months, clinical symptoms of infection reappeared and work-ups, no secondary neurological, digestive or haematological started to diminish and, after 17 days, cultures of the exudate were identified in samples of articular fluid from the periprosthetic tissue. Staphylococcus lugdunensis was once again isolated. Treatment was reinitiated with linezolid at a dose of 600 mg every 12 h. In the first few days of treatment the exudate was infused with abundant purulent secretion. The abscess ultimately received an iliac allograft. Three years later he received a right iliac replacement due to failure of the allograft. During this period, chemotherapy had caused acetabular fracture associated with hip dislocation and initially received an iliac allograft. Three years later he received a right iliac replacement due to failure of the allograft. During this time, radiotherapy and chemotherapy cycles continued because of spread of the neoplastic disease to other parts of the body. Ten years later, septic loosening of the prosthesis was detected. Staphylococcus lugdunensis sensitive to oxacillin, ciprofloxacin, trimethoprim/sulfamethoxazole, rifampicin and linezolid was identified in samples of articular fluid from the periprosthetic tissue. Re-implantation was ruled out due to the patient's condition. Surgical debridement was performed and antibiotic therapy was started with ciprofloxacin and rifampicin. The patient showed no clinical improvement and it proved impossible to eradicate the bacteria. The course of infection was complicated by the appearance of a fistulous tract with abundant purulent secretion. The abscess was drained surgically on several occasions and despite various courses of antibiotics with ciprofloxacin and rifampicin at standard doses lasting 21 days the infection persisted.

It was decided to initiate treatment with linezolid at a dose of 600 mg every 12 h. In the first few days of treatment the exudate started to diminish and, after 17 days, cultures of the exudate were sterile. During follow-up of the patient, including weekly blood work-ups, no secondary neurological, digestive or haematological effects were detected. Importantly, at no time did the platelet count drop below 120 000/mL. After 3 months of treatment, linezolid was withdrawn in the face of clinical and microbiological cure. After 6 months, clinical symptoms of infection reappeared and S. lugdunensis was once again isolated. Treatment was reinitiated with linezolid with slow improvement. After 1 year of antibiotics, the patient was finally cured. During this interval, no side effects were reported.

In the setting of deep prosthetic joint infections, one of the most widely used regimens of antibiotics is a combination of ciprofloxacin and rifampicin, although failure rates are high. Our patient failed therapy with ciprofloxacin and rifampicin, even though cultures of articular fluid were susceptible to these antibiotics. Linezolid, which acts by inhibiting bacterial protein synthesis at an early stage, is thought to have high penetration into bone and joint tissues [5], prompting some authors to try linezolid in patients with deep prosthetic joint infections [4].

A major issue with linezolid, particularly in long-term treatment lasting more than 28 days, is myelosuppression, which has been linked to certain haematological events such as thrombocytopenia and anaemia [6]. Our patient was at particular risk of such events given the presence of haematological disease for which he was receiving chemotherapy, making haematological safety of long-term therapy a particular concern and close vigilance was necessary to detect abnormal blood counts early.

Of particular note in this case was the extremely long duration of treatment (1 year). Previously, the longest duration of treatment reported was 37 weeks, in a patient in the series reported by Garazzino et al. [7]. During treatment of our patient, no haematological abnormalities were detected and the platelet count was never <120 000/mL.

In conclusion, the case reported here illustrates that long-term administration of linezolid for up to 1 year can be safe despite the presence of risk factors for adverse haematological events. The anecdotal evidence of this case would also suggest that linezolid is more effective than the conventional combination of ciprofloxacin and rifampicin in the treatment of prosthetic joint infections despite similar in vitro susceptibility, probably because of better penetration into the target tissue in the case of linezolid.

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**Influence of the AtlE autolysin on the activity of cell wall-active agents against Staphylococcus epidermidis**

Sir,

Two autolysins, AtlE and Aae, have been identified in Staphylococcus epidermidis [1,2]. AtlE shares 61% homology with Staphylococcus aureus Atl, which is involved in penicillin-induced bacterial lysis [1,3]. The role of AtlE in the killing of S. epidermidis

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by cell wall-active agents has not been described. The objective of this work was to study the impact of the inactivation of atlE on the activities of cloxacillin and vancomycin against *S. epidermidis*.

Two isogenic strains of *S. epidermidis* were used, a wild-type *atlE(+) O-47* strain and its Tn917 insertion mutant *atlE(−) O-47mut1* [1]. Overnight cultures were diluted to an optical density at 620 nm of 0.005 in trypticase soy broth and were incubated at 37 °C with agitation. Samples were collected at 1-h intervals for the first 9 h. Growth rates were estimated using curve fitting with the Gompertz model.

Minimum inhibitory concentrations (MICs) of vancomycin and cloxacillin were determined by the agar dilution method. The bactericidal activities of serial concentrations of vancomycin and cloxacillin were assessed in Mueller–Hinton broth in triplicate. Following 24 h incubation at 37 °C, cultures were diluted and plated. The Hill equation with variable slope was used to fit the relationship between the log10 of antibiotic concentration (C) and bacterial killing defined as the variation in bacterial counts in 24 h (\(\Delta V\)) [expressed as log colony-forming units (CFU)/mL]. The equation is:

\[
V = \frac{E_{\text{max}} + (E_{\text{min}} - E_{\text{max}})}{1 + 10^{\log EC_{50} - C} \text{HillSlope}},
\]

where the maximal effect, \(E_{\text{max}}\), is a negative number expressing the maximal bactericidal killing and \(EC_{50}\) is the median effective concentration.

Neutropenia was induced in female Swiss mice weighing 20 g by intraperitoneal injection of 150 mg/kg cyclophosphamide on Days 5, 3 and 1 before inoculation. Inocula ranging from 3 log10 CFU to 8 log10 CFU of *S. epidermidis* were injected intraperitoneally. The median lethal dose (LD50) was assessed at Day 6.

Cyclophosphamide-treated mice were injected intraperitoneally with an inoculum of 7 log10 CFU/mouse. Antibiotics (0.1, 0.3, 1, 3, 10, 30 and 100 mg/kg) were administered subcutaneously immediately after inoculation as well as 4 h and 8 h later. For each strain, eight mice were inoculated at each dose level. The median effective dose (ED50) was assessed at Day 6.

Mean maximum growth rates (standard error (S.E.)) of the *atlE(+) and atlE(−)* strains were 0.470 h\(^{-1}\) (0.077 h\(^{-1}\) and 0.476 h\(^{-1}\) (0.022 h\(^{-1}\), respectively (\(P = 0.9\)). MICs of cloxacillin for the *atlE(+) and atlE(−) strains were 0.25 mg/L and 0.5 mg/L, respectively; the MIC of vancomycin was 1 mg/L for both strains. Dose–response curves of the bactericidal activity of cloxacillin and vancomycin are shown in Fig. 1. Maximal bacterial killing in broth \((E_{\text{max}} \pm \text{S.E.})\) against the *atlE(+) and atlE(−) strains was, respectively, \(-2.74 \pm 0.14\) log CFU/mL and \(-1.22 \pm 0.07\) log CFU/mL for cloxacillin \((P < 0.0001)\) and \(-3.34 \pm 0.15\) log CFU/mL and \(-1.16 \pm 0.14\) log CFU/mL for vancomycin \((P = 0.0087)\).

Median lethal doses of *atlE(+) and atlE(−) strains were 4.3 log10 CFU/mouse and 4.7 log10 CFU/mouse, respectively. All mice died at the 6-day follow-up for inocula >6 log10 CFU/mouse. The ED50 values of cloxacillin for the *atlE(+) and atlE(−) strains were, respectively, 226 mg/kg and 205 mg/kg and those of vancomycin were 9 mg/kg and 6 mg/kg.

Although inactivation of *atlE* did not alter the MICs of cloxacillin and vancomycin, it was associated with decreased bactericidal activity of these antibiotics. Similarly, inactivation of the *S. aureus atl* gene is associated with decreased penicillin-induced lysis [3]. Furthermore, inactivation of the genes of the *S. aureus* transcriptional regulators SarA and Mgra enhances Triton- and oxacillin-induced lysis, whilst inactivation of *S. aureus* ciaA decreases both autolysis and the bactericidal activity of vancomycin [4,5]. Finally, downregulation of *S. aureus* gcp decreases autolysis as well as penicillin- and vancomycin-induced lysis [6].

The sepsis/peritonitis model in neutropenic mice is an infrequently used model of *S. epidermidis* infection. The fact that the *atlE(+) and atlE(−)* strains exhibited similar virulence in this model was its major advantage. In contrast, the loss of *atlE* was associated with a dramatically decreased virulence in a rat central venous catheter infection model owing to the role of AtlE in the initial adherence of *S. epidermidis* to polymer surfaces [7]. The mouse peritonitis model offered the unique opportunity to assess the efficacy of cloxacillin and vancomycin against the *atlE(+) and atlE(−) strains*. Loss of AtlE did not alter the efficacy of cloxacillin and vancomycin in this murine model.

In conclusion, although the loss of *atlE* is associated with decreased bactericidal activity of cloxacillin and vancomycin against *S. epidermidis*, there is no evidence that it alters the activity of these agents in a murine sepsis/peritonitis model.

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References


Fig. 1. Dose–response curves of the activity of (A) cloxacillin and (B) vancomycin against wild-type *atlE(+) Staphylococcus epidermidis (■)* and its *atlE(−) counterpart (♦). The graphs show the change of log colony-forming units (CFU)/mL following 24 h incubation in broth. Data are plotted as a function of the drug concentration expressed in mg/L.


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Trotondi MP, Xiong YQ, Memmi G, Bayer AS, Cheung AL. Role of β-lactam-β-lactamase type-1-lactamases have spread among Enterobacteriaceae in most countries, including Nigeria [1,3]. CTX-M-type ESBLs have been described in the city of Osogbo and numerous adjacent surrounding communities covering an area of ca. 800 km². Acute care is provided principally through one paediatric ward and three large adult wards. A centralised laboratory performs routine clinical microbiology services for both the community clinics and hospitals within the region.

From a total of 906 diagnostic samples obtained between January 2006 and January 2007 within the hospital, 116 E. coli were isolated. Of the 116 E. coli, 79 were ampicillin-resistant and were selected for phenotypic testing for the presence of ESBLs, of which 12 were positive in the screening and confirmatory test [8] from urinary tract infection (UTI). 3 from gastrointestinal infection and 1 from septicaemia]. Genotypic investigation via polymerase chain reaction (PCR) showed blaCTX-M genes in nine of these strains, which were therefore included in further analyses. These findings were found by PCR to carry genes coding for bla_CTX-M-1 group β-lactamases, and sequencing revealed all to be CTX-M-15. These E. coli strains were recovered from the urine of patients presenting with UTI (8/9), except for 1 case of septicaemia in a paediatric patient.

Multiresistance has often been described for ESBL-producing isolates (particularly CTX-M ESBLs) [5]. Therefore, the nine isolates were tested for their susceptibility to 18 antimicrobial agents. All susceptibility tests, including the ESBL screening and confirmatory test, were performed according to Clinical and Laboratory Standards guidelines [6]. The nine CTX-M ESBL-producing strains showed similar phenotypic resistance patterns. All were resistant to the tested cephalosporins (ceftaxime, cefazidime, cefpodoxime, ceftriaxone and cefalexin) but susceptible to the combinations of cefotaxime or ceftazidime with clavulanic acid. They were also resistant to other β-lactams such as ampicillin and aztreonam as well as the non-β-lactam antibiotics ciprofloxacin, gentamicin, tetracycline and tobramycin. None of the isolates showed imipenem resistance. This is important to mention, as carbapenems are the drug of choice for therapy of an infection with bacteria that produce ESBLs, but overuse of these agents is of concern and resistant isolates have already been observed [7]. The only differences in resistance were found against chloramphenicol (7/9) and sulfamethoxazole/trimethoprim (8/9) (Table 1).

PCR amplification further revealed blaTEM and blaOXA group III genes in all nine isolates, whilst genes coding for SHV enzymes were not present in these strains. All relevant results are summarised in Table 1.

The nine CTX-M ESBL-producing E. coli were compared by pulsed-field gel electrophoresis (PFGE) of XbaI-digested genomic DNA. Four isolates were closely related (IMT12574, IMT13267 and IMT13268) and formed a separate cluster with >90% similarity in PFGE profiles, designated IMT A. The remaining five strains were not closely related to strains of clonal group IMT A or to each other and were assigned to clonal groups IMT B–F. The existence of different clonal groups points out the opportunity of horizontal and vertical transmission of CTX-M in this region.

Interestingly, serotyping revealed that only one of the strains belonged to the pandemic O25:H4 serogroup. As shown in Table 1, the nine strains were assigned to a total of five different serotypes, namely O8:Hnt. (n = 4), O102:H6 (n = 2) and O25:H4, O8:H23 and O86:Hnt. (1 of each serotype). The four O8:Hnt. strains formed clonal group IMT A.

As CTX-M-15 enzymes found in E. coli are associated with nosocomial as well as community-acquired infections, these findings underline the importance of further investigations of the ESBL situation in Nigeria. It is necessary to characterise whether there is a certain E. coli clone spreading in the community, such as described for other CTX-M ESBL-producing bacteria, or whether there is a specific problem in this particular hospital. In particular, CTX-M-15-positive E. coli with serotype O25:H4, as found in one of the isolates from a UTI, is described a as new worldwide emerging pathogen [4].

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