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 -lactamase (ESBL) by these two isolates was investigated by comparing zone sizes surrounding cefepime and cefepime/clavulanate combined discs (Drieux *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 *bla*_{CTX-M-15}-like gene was amplified from both organisms and a *bla*_{TEM} gene was detected in *C. freundii*. The chromosomal AmpC genes *bla*_{DHA} and *bla*_{CIT} 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 *bla*_{CTX-M-15} gene is available with the online version of this paper.

cefotaxime 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*_{TEM} and *bla*_{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*_{CTX-M-15} gene. The *C. freundii* also contained *bla*_{TEM-1}. Analysis of the genetic environment surrounding the CTX-M genes using primers hybridizing to the insertion sequences *ISEc1*, *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*_{CTX-M-15} gene was flanked by a disrupted *ISEc1* sequence containing a promoter inserted 48 bp upstream of the start of the *bla*_{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*_{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*_{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*_{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*_{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

- Aibinu, I. E., Ohaegbulam, V. C., Adenipekun, E. A., Ogunola, F. T., Odugbemi, T. O. & Mee, B. J. (2003). Extended-spectrum β -lactamase enzymes in clinical isolates of *Enterobacter* species from Lagos, Nigeria. *J Clin Microbiol* **41**, 2197–2200.
- Blomberg, B., Jureen, R., Manji, K. P., Tamim, B. S., Mwagagile, D. S., Urassa, W. K., Fataki, M., Msangi, V., Tellevik, M. G. & other authors (2005). High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. *J Clin Microbiol* **43**, 745–749.
- Brasme, L., Nordmann, P., Fidel, F., Lartigue, M. F., Bajolet, O., Poirel, L., Forte, D., Vernet-Garnier, V., Madoux, J. & other authors (2007). Incidence of class A extended-spectrum β -lactamases in Champagne-Ardenne (France): a 1 year prospective study. *J Antimicrob Chemother* **60**, 956–964.
- Dallenne, C., Da Costa, A., Decré, D., Favier, C. & Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important β -lactamases in *Enterobacteriaceae*. *J Antimicrob Chemother* **65**, 490–495.
- Drieux, L., Brossier, F., Sougakoff, W. & Jarlier, V. (2008). Phenotypic detection of extended-spectrum beta-lactamase production in

- Enterobacteriaceae: review and bench guide. *Clin Microbiol Infect* **14**, 90–103.
- Falagas, M. E. & Karageorgopoulos, D. E. (2009). Extended-spectrum β -lactamase-producing organisms. *J Hosp Infect* **73**, 345–354.
- Frank, T., Arlet, G., Gautier, V., Talarmin, A. & Bercion, R. (2006). Extended-spectrum beta-lactamase-producing Enterobacteriaceae, Central African Republic. *Emerg Infect Dis* **12**, 863–865.
- Gangoué-Piéboji, J., Bedenic, B., Koulla-Shiro, S., Randegger, C., Adiogo, D., Ngassam, P., Ndumbe, P. & Hächler, H. (2005). Extended-spectrum- β -lactamase-producing Enterobacteriaceae in Yaounde, Cameroon. *J Clin Microbiol* **43**, 3273–3277.
- Kariuki, S., Revathi, G., Corkill, J., Kiiru, J., Mwituria, J., Mirza, N. & Hart, C. A. (2007). *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams. *J Infect Dev Ctries* **1**, 257–262.
- Lartigue, M. F., Poirel, L. & Nordmann, P. (2004). Diversity of genetic environment of *bla*_{CTX-M} genes. *FEMS Microbiol Lett* **234**, 201–207.
- Legbo, J. N. & Legbo, J. F. (2007). Bacterial isolates from necrotizing fasciitis: a clinico-pathological perspective. *Niger J Med* **16**, 143–147.
- Peirano, G. & Pitout, J. D. (2010). Molecular epidemiology of *Escherichia coli* producing CTX-M β -lactamases: the worldwide emergence of clone ST131 O25:H4. *Int J Antimicrob Agents* **35**, 316–321.
- Pérez-Pérez, F. J. & Hanson, N. D. (2002). Detection of plasmid-mediated AmpC β -lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* **40**, 2153–2162.
- Pitout, J. D., Reisbig, M. D., Venter, E. C., Church, D. L. & Hanson, N. D. (2003). Modification of the double-disk test for detection of Enterobacteriaceae producing extended-spectrum and AmpC β -lactamases. *J Clin Microbiol* **41**, 3933–3935.
- Pitout, J. D., Campbell, L., Church, D. L., Gregson, D. B. & Laupland, K. B. (2009). Molecular characteristics of travel-related extended-spectrum- β -lactamase-producing *Escherichia coli* isolates from the Calgary Health Region. *Antimicrob Agents Chemother* **53**, 2539–2543.
- Rossolini, G. M., D'Andrea, M. M. & Mugnaioli, C. (2008). The spread of CTX-M-type extended-spectrum beta-lactamases. *Clin Microbiol Infect* **14**, 33–41.
- Sarani, B., Strong, M., Pascual, J. & Schwab, C. W. (2009). Necrotizing fasciitis: current concepts and review of the literature. *J Am Coll Surg* **208**, 279–288.
- Soge, O. O., Queenan, A. M., Ojo, K. K., Adeniyi, B. A. & Roberts, M. C. (2006). CTX-M-15 extended-spectrum β -lactamase from Nigerian *Klebsiella pneumoniae*. *J Antimicrob Chemother* **57**, 24–30.