AAC Journals.ASM.org

In Vivo Emergence of Tigecycline Resistance in Multidrug-Resistant *Klebsiella pneumoniae* and *Escherichia coli*

Teresa Spanu,[a] Giulia De Angelis,[b] Michela Cipriani,[b] Barbara Pedruzzi,[b] Tiziana D'Inzeo,[a] Maria Adriana Cataldo,[b]* Gabriele Sganga,[c] and Evelina Tacconelli[b]

Institute of Microbiology,[a] Infectious Diseases Unit,[b] and Department of Surgery,[c] Catholic University, Rome, Italy

Although resistance to tigecycline has been reported in surveillance studies, very few reports have described the emergence of resistance *in vivo*. We report two cases of patients with infections due to SHV-12-producing *Klebsiella pneumoniae* and *K. pneumoniae* carbapenemase-3 (KPC-3)-producing *Escherichia coli*, which developed tigecycline resistance *in vivo* after treatment. The reported limited experience underlines the risk of occurrence of a tigecycline MIC increase under treatment pressure.

Developed in 1993, tigecycline is a semisynthetic analogue of earlier tetracyclines and represents the first member of the glycylcyclines. It generally exhibits bacteriostatic activity against a wide spectrum of aerobic and anaerobic bacteria, including multidrug-resistant (MDR) organisms, but it consistently shows little or no activity against *Pseudomonas aeruginosa* (6, 8, 11).

Pharmacokinetic studies show that tigecycline produces relatively low mean steady-state serum concentrations of 0.4 to 0.6 mg/liter with an extensive volume of distribution (7, 18, 19, 33). Excretion is predominantly biliary and is renal only as a secondary pathway, accounting for up to 59% and 33% of the administered dose, respectively.

In 2005, tigecycline was approved by the Food and Drug Administration to treat complicated intra-abdominal (cIAI) and complicated skin and skin structure (cSSSI) infections and, in 2009, community-acquired bacterial pneumonia. The compound has shown equivalence to imipenem/cilastatin in cIAIs and to vancomycin plus aztreonam in cSSSIs (5, 21, 27). The antibiotic is a protein synthesis inhibitor able to evade the major determinants of tetracycline resistance, such as Tet(A)- to Tet(E)-mediated-efflux and ribosomal protection conferred by Tet(M), but it is a substrate for chromosomally encoded resistance-nodulation-division efflux pumps (10, 25).

Tigecycline-resistant strains have been detected since phase III trials (5, 21). Resistance in the *Enterobacteriaceae* appears to be mediated via upregulation of efflux pumps that are controlled by certain regulatory loci (16, 25). Increased expression of *marA*, a global activator that affects the expression of the AcrAB efflux system and the porin OmpF, has been found in clinical *Escherichia coli* isolates displaying decreased susceptibility to tigecycline (16), and another positive regulator of the AcrAB efflux system, *ramA*, was shown to be overexpressed in tigecycline-resistant strains of *Klebsiella pneumoniae* (25, 26).

The first cautionary report on the occurrence of bacteremia caused by tigecycline-nonsusceptible *Acinetobacter baumannii* in two patients receiving tigecycline appeared in 2007 (22). Since then, 13 other cases have been reported, involving mostly *A. baumannii* and *Klebsiella pneumoniae* (2, 3, 9, 12, 15, 20, 23, 24, 28, 30, 32). Recently, Rodríguez-Avial et al. reported the first detection of tigecycline resistance in SHV-12-producing *K. pneumoniae* during tigecycline treatment (24).

We report two cases of emergence of tigecycline resistance *in vivo* after treatment. *In vitro* antibiotic susceptibility profiling was performed with the Vitek-2 system (bioMérieux, Marcy l'Etoile, France), and the results were confirmed by Etest (bioMérieux) determination of MICs (4) in all cases. Tigecycline MICs were identified with the Sensititre broth microdilution method (Trek Diagnostic Systems, Cleveland, OH). All MICs were interpreted according to EUCAST breakpoints (http://www.eucast.org/clinical_breakpoints).

The first patient was a 55-year-old man, affected by Down syndrome, who developed a urinary tract infection (UTI) caused by tigecycline-resistant SHV-12-producing *K. pneumoniae.* He was admitted to the intensive care unit for respiratory failure secondary to aspiration pneumonia and intubated. He was placed on amoxicillin/clavulanate (1,200 g/8 h intravenously [i.v.]) and azithromycin (500 mg/24 h i.v.). After 4 days, the patient's clinical condition improved; he was extubated and then transferred to the clinical ward. On hospital day 14, he presented with fever, chills, and hypotension; the leukocyte count was 9.2×10^6 cells per liter, and the C-reactive protein (CRP) level was 169 mg/liter. Chest X-ray was negative. Empirical therapy was instituted with piperacillin-tazobactam (4,500 g/8 h i.v.). Blood cultures grew MDR *A. baumannii*. The strain retained susceptibility to colistin (MIC, 1 mg/liter) and tigecycline (MIC, 0.5 mg/liter). Urine cultures were positive for *K. pneumoniae* (isolate P1-KP1) susceptible to amikacin, carbapenems, levofloxacin, colistin, and tigecycline (Table 1). SHV-12 extended-spectrum β-lactamase (ESBL) was identified by PCR and sequencing (1). Colistin (100,000 U/kg of body weight/24 h i.v.) and tigecycline (100 mg for the first dose and then 50 mg/12 h i.v.) were administered for 14 days, with improvement of clinical conditions. Three days after the end of therapy, the patient complained of symptoms of UTI, and a tigecycline-resistant strain of *K. pneumoniae* was isolated from urine (isolate P1-KP2; MIC of 16 mg/liter by both Etest and broth microdilution

Received 31 January 2012 Returned for modification 26 February 2012
Accepted 19 May 2012
Published ahead of print 29 May 2012
Address correspondence to Evelina Tacconelli, etacconelli@rm.unicatt.it.
* Present address: Maria Adriana Cataldo, 2nd Infectious Diseases Division, National Institute for Infectious Diseases, Lazzaro Spallanzani, Rome, Italy.

doi:10.1128/AAC.00234-12

TABLE 1 Microbiological characteristics of *Klebsiella pneumoniae* and *Escherichia coli* isolates recovered before and after a tigecycline-containing regimen

Isolate[a]	Source	β-Lactamase gene	DL type[b]	MIC[c] (mg/liter)												
				AMK	GEN	AMC	FEP	CTX	CAZ	TZP	ERT	IPM	MEM	LVX	SXT	CST	TGC
P1-KP1	Urine	SHV-12	A	≤2	≥32	≥32	>64	8	>64	>128	0.12	0.12	0.03	≤0.12	≥320	0.5	0.5
P1-KP2	Urine	SHV-12	A	≤2	≥32	≥32	>64	8	>64	>128	0.12	0.12	0.03	≤0.12	≥320	0.5	16
P2-EC1	Liver aspirate	KPC-3	B	16	≥32	≥32	>64	>64	>64	>128	>32	4	8	≥8	≥320	0.5	0.5
P2-EC2	Blood	KPC-3	B	16	≥32	≥32	>64	>64	>64	>128	>32	4	8	≥8	≥320	0.5	0.5
P2-EC3	Rectal swab	KPC-3	B	16	≥32	≥32	>64	>64	>64	>128	>32	4	8	≥8	≥320	0.5	0.5
P2-KP1	Rectal swab	KPC-3	C	≥64	4	≥32	>64	>64	>64	>128	>32	>32	>32	≥8	≥320	≥16	0.5
P2-KP2	Urine	KPC-3	C	≥64	4	≥32	>64	>64	>64	>128	>32	>32	>32	≥8	≥320	≥16	0.5
P2-KP3	Blood	KPC-3	C	≥64	4	≥32	>64	>64	>64	>128	>32	>32	>32	≥8	≥320	≥16	0.5
P2-EC4	Blood	KPC-3	B	≥64	≥32	≥32	>64	>64	>64	>128	>32	>32	>32	≥8	≥320	0.5	24
P2-EC5	Liver aspirate	KPC-3	B	≥64	≥32	≥32	>64	>64	>64	>128	>32	>32	>32	≥8	≥320	0.5	24
P2-KP4	Blood	KPC-3	C	≥64	4	≥32	>64	>64	>64	>128	>32	>32	>32	≥8	≥320	≥16	0.5

[a] P1-KP, patient 1 *K. pneumoniae* isolates recovered before (KP1) or after (KP2) a tigecycline-containing regime; P2-EC, patient 2 *E. coli* isolates recovered before (EC1 to -3) or after (EC4 and -5) a tigecycline-containing regimen; P2-KP1 to -4, patient 2 *K. pneumoniae*.

[b] DL, DiversiLab microbial typing system.

[c] AMK, amikacin; GEN, gentamicin; AMC, amoxicillin-clavulanate; ERT, ertapenem; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; TZP, piperacillin-tazobactam; CST, colistin; TGC, tigecycline.

[Table 1]). The DiversiLab (DL) microbial typing system (bioMérieux) was used to characterize strain relatedness. Isolates with a similarity of >98% were considered indistinguishable (13). Strains KP1 and -2 had a unique DL type. Levofloxacin (500 mg/24 h i.v.) was administered for 8 days, with resolution of UTI symptoms, and the patient was discharged in good clinical condition.

The second case describes the first detection of tigecycline resistance in KPC-3 producing *Escherichia coli* during a tigecycline-containing treatment. A 56-year-old man underwent liver transplantation because of a hepatocarcinoma arisen on hepatitis B/D-induced liver cirrhosis. One month later, the patient was readmitted to the transplant unit with fever (39°C) and chills; the leukocyte count was 12.2×10^6 cells per liter (90% neutrophils), and the CRP value was 160 mg/liter. An abdominal computed tomography (CT) scan revealed multiple liver abscesses, with diameters ranging from a few millimeters to 6 cm. The largest abscess was subjected to continuous percutaneous drainage. Blood and abscess drainage fluid cultures yielded *E. coli* (isolates P2-EC1 and -2) susceptible to colistin and tigecycline only (Table 1). Surveillance rectal swab cultures (plated on MacConkey agar and ChromID ESBL plates; bioMérieux, La Balme-les-Grottes, France) yielded *E. coli* (isolate P2-EC3) and *K. pneumoniae* (isolate P2-KP1). All isolates from blood, abscess fluid, and rectal swab produced KPC-3 carbapenemases, as identified by PCR and sequencing analysis (1, 14) (Table 1). Empirical therapy with imipenem (1 g/6 h i.v.) was then switched to tigecycline (100 mg for the first dose and then 50 mg/12 h i.v.), with improvement of clinical condition and slight reduction of liver abscesses, as revealed by the CT scan. On day 12, the patient again developed fever (38.5°C) and chills. Blood and urine cultures grew *K. pneumoniae* (isolates P2-KP2 and -3) producing KPC-3 carbapenemases, susceptible to gentamicin and tigecycline (Table 1). Gentamicin (7 mg/kg/24 h i.v.) was added to tigecycline, with improvement of clinical conditions. On tigecycline-containing regimen day 21, a CT scan revealed enlargement of liver abscesses. Blood cultures and liver aspirates were performed, and after 48 to 72 h of incubation, tigecycline-resistant *E. coli* (isolate P2-EC4-5) grew. Pinpoint colonies grew from the abscess drainage fluid cultures. The heterogeneity of colonies' morphology suggested small-colony variants with no auxotrophism to hemin, menadione, or thymidine (29). All *E. coli* (isolates EC1 to -5) yielded a unique type with DL. The patient was switched to meropenem (2 g/8 h i.v.) and colistin (100,000 U/kg every 24 h i.v.), but he experienced a second episode of bacteremia, this time caused by a colistin-resistant, tigecycline-sensitive strain of *K. pneumoniae* (isolate P2-KP4; Table 1). He expired shortly thereafter of multiorgan failure.

In summary, this report illustrates the occurrence of a tigecycline MIC increase in SHV-12-producing *K. pneumoniae* and KPC-3-producing *E. coli* under treatment pressure (8). The mechanisms underlying this resistance have yet to be fully explored, but DL typing seems to indicate that tigecycline-resistant strains arose from the normal-phenotype parent strains isolated before tigecycline therapy. Our report suggests that first, in the *Enterobacteriaceae*, as has been reported for Gram-positive bacteria, development of resistance might be strictly related to antibiotic pressure (24, 31). Second, the development of resistance might be associated with unsuccessful microbiological eradication when tigecycline is administered for infections where microorganisms are exposed to subinhibitory concentrations of the drug, such as UTI, septicemia, and abdominal abscess (7, 12, 17, 23, 32). We therefore underline the importance of supporting international surveillance reporting on the susceptibility of members of the *Enterobacteriaceae* to tigecycline in parallel with clinical monitoring of the use of this antibiotic nationwide. Clinicians should be aware of the risk of emergence of tigecycline resistance in Gram-negative bacteria during drug exposure.

ACKNOWLEDGMENT

This work was supported by Università Cattolica del Sacro Cuore (Fondi Ateneo, Linea D1-2010).

REFERENCES

1. **Al Naiemi N, Schipper K, Duim B, Bart A.** 2006. Application of minimal sequence quality values prevents misidentification of the *bla*SHV type in single bacterial isolates carrying different SHV extended-spectrum β-lactamase genes. J. Clin. Microbiol. **44**:1896–1898.
2. **Al-Qadheeb NS, Althawadi S, Alkhalaf A, Hosaini S, Alrajhi AA.** 2010.

Evolution of tigecycline resistance in *Klebsiella pneumoniae* in a single patient. Ann. Saudi Med. **30**:404–407.
3. **Anthony KB, et al.** 2008. Clinical and microbiological outcomes of serious infections with multidrug-resistant gram-negative organisms treated with tigecycline. Clin. Infect. Dis. **46**:567–570.
4. **Bolmström A, et al.** 2007. Validation and reproducibility assessment of tigecycline MIC determinations by Etest. J. Clin. Microbiol. **45**:2474–2479.
5. **Breedt J, et al.** 2005. Safety and efficacy of tigecycline in 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.
6. **Cunha BA, McDermott B, Nausheen S.** 2007. Single daily high-dose tigecycline therapy of a multidrug-resistant (MDR) *Klebsiella pneumoniae* and *Enterobacter aerogenes* nosocomial urinary tract infection. J. Chemother. **19**:753–754.
7. **Curcio D.** 2008. Treatment of recurrent urosepsis with tigecycline: a pharmacological perspective. J. Clin. Microbiol. **46**:1892–1893.
8. **Daly MW, Riddle DJ, Ledeboer NA, Dunne WM, Ritchie DJ.** 2007. Tigecycline for treatment of pneumonia and empyema caused by carbapenemase-producing *Klebsiella pneumoniae*. Pharmacotherapy **27**:1052–1057.
9. **Daurel C, Fiant AL, Brémont S, Courvalin P, Leclercq R.** 2009. Emergence of an *Enterobacter hormaechei* strain with reduced susceptibility to tigecycline under tigecycline therapy. Antimicrob. Agents Chemother. **53**:4953–4954.
10. **Dean CR, Visalli MA, Projan SJ, Sum PE, Bradford PA.** 2003. Efflux-mediated resistance to tigecycline (GAR-936) in *Pseudomonas aeruginosa* PAO1. Antimicrob. Agents Chemother. **47**:972–978.
11. **Doan TL, Fung HB, Mehta D, Riska PF.** 2006. Tigecycline: a glycylcycline antimicrobial agent. Clin. Ther. **28**:1079–1106.
12. **Elemam A, Rahimian J, Mandell W.** 2009. Infection with panresistant *Klebsiella pneumoniae*: a report of 2 cases and a brief review of the literature. Clin. Infect. Dis. **49**:271–274.
13. **Fluit AC, et al.** 2010. Evaluation of the DiversiLab system for detection of hospital outbreaks of infections by different bacterial species. J. Clin. Microbiol. **48**:3979–3989.
14. **Gootz TD, et al.** 2009. Genetic organization of transposase regions surrounding bla_{KPC} carbapenemase genes on plasmids from *Klebsiella* strains isolated in a New York City hospital. Antimicrob. Agents Chemother. **53**:1998–2004.
15. **Gordon NC, Wareham DW.** 2009. A review of clinical and microbiological outcomes following treatment of infections involving multidrug-resistant *Acinetobacter baumannii* with tigecycline. J. Antimicrob. Chemother. **63**:775–780.
16. **Keeney D, Ruzin A, Bradford PA.** 2007. RamA, a transcriptional regulator, and AcrAB, an RND-type efflux pump, are associated with decreased susceptibility to tigecycline in *Enterobacter cloacae*. Microb. Drug Resist **13**:1–6.
17. **Livermore DM, et al.** 2011. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. Int. J. Antimicrob. Agents **37**:415–419.
18. **MacGowan AP.** 2008. Tigecycline pharmacokinetic/pharmacodynamic update. J. Antimicrob. Chemother. **62**(Suppl 1):i11–i16.
19. **Meagher AK, Ambrose PG, Grasela TH, Ellis-Grosse EJ.** 2005. The pharmacokinetic and pharmacodynamic profile of tigecycline. Clin. Infect. Dis. **41**(Suppl. 5):S333–S340.
20. **Neonakis IK, Stylianou K, Daphnis E, Maraki S.** 2011. First case of resistance to tigecycline by *Klebsiella pneumoniae* in a European university hospital. Indian J. Med. Microbiol. **29**:78–79.
21. **Oliva ME, et al.** 2005. A multicenter trial of the efficacy and safety of tigecycline versus imipenem/cilastatin in patients with complicated intra-abdominal infections. BMC Infect. Dis. **5**:88. doi:10.1186/1471-2334-5-88.
22. **Peleg AY, et al.** 2007. *Acinetobacter baumannii* bloodstream infection while receiving tigecycline: a cautionary report. J. Antimicrob. Chemother. **59**:128–131.
23. **Reid GE, Grim SA, Aldeza CA, Janda WM, Clark NM.** 2007. Rapid development of *Acinetobacter baumannii* resistance to tigecycline. Pharmacotherapy **27**:1198–1201.
24. **Rodríguez-Avial C, Rodríguez-Avial I, Merino P, Picazo JJ.** 2012. *Klebsiella pneumoniae*: development of a mixed population of carbapenem and tigecycline resistance during antimicrobial therapy in a kidney transplant patient. Clin. Microbiol. Infect. **18**:61–66.
25. **Ruzin A, Visalli MA, Keeney D, Bradford PA.** 2005. Influence of transcriptional activator RamA on expression of multidrug efflux pump AcrAB and tigecycline susceptibility in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **49**:1017–1022.
26. **Ruzin A, Immermann FW, Bradford PA.** 2008. Real-time PCR and statistical analyses of *acrAB* and *ramA* expression in clinical isolates of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **52**:3430–3432.
27. **Sacchidanand S, et al.** 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.
28. **Schafer JJ, Goff DA, Stevenson KB, Mangino JE.** 2007. Early experience with tigecycline for ventilator-associated pneumonia and bacteremia caused by multidrug-resistant *Acinetobacter baumannii*. Pharmacotherapy **27**:980–987.
29. **Sendi P, et al.** 2010. *Escherichia coli* variants in periprosthetic joint infection: diagnostic challenges with sessile bacteria and sonication. J. Clin. Microbiol. **48**:1720–1725.
30. **Stone NR, et al.** 2011. Breakthrough bacteraemia due to tigecycline-resistant *Escherichia coli* with New Delhi metallo-β-lactamase (NDM)-1 successfully treated with colistin in a patient with calciphylaxis. J. Antimicrob. Chemother. **66**:2677–2678.
31. **Tacconelli E, et al.** 2009. Antibiotic usage and risk of colonization and infection with antibiotic-resistant bacteria: a hospital population-based study. Antimicrob. Agents Chemother. **53**:4264–4269.
32. **Werner G, Gfrörer S, Fleige C, Witte W, Klare I.** 2008. Tigecycline-resistant *Enterococcus faecalis* strain isolated from a German intensive care unit patient. J. Antimicrob. Chemother. **61**:1182–1183.
33. **Wyeth Pharmaceuticals. Tygacil (package insert).** 2005. Wyeth Pharmaceuticals. Philadelphia, PA.