GenBank accession number

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Transparency declarations

None to declare.

References

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sequenced and share >99% nucleotide sequence identity with tet(39) from Acinetobacter sp. LUHS605 (AY743590.1) (data not shown). The two P. rettgeri (Q52 and Q61), the Pseudomonas putida (O205) and the Stenotrophomonas sp. (C8) were tet(39) negative. Previously both P. rettgeri and Stenotrophomonas maltophilia, from a polluted river, were reported to be tet(39) positive.8 None of the isolates tested in the study were positive for either the tet(W) gene or the tet(X) gene.

In the first study the mobilities of L7, O193, O213, O205 and O61 were tested using agar mating methods as previously described (Table 1).2 In this study, the mobilities of isolates CH90 and O275 were tested to determine their ability to transfer their tet(39) gene to a HB101 Escherichia coli recipient as previously described.2 We were unable to show transfer of the tet(39) gene for either donor, although the rate of transfer could be <1×10⁻¹⁰ per recipient (Table 1). However, a previous report showed the tet(39) gene on transferable plasmids in 10 of 11 Acinetobacter spp. isolates.6 However the previous report used Acinetobacter spp. recipients, while we used E. coli, which could explain the differences in results.2,6

In the current study, the six tetracycline-resistant tet(39)-positive isolates represented four different genera, of which three, Corynebacterium, Pseudomonas and Psychrobacter, had not been previously identified as carrying the tet(39) gene. The tet(39) gene was as common (n=6) as the tet(A) gene was in the original study of 25 tetracycline-resistant Chilean fish farm isolates. The tet(39) gene was first identified in Acinetobacter spp. samples from Europe between 1986 and 1997.6–10 Since then the tet(39) gene has been identified in fish farms in Thailand, a polluted river in Nigeria and clinical samples in the USA.8–10 This study illustrates that the tet(39) gene has been present in Chilean bacteria since 1999 and has now been identified on four continents, in four different Gram-positive genera and eight Gram-negative genera. This makes the tet(39) gene the eighth most commonly found among the 29 characterized tet efflux genes and the 12th most common among all 45 currently identified tet genes.6 Most of the tet(39)-positive genera have been isolated from water sources, including aquaculture, suggesting that these resistant bacteria have the potential to contaminate the food chain and ultimately impact humans.

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Mutant prevention concentration of tigecycline for Acinetobacter baumannii and Klebsiella pneumoniae clinical isolates

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Sir,
Tigecycline is a therapeutic option for multidrug-resistant Acinetobacter baumannii and Klebsiella pneumoniae infections.1 Recently, tetracycline-non-susceptible A. baumannii and K. pneumoniae have been reported in patients receiving tigecycline.2 Despite several reports concerning in vitro and in vivo susceptibility of bacterial pathogens to tigecycline, there is a paucity of data regarding its effect on the development of resistant single-step mutants within the mutant selection window (MSW) during clinical tigecycline dosing.3

In this study, we investigated the mutant prevention concentrations (MPCs) of tigecycline along with the effect of efflux pumps such as AdeABC and AcrAB-ToIC on the emergence of tigecycline-resistant mutants in A. baumannii and K. pneumoniae, respectively.

Thirty A. baumannii and 30 K. pneumoniae isolates (15 colistin-resistant and 15 colistin-susceptible, respectively), obtained from six Korean hospitals, were investigated. The MICs of tigecycline were determined by a broth microdilution method according to CLSI 2013 guidelines.4 The clinical isolates of A. baumannii and K. pneumoniae were considered susceptible to tigecycline at breakpoints of 1 mg/L according to EUCAST.3 The MPC of tigecycline was determined as described previously and is defined as the lowest antimicrobial concentration at which the growth of colonies on an agar plate is completely inhibited after 24 h of incubation.5 Single-step mutants were selected by plating the wild-type strain onto LB agar plates containing tigecycline within the concentration range of the MSW (0.5–4 mg/L). Amino acid substitutions in the AdeABC efflux pump system or the AcrAB-ToIC efflux pump system and their respective local repressor genes, such as adeRS or acrR, were examined in single-step mutants of five A. baumannii and K. pneumoniae isolates.5–8 The expression levels of adeB or acrA were also investigated using quantitative real-time PCR in seven pairs of isogenic A. baumannii and K. pneumoniae strains, respectively.

The MICs for A. baumannii and K. pneumoniae isolates ranged from 0.5 to 2 mg/L and from 0.5 to 1 mg/L, respectively. For A. baumannii isolates, tigecycline MPCs ranged from 1 to 4 mg/L (Table 1) and the MPC/MIC ratio was in the range of 4–16. Higher MPC values (≥8 mg/L) were observed only in four colistin-resistant A. baumannii isolates, which suggests that careful consideration should be given to use tigecycline as an alternative for the treatment of colistin-resistant A. baumannii infections. With one exception, in all other K. pneumoniae isolates the tigecycline MPCs were between 4 and 16 mg/L and the MPC/MIC ratio was in the range 4–16. The MPCs and MIC/MIC for K. pneumoniae isolates were unaffected by their colistin susceptibility. Tigecycline MICs for all single-step mutants exhibited a 2- to 16-fold increase compared with the parental isolates of both A. baumannii and K. pneumoniae. A similar phenomenon was described previously for fluoroquinolones against Escherichia coli.9 adeABC and adeRS were not detected in some of the seven isolates of A. baumannii under investigation. adeABC and adeRS was not identified in 07AC-029. adeC was identified in only three isolates: E07–612, E10–93 and H09–504. Four isolates harboured adeRS (Table 1). Although the AcrAB-ToIC efflux pump and acrR were identified in all seven K. pneumoniae isolates, no amino acid alterations were found. Quantitative real-time PCR analysis revealed that the expression of adeB or acrA in single-step mutants of A. baumannii and K. pneumoniae was different between isolates that had high and low MPCs (Table 1). Compared with their parental isolates, in A. baumannii the expression of adeB in single-step mutants with MPC of ≥4 mg/L or MPC/MIC of ≥8 was 2.4- to 71.6-fold higher. However, a single-step mutant, E07–612 (MPC/MIC, 2), showed no increase in adeB expression. 07AC–029, which possessed no AdeABC efflux pump, showed a low MPC and a low MPC/MIC. 06AC–108, with no adeRS, showed a very high expression level of adeB, and adeC may affect the increased adeB expression in E10–93 and H09–504, compared with 06AC–23 and 06AC–66. However, further studies may be required. Five out of the seven single-step mutants of K. pneumoniae, which had MPCs ≥8 mg/L or MPC/MIC ratios ≥8, showed a 3.3- to 17.2-fold increase in acrA expression compared with their parental isolates. In the other two single-step mutants, K01–08–10058 and LJA (MPC/MIC, 2 and 4, respectively), acrA expression increased by only 1.7-fold.

These results are consistent with the notion that up-regulation of efflux pumps is associated with tigecycline resistance in clinical isolates.4,7 A plasma Cmax of 0.87 mg/L tigecycline in the recommended dose regimen (loading dose of 100 mg followed by multiple doses of 50 mg every 12 h) is within the MSW in most