(debridement, antibiotics and implant retention) approach may be used [3].

The antibiotic regimen must be well tolerated. For staphylococcal infections, rifampicin alone or in combination with a fluoroquinolone such as ciprofloxacin is often used, although success rates for the overall procedure are variable. Recently, promising outcomes in patients with prosthetic joint infections caused by Gram-positive bacteria, including meticillin-resistant *Staphylococcus aureus* (MRSA), have been reported with the oxazolidinone antibacterial agent linezolid [4]. Given that one of the safety concerns of linezolid is myelosuppression with subsequent haematological abnormalities, all linezolid-treated patients should undergo rigorous blood work-ups to detect possible low cell counts.

We report the successful treatment with linezolid of a prosthetic joint infection caused by the coagulase-negative bacterium *Staphylococcus lugdunensis* following treatment failure with a combination of rifampicin and ciprofloxacin in a patient receiving chemotherapy for malignant haematological disease. A 51-year-old man was diagnosed with multiple myeloma, located exclusively in the pelvis, and received radiotherapy, chemotherapy and bone marrow transplant. During the third cycle of chemotherapy he suffered 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 bacterium. 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*

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 Tn*917* insertional 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 $\log_{10}$ of antibiotic concentration (C) and bacterial killing defined as the variation in bacterial counts in 24 h (V) [expressed as log colony-forming units (CFU)/mL]. The equation is:

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

where the maximal effect, E_{max}, is a negative number expressing the maximal bacterial 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 $\log_{10}$ CFU to 8 $\log_{10}$ CFU of *S. epidermidis* were injected intraperitoneally. The median lethal dose (LD_{50}) was assessed at Day 6.

Cyclophosphamide-treated mice were injected intraperitoneally with an inoculum of 7 $\log_{10}$ 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 (ED_{50}) 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 ($E_{max} \pm$ S.E.) against the *atlE*(+) and *atlE*(−) strains was, respectively, -2.74 ± 0.14 log CFU/mL and -1.22 ± 0.07 log CFU/mL for cloxacillin ($P < 0.0001$) and -3.34 ± 0.15 log CFU/mL and -1.16 ± 0.14 log CFU/mL for vancomycin ($P = 0.0087$).

Median lethal doses of *atlE*(+) and *atlE*(−) strains were 4.3 $\log_{10}$ CFU/mouse and 4.7 $\log_{10}$ CFU/mouse, respectively. All mice died at the 6-day follow-up for inocula >6 $\log_{10}$ CFU/mouse. The ED_{50} 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 cidA* 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].

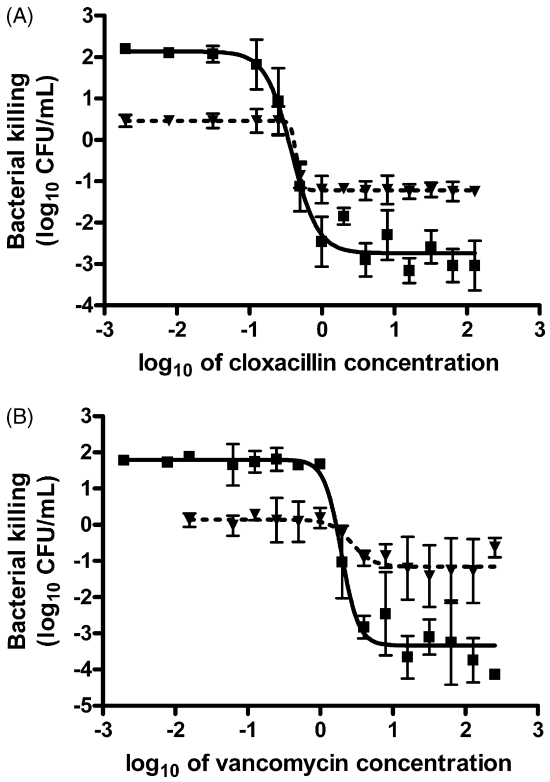

Fig. 1. Dose–response curves of the activity of (A) cloxacillin and (B) vancomycin against wild-type *atlE*(+) *Staphylococcus epidermidis* (■) and its *atlE*(−) counterpart (▼). The graphs show the change of log colony-forming units (CFU)/mL following 24 h incubation in broth. Data are plotted as a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

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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Detection of *bla*$_{\text{CTX-M-15}}$ extended-spectrum β-lactamase genes in *Escherichia coli* from hospital patients in Nigeria

Sir,

Extended-spectrum β-lactamases (ESBLs) have the ability to hydrolyse the majority of β-lactam antibiotics currently in use, including oxyimino-cephalosporins, and were first identified in a *Klebsiella pneumoniae* isolate in Germany in 1983. Since then, ESBLs found in Enterobacteriaceae have been isolated worldwide, mostly from hospitalised patients but also in the community [1]. The classical ESBLs are mutants of the TEM and SHV enzymes, but other classes are emerging, most importantly CTX-M ESBLs. First described in the late 1990s, these novel types of ESBLs emerged worldwide, mostly from *Escherichia coli*. In recent years, CTX-M-type β-lactamases have spread among Enterobacteriaceae in most parts of the world [2]. Outbreaks with producers of CTX-M ESBLs have been reported in hospitals across Europe, the Far East and South America, whilst the emergence of CTX-M ESBL-producing Enterobacteriaceae in the community has been reported in several countries, including Nigeria [1,3]. CTX-M-type ESBLs have been described from all continents, and a specific ST131 O25:H4 *E. coli* clone is described as pandemic [4]. In this study, we investigated the occurrence and relatedness of CTX-M ESBL-producing *E. coli* isolates from patients in a Nigerian hospital.

Ladoke Akintola University Teaching Hospital (LAUTECH) in Osogbo, Nigeria, provides healthcare services to people residing in the city of Osogbo and numerous adjacent surrounding communities covering an area of ca. 800 km^2. 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 *bla*$_{\text{CTX-M}}$ genes in nine of these strains, which were therefore included in further analyses. These strains were found by PCR to carry genes coding for *bla*$_{\text{CTX-M-1}}$-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 (cefotaxime, ceftazidime, 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 *bla*$_{\text{TEM}}$ and *bla*$_{\text{OXA}}$ 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 *Xba*I-digested genomic DNA. Four isolates were closely related (IMT12574, IMT13264, 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:Hn.t. (n = 4), O102:H6 (n = 2) and O25:H4, O8:H23 and O86:Hn.t. (1 of each serotype). The four O8:Hn.t. 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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