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

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Running title
Emergence of tigecycline resistance under treatment.

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Although resistance to tigecycline has been reported in surveillance studies, a very few reports described the emergence of resistance \textit{in vivo}. We report two cases of patients with infections due to SHV-12 producing \textit{Klebsiella pneumoniae} and \textit{K. pneumoniae} carbapenemase-3 (KPC-3) producing \textit{Escherichia coli}, which developed tigecycline resistance \textit{in vivo} after treatment. The reported limited experience underlines the risk of occurrence of tigecycline MIC increase under treatment pressure.
Developed in 1993, tigecycline is a semisynthetic analogue of earlier tetracyclines and represents the first member of glycyccyclines. 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 (7,18,19,32). Excretion is predominantly biliary and renal only as a secondary pathway, accounting up to 59% and 33% of 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–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 *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 *E. 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 *K. pneumoniae* (25, 26).

The first cautionary report on occurrence of bacteremia caused by tigecycline-non-susceptible *Acinetobacter baumannii* in two patients receiving tigecycline appeared in 2007 (22). Since then, other 13 cases have been reported, involving mostly *A. baumannii* and *Klebsiella pneumoniae* (2,3,9,12,15,20,23, 24, 28, 30, 33). Recently, Rodriguez-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 is 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 [1200 g/8 h intravenously (IV)] and azithromycin (500 mg/24h IV). After four days the patient’s clinical condition improved, he was extubated and then transferred to the floor. On hospital day 14, he presented with fever, chills and hypotension; leukocyte count was 9.2 x 10⁶ cells per liter and C-reactive protein (CRP) level was 169 mg/L. Chest X-ray was negative. Empirical therapy was instituted with piperacillin/tazobactam (4500 g/8 h IV). 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 AB-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 (100000 U/kg/24h IV) and tigecycline (100 mg for the first dose and then 50 mg/12h IV) were administered for 14 days with improvement of clinical conditions. Three days after ending of therapy, the patient complained of symptoms of UTI and a tigecycline-resistant strain of *K. pneumoniae* was isolated from urine (isolate AB-KP2, MICs, 16 mg/liter by both Etest and broth microdilution; Table 1). DiversiLab (DL) Microbial Typing System (bioMérieux) was used to characterise strain relatedness. Isolates with similarity of > 98% were considered indistinguishable (13). Strains KP1 and 2 had an unique DL type. Levofloxacin (500 mg/24h IV) was administered for 8 days with resolution of UTI symptoms and the patient was discharged in good clinical conditions.

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 an hepatocarcinoma arisen on hepatitis B/D-induced liver cirrhosis. One month later, the patient was readmitted to Transplant Unit with fever (39°C) and chills; leukocyte count was 12.2 x 10⁶ cells per liter (90% neutrophils) and CRP value was 160 mg/L. Abdominal computed tomography (CT) scan revealed multiple liver abscesses, with diameter ranging from a few millimetres to 6 cm. The largest abscess was subjected to continuous percutaneous drainage. Blood and abscess drainage fluid cultures yielded *E. coli* (isolate JC-EC1 and 2) susceptible to colistin and tigecycline only (Table 1). Surveillance rectal swab cultures (plated onto MacConkey agar and ChromID ESBL plates, bioMérieux, La Balme-les-Grottes, France) yielded *E. coli* (isolate JC-EC3) and *K. pneumoniae* (isolate JC-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 (1g/6h IV) was then switched to tigecycline (100 mg for the
first dose and then 50 mg/12h IV) with improvement of clinical condition and slight reduction of liver abscesses, as revealed by the CT scan. On day 12, the patient developed again fever (38.5°C) and chills. Blood and urine cultures grew *K. pneumoniae* (isolates JC-KP2-3) producing KPC-3 carbapenemases, susceptible to gentamicin and tigecycline (Table 1). Gentamicin (7 mg/kg/24h IV) was added to tigecycline with improvement of clinical conditions. On tigecycline-containing regimen day 21 CT scan revealed enlargement of liver abscesses. Blood cultures and liver aspirates were performed and, after 48–72 hours of incubation, tigecycline-resistant *E. coli* (isolate JC-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 EC1-5) yielded a unique type with DL. The patient was switched to meropenem (2 g/8 h 8h IV) and colistin (100,000 U/kg every 24h IV), but he experienced a second episode of bacteremia, this time caused by a colistin-resistant, tigecycline-sensitive strain of *K. pneumoniae* (isolate JC-KP4; Table 1). He expired shortly thereafter of multiorgan failure.

In summary, this report illustrates the occurrence of 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 to be fully explored yet, 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 of all, in *Enterobacteriaceae* as well as 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 to the unsuccessful microbiological eradication when tigecycline is administered for infections where microorganisms are exposed to sub-inhibitory concentration of the drug, such as UTI, septicemia and abdominal abscess (7,12,17, 23,33). We therefore underline the importance to support international surveillance reporting on the susceptibility of *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.

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Potential conflict of interest. All authors: no conflict.
REFERENCES


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<sup>a</sup> AB-KP, patient 1-<i>K. pneumoniae</i> isolates recovered before (KP1) and after tigecycline-containing regime (KP2); JC-EC, patient 2-<i>E. coli</i> isolates recovered before (EC1-3) and after tigecycline-containing regimen (EC4-5); JC-KP 1-4, patient 2-<i>K. pneumoniae</i>.

<sup>b</sup> DL, DiversiLab (DL) Microbial Typing System.
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; and TGC,