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Relationship between autolysis and teicoplanin activity against *Staphylococcus epidermidis*

Sir,

Reduced autolytic activity is a common feature of vancomycin-intermediate *Staphylococcus aureus* (VISA) strains and may precede the VISA state [1]. However, the relationship between autolysis and susceptibility to glycopeptide agents is poorly documented for coagulase-negative staphylococci (CoNS). Decreased susceptibility to teicoplanin is more frequent than decreased susceptibility to vancomycin amongst clinical isolates of *Staphylococcus epidermidis* [2]. The objectives of this study were to assess the relationship between rate of autolysis and the minimum inhibitory concentration (MIC) of teicoplanin amongst unrelated clinical strains of *S. epidermidis* and to determine whether inactivation of the *atlE* gene, encoding the *S. epidermidis* major autolysin, affects the activity of teicoplanin.

Ten clinical strains of *S. epidermidis* were selected to cover the range of teicoplanin MICs commonly observed in clinical isolates. These strains were not clonally related as shown by pulsed-field gel electrophoresis (PFGE) following restriction of DNA with *Sma*I (data not shown). Wild-type *atlE*(+) O-47 *S. epidermidis* and its Tn*917* insertional mutant *atlE*(−) *O-47*mut1, respectively named *atlE*(+) and *atlE*(−), were also studied [3]. MICs of teicoplanin were determined by agar dilution. Triton-induced autolysis was quantified as a percentage of the initial optical density at 620 nm (%OD) after 4 h [4]. Bactericidal activity of serial concentrations of teicoplanin was assessed in Mueller–Hinton broth as previously described [4]. The relationship between the teicoplanin concentration (C) and bacterial killing (K), defined as the variation in bacterial counts between 0 h and 24 h, was fitted using the Hill equation: $K = E_{max} + \{(E_{min} - E_{max})/[1 + 10^{(\log EC_{50} - C) \times n_H}]\}$. In this model, E_{min} and E_{max} are, respectively, the minimal and maximal effects; negative and positive values of these parameters indicate, respectively, bacterial killing and bacterial growth. Log EC_{50} is the log of the concentration that produces a half-maximal response and n_H is the Hill slope [4]. All experiments were performed in triplicate.

Neutropenic mice were injected intraperitoneally with an inoculum of 7 $\log_{10}$ colony-forming units (CFU)/mouse, which achieved a 100% mortality rate in control mice [4]. Antibiotics were administered subcutaneously immediately after inoculation as well as 4 h and 8 h later. The median effective dose (ED_{50}) was assessed at Day 6.

MICs of teicoplanin for clinical strains #1 to #10 were, respectively, 0.25, 0.5, 0.5, 2, 2, 4, 4, 8, 8 and 8 mg/L. MICs of teicoplanin were 0.5 mg/L for strains *atlE*(+) and *atlE*(−). Following exposure to Triton, %OD of strains #1 to #10 were, respectively, 20 ± 4, 18 ± 9, 10 ± 2, 21 ± 5, 23 ± 16, 12 ± 2, 13 ± 5, 31 ± 9, 10 ± 5 and 29 ± 7. There was no significant correlation between the %OD and the teicoplanin MIC amongst clinical isolates (Spearman correlation coefficient $r = 0.21$; $P = 0.56$). %OD following Triton exposure for strains *atlE*(+) and *atlE*(−) were, respectively, 34 ± 8 and 89 ± 1.

In the bactericidal activity experiment, best-fit values ± standard error (S.E.) of E_{max}, log EC_{50} and Hill slope for strain *atlE*(+) were, respectively, -2.1 ± 0.2 log CFU/mL, 0.4 ± 0.1 and -1.9 ± 0.6 (Fig. 1). Best-fit values ± S.E. of E_{max}, log EC_{50} and Hill slope for strain *atlE*(−) were, respectively, -1.7 ± 0.3 log CFU/mL, 0.7 ± 0.2 and -1.2 ± 0.6. Globally, the three parameters were significantly different ($P = 0.009$), indicating lower bactericidal activity of teicoplanin against the mutant strain. ED_{50} values of *atlE*(+) and *atlE*(−) strains were, respectively, 2 mg/kg and 8 mg/kg in the mouse sepsis/peritonitis model.

There was high interstrain variation of autolysis rates between unrelated clinical strains of *S. epidermidis*, including teicoplanin-susceptible strains. This phenomenon has been observed amongst

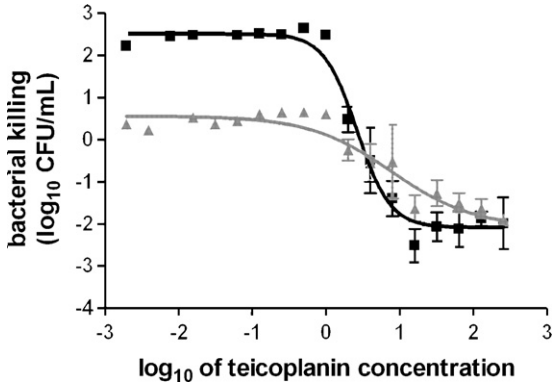

Fig. 1. Dose–response curves of the bactericidal activity of teicoplanin against a wild-type *atlE*(+) *Staphylococcus epidermidis* (■) and its *atlE*(−) counterpart (▲), showing the change in log CFU/mL after 24 h incubation in broth. Data are plotted as a function of log drug concentration in mg/L. CFU, colony-forming units.

teicoplanin-resistant CoNS by Sieradzki et al. [5]. Interestingly, the present strains with lower teicoplanin MICs showed autolysis rates varying from 10% to 20%, which were similar to autolysis rates of strains with higher MICs. As *atlE*(+) and *atlE*(−) strains had similar virulence in the mouse sepsis/peritonitis, this model was well suited to study the activity of teicoplanin against these strains [4]. Decreased activity of teicoplanin against the *atlE*(−) strain in the sepsis/peritonitis model, shown by the four-fold increase in ED_{50}, contrasts with what we previously observed with vancomycin, hence highlighting that these two glycopeptide antibiotics have different pharmacodynamic properties [4].

The MIC of teicoplanin fails to predict its activity against *S. aureus* in the rabbit endocarditis model and against *Streptococcus pneumoniae* in the mouse sepsis/peritonitis [6,7]. Similar observations were made with *S. epidermidis* in the mouse sepsis/peritonitis (unpublished data). The present work raises the hypothesis that reduced autolysis may at least partly explain low in vivo activity of teicoplanin against some teicoplanin-susceptible strains of *S. epidermidis*. In conclusion, there is no correlation between autolysis rate and the MIC of teicoplanin against clinical strains of *S. epidermidis*. Inactivation of *atlE* did not alter the MIC of teicoplanin, but it did decrease the bactericidal activity of teicoplanin and its activity in the mouse sepsis/peritonitis model.

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Emergence of carbapenem resistance due to porin loss in an extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* strain during meropenem therapy

Sir,

Here we report the emergence of carbapenem resistance in an extended-spectrum β-lactamase-producing strain of *Klebsiella pneumoniae* (ESBL-KP) owing to loss of outer membrane proteins (OMPs) (or porins). Emergence of resistance occurred during prolonged treatment with meropenem and in the absence of an apparent focus of infection.

The patient was a 49-year-old male in a Haematology Unit undergoing chemotherapy for progressive diffuse large B-cell lymphoma. He had an episode of neutropenic fever treated initially with piperacillin/tazobactam and gentamicin, during which an ESBL-KP was isolated from blood cultures (Isolate 1a). Antibiotic therapy was changed to meropenem (500 mg four times a day). Following initial improvement, the patient became persistently febrile despite normalisation of his neutrophil count and treatment with meropenem, vancomycin and amphotericin. Sixteen sets of blood cultures obtained whilst he was receiving meropenem over the next 26 days were sterile, before a carbapenem-resistant *K. pneumoniae* (Isolate 1b) was cultured from blood (with a vancomycin-resistant *Enterococcus* spp.) during a second period of neutropenia. The patient was treated with ciprofloxacin and linezolid that led to microbiological cure, although death occurred 25 days later due to progressive haematological malignancy.

Minimum inhibitory concentrations (MICs) for the pre- and post-therapy isolates (Isolates 1a and 1b) were determined by British Society for Antimicrobial Chemotherapy (BSAC) methodology. Isolate 1a was susceptible to meropenem, imipenem and ertapenem and demonstrated ESBL activity, with relative MICs of cefotaxime and ceftazidime consistent with a CTX-M-type enzyme, which was confirmed by polymerase chain reaction (PCR) for ESBL genes. In contrast, Isolate 1b was fully resistant to all three carbapenems tested. Pulsed-field gel electrophoresis (PFGE) showed that both isolates belonged to the same strain, suggesting that the carbapenem-resistant isolate was derived from the original susceptible isolate and did not represent a superinfecting second strain. Isolate 1b was negative by PCR for genes encoding KPC and OXA-48 carbapenemases and its antibiogram was inconsistent with metallo-β-lactamase production. Rather, loss of synergy between oxyimino-cephalosporins and clavulanic acid was demonstrated, consistent with the emergence of resistance through impermeability owing to porin loss. Analysis of OMPs confirmed that Isolate 1b did not express a full complement of porins compared with Isolate 1a or with other carbapenem-susceptible *K. pneumoniae* isolates (Fig. 1). A band of ca. 40 kDa was absent on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) from Isolate 1b that was present in Isolate 1a and in the carbapenem-susceptible control isolates. This same band, which is likely to represent co-migration of OmpK35 and OmpK36, was absent in a known porin-deficient *K. pneumoniae* strain (Fig. 1).

Carbapenem antibiotics are regarded as a good last line of defence in the treatment of resistant Gram-negative infections such as those caused by Enterobacteriaceae producing ESBLs. Emergence of resistance to these agents leaves few therapeutic options and