSA throughout the world is rising; however, regionally the rates differ dramatically (5, 8, 16, 54). Current studies place the incidence of MRSA in blood isolates from Europe at 20 to 35% (16, 19, 44, 54). A recent analysis revealed that when the incidence is broken down geographically, the incidence of MRSA was significantly higher in Southern Europe than in Northern Europe, with rates ranging from a high of 44% to a low of 0.5%, respectively (16, 54). Consistent with this finding, the recent British Society for Antimicrobial Chemotherapy study found that in the United Kingdom and Ireland the incidence of MRSA among blood isolates was 42% (44). Among skin isolates collected between 1997 and 2000 in Latin America, the rate of occurrence of MRSA was 28.4% (49). The rates of occurrence of MRSA among isolates from all clinical sources from pan-Asian sites are reported to vary from a low of 5% in the Philippines to a high of 69.5% in Japan (3). The SENTRY study (isolates collected in 2000) found that 29.5% of skin and skin structure infection isolates in the United States and Canada were resistant to methicillin, an increase from the rate of 24% reported in the 1999 study (isolates collected in 1997) (17, 43). A considerable rise in the rates of MRSA infection among patients in intensive care units was reported by the National Nosocomial Infections Surveillance report in 2003 (12). That analysis found an incidence of 57.1%, an increase of 13% over that reported in the previous 5-year period (1997 to 2002).

Currently, a number of technologies are available for the detection and confirmation of MRSA (2, 28, 37). Several commercial kits have been developed for the rapid confirmation of MRSA following growth on oxacillin (6 μg/ml)-supplemented agar. However, multiplex PCR still remains the "gold standard" for the confirmation of MRSA through direct detection of the *mecA* gene in clinical isolates and distinguishing between MRSA and borderline oxacillin-resistant strains (37).

The growth in the rates of resistance to tetracyclines has been aided by antibiotic misuse and overuse in the human population, in addition to the widespread use of the agent both as an animal growth promoter and for infection control in agriculture and aquaculture (13, 27). Tetracyclines still remain first-line agents of choice in many parts of the world, including the United States, for a number of human infections, including acne and prophylaxis and treatment of protozoan diseases as

* Corresponding author. Mailing address: Infectious Diseases Discovery Research, Wyeth Research, 401 N. Middletown Rd., Bldg. 200, Rm. 3219, Pearl River, NY 10965. Phone: (845) 602-4612. Fax: (845) 602-5671. E-mail: jonesh3@wyeth.com.

TABLE 1. *S. aureus* control strains used in study

Strain	Resistance determinant(s)	Source[a]	Reference or source
NRS1 (Mu50, ATCC 700699)	*tet*(M), *mecA*	NARSA	24
GAR 1497	*tet*(K)	Wyeth Strain Collection	This study
NRS71 (Sanger 252)	*mecA*	NARSA	25
NRS72 (Sanger 476)	*mecA* negative	NARSA	25
ATCC 29213	None	ATCC	14

[a] NARSA, Network on Antimicrobial Resistance in *S. aureus* (www.narsa.net); ATCC, American Type Culture Collection.

well as periodontal diseases (13, 45). The rate of tetracycline resistance concomitant with methicillin resistance has been reported to be as high as 57.1% across Europe and as low as 1.5% in the United Kingdom and Ireland (44, 51).

The isolates in the present study were obtained from the recent worldwide phase 3 clinical trials of tigecycline, a novel glycylcycline antibiotic (10, 26, 41, 52). Tigecycline has been shown to have activity against resistant gram-negative and gram-positive pathogens, including MRSA, glycopeptide-intermediate *S. aureus*, penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus* spp., and extended-spectrum β-lactamase-expressing *Escherichia coli* and *Klebsiella pneumoniae* (4, 6, 7, 10, 20, 29, 32, 40). Additionally, tigecycline has demonstrated potency against organisms expressing tetracycline resistance determinants specifying either ribosomal protection [e.g., *tet*(M)] or efflux [e.g., *tet*(K)] (18, 41).

This study was conducted to investigate the incidence of *mecA* and several common tetracycline resistance determinants among *S. aureus* strains isolated during phase 3 clinical trials of tigecycline for skin and skin structure infections (11, 48).

MATERIALS AND METHODS

Bacterial strains. Clinical isolates of *S. aureus* were from phase 3 double blind clinical trials comparing the safety and efficacy of tigecycline to those of an active comparator for the treatment of complicated skin and skin structure infections (2002 to 2004) (11, 48). Patient specimens were processed and bacterial pathogens were cultured by each site laboratory according to local practices. Individual investigators sent all bacterial isolates to a central laboratory for identification and susceptibility testing. Primary cultures were provided to Wyeth Research in frozen vials. Upon receipt, the isolates were plated onto blood agar, checked for purity, and frozen in skim milk-glycerol (50:50) at −70°C. Confirmation of species identity, when necessary, was by standard microbiological methods (33). In instances in which multiple isolates from a single patient were collected, Riboprinting (Qualicon; Dupont, Wilmington, DE) was used, according to the manufacturer's instructions, to determine if the isolates were serial isolates of the same strain or represented different strains. If the isolates were identical, only the first isolate, based on the hospital collection date, was included in the analysis. The *S. aureus* strains used as controls for the PCR assays are shown in Table 1.

Susceptibility determination. Tests for susceptibility to tigecycline, minocycline, and tetracycline were performed by the broth microdilution method with fresh Mueller Hinton II broth, as recommended by the Clinical Laboratory Standards Institute (CLSI; formerly NCCLS) (14, 35). The methicillin resistance of staphylococci was determined by MIC tests for oxacillin supplemented with 2% NaCl and was interpreted according to the criteria of CLSI (35). A screening test for resistance to tetracycline was performed by the disk diffusion method, according to standard protocols (34).

Whole-cell lysates. *S. aureus* isolates were plated on Trypticase soy agar (Becton Dickinson and Company, Cockeysville, MD). Following overnight incubation, several colonies were collected with a 10-μl loop and resuspended in 250 μl 50 mM Tris (pH 8) supplemented with 50 μg lysostaphin (glycyl-glycine endopeptidase) (Sigma, St. Louis, MO). The cell suspension was incubated for 15 min at 37°C, followed by placement in a boiling water bath for 5 min. The lysate was subjected to brief centrifugation at 14,000 × *g*, and 1 μl of the supernatant was used as the template for amplification.

Amplification of *mecA*, *tet*(K), and *tet*(M) genes. Primers were designed from the respective published sequences (36, 42, 47) as well as from the sequence information directly deposited in GenBank (www.ncbi.nlm.nih.gov) (Table 2). The primer sequences, the primer location (starting base pair), and the expected amplicon size are shown in Table 2. In addition to the resistance determinant, a primer set specific for the 16S rRNA-coding sequence was also added for internal standardization and quality control of the assay (42). Control strains that were previously characterized, based on antibiotic resistance and the presence of specific determinants, were used for assay development (Table 1). The FAIL SAFE PCR system (Epicenter Technologies, Madison, WI) was used for amplification. Buffer E, supplied by the manufacturer, was used for the *mecA*- and *tet*(M)-specific primer sets, and buffer C was used for the *tet*(K)-specific primer set. One microliter of the whole-cell lysate was used in the PCR assay in a 25-μl-volume reaction mixture. The cycling conditions were as follows: the initial denaturation step consisted of 5 min at 94°C; and amplification consisted of 30 cycles of 1 min at 56°C, 1 min at 72°C, and 1 min at 94°C, with a final 1 min at 45°C followed by an extension step of 5 min at 75°C. The reaction products were resolved on a 0.8% agarose gel containing 0.5 μg/ml ethidium bromide. Several PCR products for each target from the control strains were cloned and sequenced to confirm the identity of each amplicon (data not shown).

RESULTS

Incidence of *mecA* in *S. aureus* clinical isolates. A total of 503 *S. aureus* strains collected during worldwide phase 3 clinical trials of tigecycline for the treatment of complicated skin and skin structure infections were analyzed for resistance to oxacillin (MICs ≥ 4 μg/ml) by a standard methodology (14, 35). Isolates were predominately from skin (93%) and blood (4%). One hundred twenty strains were identified as MRSA (23.9%) and were

TABLE 2. Oligonucleotide primers used in study

Primer	Sequence (5′-3′)	Start point (bp)	Amplicon (kb)	Sequence accession no.[a]
MecA-F	GCAATACAATCGCACTACATTAATAG	910	0.9	X52593
MecA-R	CATTTTGAGTTCTGCAGTACCG	1814		
TetM-F	GGAGCGATTACAGAATTAGG	96	1.78	M21136
TetM-R	CGGGTCTGGCAAACAGGTT	1,879		
TetK-F	CCTGGAATTACAAACTGGGT	142	1.08	AJ888003
TetK-R	CCTCCTACAATTGCTATACC	1,217		
16s-F	GCCAGCAGCGCGGTAATACG	465	0.3	M87484
16s-R	GGACTACCAGGGTATCTAATCC	757		

[a] Genbank Accession numbers (www.ncbi.nlm.nih.gov).

TABLE 3. Regional distribution of MRSA and tetracycline resistance determinants

Region[a]	No. (%) of isolates					
	Unique isolates	MRSA	Total Tet^r	*tet*(M)	*tet*(K)	*tet*(M) *tet*(K)
NA	165	84 (50.9)	10 (11.9)	0	10 (100)	0
LA	42	6 (14.3)	0	0	0	0
IND	20	0	0	0	0	0
TWN	5	2 (40)	2 (100)	0	1 (50)	1 (50)
AUS	18	4 (22.2)	2 (50)	2 (100)	0	0
EE	199	13 (6.5)	6 (46.2)	0	5 (83.3)	1 (16.7)
WE	18	8 (44.5)	1 (12.5)	0	1 (100)	0
SAF	36	3 (8.3)	2 (66.7)	1 (50)	0	1 (50)
Total	503	120 (23.9)	23 (19.2)	3 (13)	17 (73.9)	3 (13)

[a] NA, North America (the United States and Canada); LA, Latin America (Argentina, Chile, Guatemala, Mexico, and Peru); IND, India; TWN, Taiwan; AUS, Australia; EE, Eastern Europe (Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Russia, Slovakia, and Ukraine); WE, Western Europe (Austria, Belgium, Germany, Spain, and the United Kingdom); SAF, South Africa.

subjected to PCR analysis to determine the presence of the *mecA* gene. PCR confirmed the presence of the *mecA* gene in all 120 of the phenotypically defined MRSA strains. The worldwide occurrence of *mecA* determined by region is shown in Table 3. Among the regions where 20 or more strains were collected, the highest incidence of MRSA was in North America (United States and Canada [50.9%]), whereas the lowest incidence was in Eastern Europe (6.5%). For a single country, the highest incidence of MRSA was found to be 63.6% in the United States.

Prevalence of tetracycline resistance determinants in *S. aureus* clinical isolates. MRSA strains were subjected to PCR analysis to determine the occurrence of the major tetracycline resistance genes, *tet*(K) and *tet*(M), coincident with the occurrence of *mecA*. Overall, the prevalence of a tetracycline resistance gene among MRSA isolates was found to be 19.2%, with *tet*(K) and *tet*(M) present in 73.9% and 13% of the tetracycline-resistant strains, respectively (Table 3). Only three strains (13%) were found to encode both genes. Regionally, the rates varied considerably; however, only the North American region had more than 20 MRSA isolates available for analysis.

Methicillin-susceptible *S. aureus* (MSSA) strains for which the minocycline MIC was ≥8 μg/ml were subjected to PCR to identify the tetracycline resistance determinant responsible. Those isolates that were minocycline susceptible (MICs ≤ 4 μg/ml) were tested for tetracycline susceptibility by the disk diffusion assay (34). An analysis of 383 MSSA strains revealed an overall prevalence of 11.0% tetracycline-resistant isolates, with regional variations consisting of a low of 2.5% in North America to a high of 16.1% in Eastern Europe (Table 4). Among the resistant strains, *tet*(K) (73.8%) was found more often than *tet*(M) (21.4%) in this study, with only two strains (4.8%) encoding both resistance determinants.

Activity of tigecycline against MRSA and tetracycline-resistant *S. aureus* isolates. Tigecycline demonstrated potent activity against MRSA and MSSA strains, with the MIC at which 50% of isolates are inhibited (MIC_{50}), the MIC_{90}, and the MIC range being 0.12 μg/ml, 0.25 μg/ml, and 0.06 to 1.0 μg/ml, respectively (Table 5). Additionally, the tigecycline MICs were unchanged for tetracycline-resistant strains (MRSA and MSSA isolates) bearing either *tet*(M) (MIC_{50} = 0.12 μg/ml, MIC_{90} = 0.25 μg/ml, MIC range = 0.12 to 0.25 μg/ml) or *tet*(K) (MIC_{50} = 0.12 μg/ml, MIC_{90} = 0.25 μg/ml, MIC range = 0.12 to 0.5 μg/ml). Likewise, the MIC range for the five strains bearing both determinants was unchanged (0.25 μg/ml) (Table 5).

DISCUSSION

The rate of methicillin resistance among *Staphylococcus aureus* isolates is clearly on the rise throughout the world,

TABLE 4. Regional distribution of tetracycline resistance determinants in MSSA strains

Region[a]	No. (%) of isolates					
	Unique isolates	MSSA	Total Tet^r	*tet*(M)	*tet*(K)	*tet*(M) *tet*(K)
NA	165	81 (49.9)	2 (2.5)	1 (50)	1 (50)	0
LA	42	36 (85.7)	4 (11.1)	1 (25)	3 (75)	0
IND	20	20 (100)	3 (15)	0	3 (100)	0
TWN	5	3 (60)	1 (33.3)	0	1 (100)	0
AUS	18	14 (77.8)	0	0	0	0
EE	199	186 (93.5)	30 (16.1)	7 (23.3)	21 (70)	2 (6.7)
WE	18	10 (55.5)	0	0	0	0
SAF	36	33 (91.7)	2 (6.1)	0	2 (100)	0
Total	503	383 (76.1)	42 (11)	9 (21.4)	31 (73.8)	2 (4.8)

[a] NA, North America (the United States and Canada); LA, Latin America (Argentina, Chile, Guatemala, Mexico, and Peru); IND, India; TWN, Taiwan; AUS, Australia; EE, Eastern Europe (Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Russia, Slovakia, and Ukraine); WE, Western Europe (Austria, Belgium, Germany, Spain, and the United Kingdom); SAF, South Africa.

TABLE 5. In vitro activities of tigecycline and early tetracyclines against *S. aureus*

Organism group	No. of strains	Antibiotic	MIC (μg/ml)		
			Range	50%	90%
All *S. aureus*	503	Tigecycline	0.06–1	0.12	0.25
		Tetracycline	0.12–>64	0.5	32
		Minocycline	<0.06–32	0.12	0.25
MRSA	120	Tigecycline	0.06–1	0.12	0.25
		Tetracycline	0.12–>64	0.5	32
		Minocycline	<0.06–32	0.12	0.25
MSSA	383	Tigecycline	0.06–1	0.12	0.25
		Tetracycline	0.12–>64	0.5	16
		Minocycline	<0.06–16	0.12	0.5
S. aureus [*tet*(M)]	12	Tigecycline	0.12–0.25	0.12	0.25
		Tetracycline	32–>64	64	>64
		Minocycline	4–8	8	8
S. aureus [*tet*(K)]	48	Tigecycline	0.12–0.5	0.12	0.25
		Tetracycline	8–>64	32	64
		Minocycline	0.12–32	0.25	0.25
S. aureus [*tet*(M) *tet*(K)]	5	Tigecycline	0.25	NA[a]	NA
		Tetracycline	>64	NA	NA
		Minocycline	4–16	NA	NA

[a] NA, not applicable (<10 strains were analyzed).

especially in the hospitalized population (16, 21). However, as a number of recent studies have shown, the basal rates and the increase thereof vary dramatically based on geographic region. Most recently, the European Antimicrobial Resistance Surveillance System demonstrated a dramatic north-south gradient of resistance rates in blood isolates from Europe, with the highest rates of MRSA reported in the southern countries (44%) and a nearly 100-fold lower rate of resistance in some northern countries (54). In North America, the incidence of MRSA increased from 24% to 29.5% for the period from 1997 to 2000, according to the ongoing SENTRY study of skin and soft tissue infections (43). By contrast, MRSA incidence rates in Canada for the study period from 1997 to 1999 were reported to be less than 10% (16).

In the present study, 50.9% of *S. aureus* isolates in North America were found to encode and express the *mecA* gene. When the occurrence was analyzed by country, the occurrence of MRSA in the United States (129 isolates) was nearly 64%, whereas the incidence in Canada (36 isolates) was 5.5%. These data continue the upward trend seen in earlier studies (5, 16, 17). Biedenbach et al. (5) reported the incidence for bloodstream isolates in North America to be 39.1%, with a 43.5% rate of occurrence among U.S. isolates. The difference in incidence is most likely affected by the demographics of the patient population in the various studies. The patients enrolled in the phase 3 clinical trial of tigecycline required hospitalization and intravenous antibiotic therapy; and this may well be the reason for the higher incidence, as the SENTRY studies did not break out incidence rates based on clinical presentation. As such, our data are more in line with the recent findings of the National Nosocomial Infections Surveillance report for 2003, in which the incidence of MRSA was reported to be 57.1% for patients in intensive care units (12).

Our data diverge considerably from those previously reported for MRSA incidence in Latin America and South Africa (3, 49). Five of the six Latin American MRSA isolates originated in Argentina; therefore, our survey was by no means comprehensive. Likewise, the 56 South African isolates represented only five institutions throughout the country. By comparison, the earlier analysis by Bell et al. (3) examined 94 clinical isolates from a single institution in South Africa.

A unique feature of this study is an examination of the incidence of MRSA from skin infections from diverse Eastern European sites. In the present study, of the countries supplying more than 20 isolates, it was noted that both Estonia (21 isolates) and Latvia (29 isolates) lacked MRSA, whereas Poland (21 isolates) and Russia (46 isolates) had MRSA incidences of 4.7% and 10.8%, respectively. This is consistent with the findings from a recent European Antimicrobial Resistance Surveillance System study (54), which reported an 11.8% incidence for MRSA in blood isolates from selected Eastern European countries, and the PEARLS (Pan European Antimicrobial Resistance Using Local Surveillance) study (8), which reported MRSA incidences of 12.1% and 11.5% for isolates from diverse clinical sites from Croatia and Slovenia, respectively.

Schmitz et al. (51) demonstrated an approximately fivefold concomitant occurrence of tetracycline resistance in MRSA strains versus MSSA strains in isolates from across Europe. The incidence of tetracycline resistance in MRSA strains was reported to be 57.1% (51). Conversely, in the recent British Society for Antimicrobial Chemotherapy study, MSSA and MRSA isolates from the United Kingdom and Ireland showed rates of tetracycline resistance of 4.3% and 1.5%, respectively (44). In North America, the rates of occurrence of tetracycline resistance among MRSA isolates from the United States and Canada were recently reported to be 15.6% and 14.8%, respectively, whereas in Latin America and the western Pacific the rates were considerably higher, exceeding 60% (16).

In this study the incidences of tetracycline resistance in North America was 11.9% and 2.5% for MRSA and MSSA isolates, respectively. It should be noted that none of the 36 isolates from Canada were tetracycline resistant. When tetracycline resistance in MSSA isolates was examined, the Eastern European region had an incidence of 16.1% and South Africa had an incidence of 6.1%. As the prior European study (19) had a predominately Western European representation, a direct comparison of the occurrence of tetracycline resistance is not possible.

In the present study the predominance of the *tet*(K) determinant in MRSA isolates was unexpected, as recent studies demonstrated that 50% of MRSA strains in Europe were minocycline resistant and encoded the *tet*(M) gene, although not exclusively (51). A recent, smaller study of tetracycline-resistant MRSA isolates from Poland revealed that approximately one-third of the isolates encoded only *tet*(M) (55). Our data indicate that 100% of tetracycline-resistant MRSA isolates from North America encoded only the *tet*(K) gene. Similarly, among MRSA isolates from Europe (Eastern and Western), 86% of the isolates encoded only *tet*(K). In concordance with previous studies, MSSA isolates from Europe predominately encoded the *tet*(K) gene. Likewise, although the numbers are small, tetracycline-resistant MSSA isolates from Latin America showed a similar bias toward encoding *tet*(K). When all isolates in the present study are considered, 73% of all tetracycline-resistant isolates encoded only *tet*(K).

The MRSA isolates in this collection were typed by staphylococcal chromosomal cassette *mec* (SCC*mec*) typing by using a published multiplex PCR methodology (38) and were found to be 84% SCC*mec* type IV (31; Wyeth Research, unpublished data); additionally, cluster analysis based on the ribotypes found that a high percentage of the SCC*mec* type IV isolates clustered with previously identified community-associated MRSA isolates (15, 30). The predominance of the SCC*mec* type IV element in these strains, which is devoid of non-β-lactam resistance determinants, suggests an explanation for the lower than expected levels of tetracycline resistance in the isolate collection.

Two additional tetracycline resistance genes have been reported in *S. aureus*, *tet*(L) and *tet*(O) (13), but they were not included in the PCR screening assays for this study. A recent study (51) of 600 tetracycline-resistant isolates (400 MRSA isolates and 200 MSSA isolates) from Europe failed to detect *tet*(O) in any isolate and found *tet*(L) in 1.5% of MRSA isolates but not at all in MSSA isolates. Whereas the incidence was low in European isolates, it is possible that these determinants may be present in the broader collection of *S. aureus* strains from this study and may contribute to tetracycline resistance in the clinic.

Tigecycline was developed to evade both the efflux and the ribosomal protection mechanisms of tetracycline resistance used by increasing numbers of bacterial pathogens. The present study represents the first prospective analysis to include tigecycline susceptibility data on MRSA isolates gathered in a clinical setting. This study is also noteworthy in that it provides details on both the genetic determinants and the ranges of MICs of *S. aureus* isolates in the population. Moreover, in contrast to previous analyses, the susceptibility data presented followed the recently approved reference methodology that instructs that MIC testing must use fresh media in order to generate accurate MIC data (9, 14, 39). The results of this study demonstrated that clinical isolates of *S. aureus* encoding *tet*(M) or *tet*(K), or both determinants, result in tigecycline MICs that were identical to those obtained with tetracycline-susceptible strains. It also demonstrated the utility of reengineering existing antimicrobial agents to evade the resistance mechanisms posed by bacterial pathogens. Moreover, with the broad-spectrum efficacy demonstrated by tigecycline in clinical trials and ongoing studies, this novel agent provides a much-needed therapeutic option for the clinic.

ACKNOWLEDGMENT

We thank Rosie Camarda for laboratory assistance.

REFERENCES

1. **Anstead, G. M., and A. D. Owens.** 2004. Recent advances in the treatment of infections due to resistant *Staphylococcus aureus*. Curr. Opin. Infect. Dis. **17:**549–555.
2. **Arbique, J., K. Forward, D. Haldane, and R. Davidson.** 2001. Comparison of the Velogene Rapid MRSA identification assay, Denka MRSA-Screen assay, and BBL Crystal MRSA ID system for rapid identification of methicillin-resistant *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **40:**5–10.
3. **Bell, J. M., and J. D. Turnidge.** 2002. High prevalence of oxacillin-resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY antimicrobial surveillance program, 1998–1999. Antimicrob. Agents Chemother. **46:**879–881.
4. **Biedenbach, D. J., M. L. Beach, and R. N. Jones.** 2001. In vitro antimicrobial activity of GAR-936 tested against antibiotic resistant gram-positive blood stream infection isolates and strains producing extended-spectrum β-lactamases. Diagn. Microbiol. Infect. Dis. **40:**173–177.
5. **Biedenbach, D. J., G. J. Moet, and R. N. Jones.** 2004. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997–2002). Diagn. Microbiol. Infect. Dis. **50:**59–69.
6. **Boucher, H. W., C. B. Wennersten, and G. M. Eliopoulos.** 2000. In vitro activities of the glycylcycline GAR-936 against gram-positive bacteria. Antimicrob. Agents Chemother. **44:**2225–2229.
7. **Bouchillon, S. K., D. J. Hoban, B. M. Johnson, T. M. Stevens, M. J. Dowzicky, D. H. Wu, and P. A. Bradford.** 2005. In vitro evaluation of tigecycline and comparative agents in 3049 clinical isolates: 2001 to 2002. Diagn. Microbiol. Infect. Dis. **51:**291–295.
8. **Bouchillon, S. K., B. M. Johnson, D. J. Hoban, J. L. Johnson, M. J. Dowzicky, D. H. Wu, M. A. Visalli, and P. A. Bradford.** 2004. Determining incidence of extended spectrum beta-lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS study 2001–2002. Int. J. Antimicrob. Agents **24:**119–124.
9. **Bradford, P. A., P. J. Petersen, M. Young, C. H. Jones, M. Tischler, and J. O'Connell.** 2005. Tigecycline MIC testing by broth dilution requires use of fresh medium or addition of the biocatalytic oxygen-reducing reagent oxyrase to standardize the test method. Antimicrob. Agents Chemother. **49:**3903–3909.
10. **Bradford, P. A., D. T. Weaver-Sands, and P. J. Petersen.** 2005. In vitro activity of tigecycline against isolates from patients enrolled in phase 3 clinical trials for complicated skin and skin structure infections and complicated intra-abdominal infections. Clin. Infect. Dis. **41**(Suppl. 5)**:**S315–S332.
11. **Breedt, J., J. Teras, J. Gardovskis, F. J. Maritz, T. Vaasna, D. P. Ross, M. Gioud-Paquet, N. Dartois, E. J. Ellis-Grosse, and E. Loh.** 2005. Safety and efficacy of tigecycline in the treatment of skin and skin structure infections: results of a double-blind phase 3 comparison study with vancomycin-aztreonam. Antimicrob. Agents Chemother. **49:**4658–4666.
12. **Centers for Disease Control and Prevention.** 2003. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2003, issued August 2003. Am. J. Infect. Control **31:**481–498.
13. **Chopra, I., and M. Roberts.** 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. **65:**232–260.
14. **Clinical Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing: M100-S15, fifteenth informational supplement, vol. 25. Clinical Laboratory Standards Institute, Wayne, Pa.
15. **Daum, R. S., T. Ito, K. Hiramatsu, F. Hussain, K. Mongkolrattanothai, M. Jamklang, and S. Boyle-Vavra.** 2002. A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds. J. Infect. Dis. **186:**1344–1347.
16. **Diekema, D. J., M. A. Pfaller, F. J. Schmitz, J. Smayevsky, J. Bell, R. N. Jones, and M. Beach.** 2001. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin. Infect. Dis. **32**(Suppl. 2)**:**S114–S132.
17. **Doern, G. V., R. N. Jones, M. A. Pfaller, K. C. Kugler, M. L. Beach, et al.** 1999. Bacterial pathogens isolated from patients with skin and soft tissue infections: frequency of occurrence and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 1997). Diagn. Microbiol. Infect. Dis. **34:**65–72.
18. **Fluit, A. C., A. Florijn, J. Verhoef, and D. Milatovic.** 2005. Presence of tetracycline resistance determinants and susceptibility to tigecycline and minocycline. Antimicrob. Agents Chemother. **49:**1636–1638.
19. **Fluit, A. C., M. E. Jones, F. J. Schmitz, J. Acar, R. Gupta, and J. Verhoef.** 2000. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY Antimicrobial Surveillance Program, 1997 and 1998. Clin. Infect. Dis. **30:**454–460.
20. **Gales, A. C., and R. N. Jones.** 2000. Antimicrobial activity and spectrum of the new glycylcycline, GAR-936 tested against 1,203 recent clinical bacterial isolates. Diagn. Microbiol. Infect. Dis. **36:**19–36.
21. **Hardy, K. J., P. M. Hawkey, F. Gao, and B. A. Oppenheim.** 2004. Methicillin resistant *Staphylococcus aureus* in the critically ill. Br. J. Anaesth. **92:**121–130.
22. **Hershow, R. C., W. F. Khayr, and P. C. Schreckenberger.** 1998. Ciprofloxacin resistance in methicillin-resistant *Staphylococcus aureus*: associated factors and resistance to other antibiotics. Am. J. Ther. **5:**213–220.
23. **Hiramatsu, K.** 1998. The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. Am. J. Med. **104:**7S–10S.
24. **Hiramatsu, K., N. Aritaka, H. Hanaki, S. Kawasaki, Y. Hosoda, S. Hori, Y. Fukuchi, and I. Kobayashi.** 1997. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet **350:**1670–1673.
25. **Holden, M. T., E. J. Feil, J. A. Lindsay, S. J. Peacock, N. P. Day, M. C. Enright, T. J. Foster, C. E. Moore, L. Hurst, R. Atkin, A. Barron, N. Bason, S. D. Bentley, C. Chillingworth, T. Chillingworth, C. Churcher, L. Clark, C. Corton, A. Cronin, J. Doggett, L. Dowd, T. Feltwell, Z. Hance, B. Harris, H.**

Hauser, S. Holroyd, K. Jagels, K. D. James, N. Lennard, A. Line, R. Mayes, S. Moule, K. Mungall, D. Ormond, M. A. Quail, E. Rabbinowitsch, K. Rutherford, M. Sanders, S. Sharp, M. Simmonds, K. Stevens, S. Whitehead, B. G. Barrell, B. G. Spratt, and J. Parkhill. 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. USA **101:**9786–9791.

26. **Jones, C. H., and P. J. Petersen.** 2005. Tigecycline: a review of preclinical and clinical studies of the first-in-class glycylcycline antibiotic. Drugs of Today **41:**637–659.
27. **Levy, S. B.** 2001. Antibiotic resistance: consequences of inaction. Clin. Infect. Dis. **33**(Suppl. 3)**:**S124–S129.
28. **Louie, L., S. O. Matsumura, E. Choi, M. Louie, and A. E. Simor.** 2000. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. **38:**2170–2173.
29. **Low, D. E., B. N. Kireiswirth, K. Weiss, and B. M. Willey.** 2002. Activity of GAR-936 and other antimicrobial agents against North American isolates of *Staphylococcus aureus*. Int. J. Antimicrob. Agents **20:**220–222.
30. **Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu.** 2002. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **46:**1147–1152.
31. **McAleese, F., E. Murphy, T. Babinchak, G. Singh, B. Said-Salim, B. Kreiswirth, P. M. Dunman, J. F. O'Connell, S. Projan, and P. A. Bradford.** 2005. Use of ribotyping to retrospectively identify methicillin-resistant *Staphylococcus aureus* isolates from phase 3 clinical trials for tigecycline that are genotypically related to community-associated isolates. Antimicrob. Agents Chemother. **49:**4521–4529.
32. **Milatovic, D., F.-J. Schmitz, J. Verhoef, and A. C. Fluit.** 2003. Activities of the glycylcycline tigecycline (GAR-936) against 1,924 recent European clinical bacterial isolates. Antimicrob. Agents Chemother. **47:**400–404.
33. **Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.).** 2003. Manual of clinical microbiology, 8th ed. ASM Press, Washington, DC.
34. **NCCLS.** 2003. Methods for dilution antimicrobial disk susceptibility tests; approved standards M2-A8, eighth ed., vol. 20. National Committee for Clinical Laboratory Standards, Wayne, Pa.
35. **NCCLS.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A6, sixth ed., vol. 23. National Committee for Clinical Laboratory Standards, Wayne, Pa.
36. **Nesin, M., P. Svec, J. R. Lupski, G. N. Godson, B. Kreiswirth, J. Kornblum, and S. J. Projan.** 1990. Cloning and nucleotide sequence of a chromosomally encoded tetracycline resistance determinant, *tetA*(M), from a pathogenic, methicillin-resistant strain of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **34:**2273–2276.
37. **Nicola, F., C. Bantar, L. F. Canigia, S. Relloso, H. Bianchini, and J. Smayevsky.** 2000. Comparison of several methods to determine methicillin-resistance in *Staphylococcus aureus* with focus on borderline strains. Diagn. Microbiol. Infect. Dis. **36:**91–93.
38. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:** 2155–2161.
39. **Petersen, P. J., and P. A. Bradford.** 2005. Effect of medium age and supplementation with the biocatalytic oxygen-reducing reagent oxyrase on in vitro activities of tigecycline against recent clinical isolates. Antimicrob. Agents Chemother. **49:**3910–3918.
40. **Petersen, P. J., P. A. Bradford, W. J. Weiss, T. M. Murphy, P. E. Sum, and S. J. Projan.** 2002. In vitro and in vivo activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate *Staphylococcus aureus* and other resistant gram-positive pathogens. Antimicrob. Agents Chemother. **46:**2595–2601.
41. **Petersen, P. J., N. V. Jacobus, W. J. Weiss, P. E. Sum, and R. T. Testa.** 1999. In vitro and in vivo antimicrobial activities of a novel glycylcycline, the 9-*t*-butylglycylamido derivative of minocycline (GAR-936). Antimicrob. Agents Chemother. **43:**738–744.
42. **Relman, D. A., T. M. Schmidt, R. P. MacDermott, and S. Falkow.** 1992. Identification of the uncultured bacillus of Whipple's disease. N. Engl. J. Med. **327:**293–301.
43. **Rennie, R. P., R. N. Jones, and A. H. Mutnick.** 2003. Occurrence and antimicrobial susceptibility patterns of pathogens isolated from skin and soft tissue infections: report from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 2000). Diagn. Microbiol. Infect. Dis. **45:** 287–293.
44. **Reynolds, R., N. Potz, M. Colman, A. Williams, D. Livermore, A. MacGowan, and BSAC Extended Working Party on Bacteraemia Resistance Surveillance.** 2004. Antimicrobial susceptibility of the pathogens of bacteraemia in the United Kingdom and Ireland 2001–2002: the BSAC Bacteraemia Resistance Surveillance Programme. J. Antimicrob. Chemother. **53:**1018–1032.
45. **Roberts, M. C.** 2003. Tetracycline therapy: update. Clin. Infect. Dis. **36:**462–467.
46. **Rybak, M. J., and R. L. Akins.** 2001. Emergence of methicillin-resistant *Staphylococcus aureus* with intermediate glycopeptide resistance: clinical significance and treatment options. Drugs **61:**1–7.
47. **Ryffel, C., W. Tesch, I. Birch-Machin, P. E. Reynolds, L. Barberis-Maino, F. H. Kayser, and B. Berger-Bachi.** 1990. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Gene **94:**137–138.
48. **Sacchidanand, S., R. L. Penn, J. M. Embil, M. E. Campos, D. Curcio, E. Ellis-Grosse, E. Loh, and G. Rose.** 2005. Efficacy and safety of tigecycline monotherapy compared with vancomycin plus aztreonam in patients with complicated skin and skin structure infections: results from a phase 3, randomized double-blind trial. Int. J. Infect. Dis. **9:**251–261.
49. **Sader, H. S., R. N. Jones, and J. B. Silva.** 2002. Skin and soft tissue infections in Latin American medical centers: four-year assessment of the pathogen frequency and antimicrobial susceptibility patterns. Diagn. Microbiol. Infect. Dis. **44:**281–288.
50. **Schmitz, F. J., A. C. Fluit, S. Brisse, J. Verhoef, K. Kohrer, and D. Milatovic.** 1999. Molecular epidemiology of quinolone resistance and comparative in vitro activities of new quinolones against European *Staphylococcus aureus* isolates. FEMS Immunol. Med. Microbiol. **26:**281–287.
51. **Schmitz, F. J., A. Krey, R. Sadurski, J. Verhoef, D. Milatovic, and A. C. Fluit.** 2001. Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates. J. Antimicrob. Chemother. **47:**239–240.
52. **Sum, P.-E., and P. Petersen.** 1999. Synthesis and structure-activity relationship of novel glycylcycline derivatives leading to the discovery of GAR-936. Bioorg. Med. Chem. Lett. **9:**1459–1462.
53. **Tenover, F. C., L. M. Weigel, P. C. Appelbaum, L. K. McDougal, J. Chaitram, S. McAllister, N. Clark, G. Killgore, C. M. O'Hara, L. Jevitt, J. B. Patel, and B. Bozdogan.** 2004. Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. Antimicrob. Agents Chemother. **48:**275–280.
54. **Tiemersma, E. W., S. L. Bronzwaer, O. Lyytikainen, J. E. Degener, P. Schrijnemakers, N. Bruinsma, J. Monen, W. Witte, and H. Grundman.** 2004. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. Emerg. Infect. Dis. **10:**1627–1634.
55. **Trzcinski, K., B. S. Cooper, W. Hryniewicz, and C. G. Dowson.** 2000. Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **45:**763–770.