Case Report

Polymicrobial necrotizing fasciitis involving enterobacteria producing CTX-M-15 extended-spectrum β-lactamases

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Necrotizing fasciitis due to multiple Gram-negative organisms in a Nigerian patient is described. Morganella morganii and Citrobacter freundii carrying the CTX-M-15 extended-spectrum β-lactamase gene were isolated, highlighting the emergence of this β-lactamase in Western Africa and its successful spread amongst a wider range of members of the Enterobacteriaceae.

Case report

A 67-year-old diabetic Nigerian gentleman presented to the emergency department with a 6 day history of right flank pain. He had arrived in the UK from Nigeria the previous day, and had not travelled to any other countries in the last 6 months. At presentation, the pain had increased in intensity and he was unable to move his right leg. On examination, there was generalized abdominal tenderness but no rigidity, and bowel sounds were diminished. The right flank was extremely tender and there was some brownish discharge noted from a 3 × 0.5 cm lesion on the flank. Necrotizing fasciitis was suspected and this was confirmed by urgent CT scanning, which revealed extensive surgical emphysema and fluid collections within the subcutaneous tissues of the right buttock, extending up to the posterolateral aspect of the chest wall. Gas-containing collections were also seen within the right iliacus and psoas muscles. The patient underwent urgent surgical exploration and thorough debridement and was initially treated with intravenous amoxicillin/clavulanate 1.2 g 8 hourly, gentamicin 5 mg kg⁻¹ once daily and metronidazole 500 mg 8 hourly. After discussion with the clinical microbiologists, the antimicrobial regime was changed to imipenem 1 g 8 hourly, clindamycin 600 mg 6 hourly and amikacin 15 mg kg⁻¹ once daily. The patient was subsequently transferred to the intensive care unit for renal, ventilatory and inotropic support.

Culture of the debrided tissue revealed the presence of Escherichia coli, Proteus mirabilis, Morganella morganii, Citrobacter freundii, Providencia rettgeri, Enterococcus faecalis and mixed anaerobes, which prompted the addition of intravenous linezolid 600 mg 12 hourly. The patient required three further debridements and clindamycin was continued for 10 days, linezolid for 14 days and imipenem for 32 days. Skin grafting was performed 6 weeks after admission, and, after 3 months of hospital care in the UK, he was transferred back to a hospital in Nigeria for rehabilitation.

Microbiology

Susceptibility testing of the enterobacteria using the MicroScan WalkAway System (Siemens Healthcare Diagnostics) showed that the M. morganii and C. freundii isolates were resistant to amoxicillin, amoxicillin/clavulanate, cefuroxime, gentamicin, ciprofloxacin, ceftazidime and aztreonam. Production of an extended-spectrum β-lactam (ESBL) by these two isolates was investigated by comparing zone sizes surrounding cefepime and cefepime/clavulanate combined discs (Driex et al., 2008). All isolates were susceptible to piperacillin/tazobactam, ertapenem, imipenem, meropenem and amikacin. The E. coli and Proteus mirabilis isolates were susceptible to all β-lactams with the exception of amoxicillin, and ESBL production by these isolates could not be detected in phenotypic tests.

The molecular mechanisms underlying ESBL production in the M. morganii and C. freundii isolates were investigated. Multiplex PCRs for genes encoding TEM, SHV, OXA-1/48-like, CTX-M-groups 1/2/9/8/25, ACC, MOX, DHA, CIT, EBC, VEB, PER and GES β-lactamases were performed as previously described (Dallenne et al., 2010). A blaCTX-M-1-like gene was amplified from both organisms and a blaTEM-like gene was detected in C. freundii. The chromosomal AmpC genes blatDHA and blatCIT were also present in the M. morganii and C. freundii isolates as expected (Pérez-Pérez & Hanson, 2002). To confirm that these contributed to β-lactam resistance in the isolates, zone sizes surrounding

Abbreviation: ESBL, extended-spectrum β-lactamase.

A schematic diagram of the genetic environment of the blaCTX-M-15 gene is available with the online version of this paper.
cefotixin discs were compared and found to be enhanced on agar supplemented with 200 mg l\(^{-1}\) of the AmpC inhibitor cloxacillin. The entire coding sequences of the bla\(_{\text{TEM}}\) and bla\(_{\text{CTX-M}}\) genes were cloned in \(E.\) coli TOP10 and sequenced. Comparison of the translated peptide sequences with those contained in the Lahey database (www.lahey.org/Studies) revealed that both isolates carried the bla\(_{\text{CTX-M-15}}\) gene. The \(C.\) freundii also contained bla\(_{\text{TEM}}\). Analysis of the genetic environment surrounding the CTX-M genes using primers hybridizing to the insertion sequences IS\(_{\text{Ecp1}},\) IS903 and Orf513, which encode transposases often found upstream and downstream of CTX-M genes (Brasme et al., 2007), revealed that in both species the bla\(_{\text{CTX-M-15}}\) gene was flanked by a disrupted IS\(_{\text{Ecp1}}\) sequence containing a promoter inserted 48 bp upstream of the start of the bla\(_{\text{CTX-M-15}}\) gene (Supplementary Fig. S1 in JMM Online). This arrangement is identical to that found in CTX-M-15-producing enterobacteria from other parts of the world (Lartigue et al., 2004). Mating experiments between the \(M.\) morganii and \(C.\) freundii isolates and a rifampicin-resistant strain of \(E.\) coli (CSH26) were carried out in broth culture. \(E.\) coli transconjugants obtained on LB agar plates supplemented with rifampicin (100 mg l\(^{-1}\)) and cefpodoxime (8 mg l\(^{-1}\)) were resistant to third-generation cephalosporins and contained the bla\(_{\text{CTX-M-15}}\) gene in the same genetic environment as the donor strains, indicating its ability to move between the species by horizontal transfer.

**Discussion**

This case describes a classical presentation of a type I polymicrobial necrotizing fasciitis, which typically affects the trunk or perineum and involves multiple Gram-negative species (Sarani et al., 2009). Although surgical debridement is the cornerstone of treatment, antimicrobials are still important in controlling systemic sepsis and further spread. The involvement of ESBL-producing organisms severely limits the treatment options available, essentially leaving the carbapenems as the only reliable therapeutic agents. Although the isolates were deemed susceptible to piperacillin/tazobactam *in vitro*, use of inhibitor combinations is not considered acceptable in severe infections (Falagas & Karageorgopoulos, 2009). The occurrence of ESBLs in organisms which also contain inducible AmpC enzymes (Morganella, Citrobacter, Serratia and Enterobacter) is another problem which leads to both difficulties in ESBL detection (Pitout et al., 2003) and the loss of fourth-generation cephalosporins (e.g. cefepime) as potential therapies.

Class A ESBLs of the CTX-M family preferentially hydrolyse cefotaxime rather than ceftazidime and in some areas have become the most widely disseminated enzymes in the Enterobacteriaceae (Rossolini et al., 2008). In both of the ESBL producers identified here, the gene carried was bla\(_{\text{CTX-M-15}}\). The spread of this gene has been well documented throughout Europe, Asia and North America, often in association with virulent clones of \(E.\) coli (Peirano & Pitout, 2010), but there are much more limited data on the epidemiology of CTX-M enzymes in Africa. A 5 year study of organisms isolated from cases of necrotizing fasciitis at a University Teaching Hospital in Nigeria did not identify resistance to cephalosporins as a significant problem in the years 2001–2005 (Legbo & Legbo, 2007), and a molecular analysis of eight Nigerian ESBL-producing *Enterobacter* species in 2001 detected only TEM and SHV-like ESBLs (Aibinu et al., 2003). In a small study of *Klebsiella pneumoniae* isolates associated with community-acquired urinary tract infections collected in Ibadan, Nigeria, CTX-M group 1-like enzymes were found in 17 (57 %), but CTX-M-15 was identified in only 2 of these (Soge et al., 2006). Reports of CTX-M-like ESBLs in other Sub-Saharan African countries include bla\(_{\text{CTX-M-15}}\) in *E. coli* and *Klebsiella* isolates in Tanzania (Blomberg et al., 2005), Cameroon (Gangoué-Pieboji et al., 2005) and the Central African Republic (Frank et al., 2006) and a bla\(_{\text{CTX-M-12}}\)-containing isolate from Kenya (Kariuki et al., 2007).

This case highlights two interesting points. Firstly, it demonstrates the increasing involvement of ESBL-producing enterobacteria in severe community-acquired infections. Secondly, it highlights that CTX-M-like genes could be emerging in Africa and are disseminating to a wider range of enterobacterial species. Travel to Africa may now need to be considered as an important risk factor for infection with ESBL-producing organisms (Pitout et al., 2009).

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**References**


