NDM-1-producing Enterobacter cloacae and Klebsiella pneumoniae from diabetic foot ulcers in India

Emerging multi-drug resistance has become a major problem in community-acquired and nosocomial infections worldwide (Shakil et al., 2008; Paterson & Bonomo, 2005; Rodriguez-Baño & Navarro, 2008). Carbapenems are used as last-resort drugs because increasing resistance against β-lactam groups of antibiotics has developed due to their excessive use in treating a wide range of infections (Paterson, 2006). One of the latest resistance enzymes, NDM-1 (New Delhi metallo-β-lactamase) was first identified in isolates from a Swedish patient of Indian origin in 2008. This patient had previously been admitted to the Ludhiana hospital in Punjab (India) and was later admitted to a hospital in New Delhi (Yong et al., 2009; Kumarsamy et al., 2010). This was the first report of blanDM-1-carrying isolates that confer resistance against all β-lactams except aztreonam (Yong et al., 2009). Since then, NDM-1 producers have been extensively described in many countries in the world but mostly those representing important bacterial populations originating from the Indian subcontinent. Widespread dissemination of NDM producers in the community, such as that found in Pakistan, indicates that prevalence of carriage of NDM in the community may be as high as 13.8% in outpatients (Perry et al., 2011).

Here, we report NDM-1 producing Enterobacter cloacae (EC15) and Klebsiella pneumoniae (KP12) strains isolated in 2010 from two patients with foot ulcers. One of the patients, a 69-year-old male (patient A), was admitted to the ICU of Aligarh Hospital, North-East India, with a diabetic foot and severe sepsis. He was treated with intravenous antibiotics (including imipenem for a week) for severe sepsis; eventually amputation at the knee had to be performed. The other patient, a 60-year-old male (patient B), was admitted to the endocrinology ward of the same hospital during the same period also with a diabetic foot ulcer. He underwent the same treatment with no recovery, finally developing severe sepsis which led to foot amputation.

Isolates were obtained from scrapings of the base of the ulcer or the deep portion of the wound edge taken from the patients on day of admission with severe sepsis. The isolates were identified as E. cloacae (EC15) and K. pneumoniae (KP12) from patients A and B, respectively, by using API 20 E strips (bioMérieux). The MICs of β-lactams were determined by using Etest strips (bioMérieux) on Mueller–Hinton agar plates incubated at 37°C and results were recorded according to updated CLSI guidelines (CLSI, 2011). The two isolates were resistant to imipenem, meropenem, doripenem, etrapenem (Table 1), all β-lactams (MICs >250 μg ml⁻¹), gentamicin, amikacin and tobramycin (MICs between 8 and 250 μg ml⁻¹) but remained susceptible to colistin and tigecycline (data not shown).

Detection of metallo-β-lactamase activity was performed by using two imipenem disks (10 mg), one containing 292 mg (10 ml of 0.1 M) anhydrous EDTA, which were placed 25 mm apart on Mueller–Hinton plates (Franklin et al., 2006). PCR amplification and sequence analysis of DNA from both of the isolates, using primers as described previously (Diancourt et al., 2005; Poirel et al., 2004, 2011a), revealed the presence of the blanDM-1 gene with additional blanCTX-M-15, blanOXA-1, blanTEM-1 β-lactamase genes (and blanSHV-1 for K. pneumoniae KP12 only). In addition, a 16s rRNA methylase gene (armA) was also detected. No blanOXA-48, blanIMP, blanVIM or blanGES genes were amplified from DNA of the isolates, whereas they have been reported previously in enterobacterial isolates from India (Poirel et al., 2011b). Conjugal transfers were performed using E. cloacae (EC15) and K. pneumoniae (KP12) isolates as donors and azide-resistant Escherichia coli strain J53 as the recipient strain, with selection based on growth on agar in the presence of ceftazidime (30 μg ml⁻¹) and azide (100 μg ml⁻¹) (Poirel et al., 2011b). Three types of transconjugants were obtained for K. pneumoniae KP12 and two types were obtained for E. cloacae EC15, according to their resistance phenotypes. One of the transconjugants of K. pneumoniae strain KP12 showed extended-spectrum β-lactamase (ESBL)- and NDM-1-producing phenotypes, one showed only an ESBL-producing phenotype and the third showed only the NDM-1-producing phenotype. Two types of transconjugants, displaying an ESBL- and NDM-1-producing phenotype and the other displaying an NDM-1-producing phenotype, were obtained for E. cloacae strain EC15. Plasmid profiling, performed using the Kieser technique (Kieser, 1984), revealed that K. pneumoniae KP12 harboured three plasmids whereas E. cloacae EC15 carried two plasmids, all ranging from 66 to 154 kb in size. Tests using a PCR-based replicon typing method (PBRT) (Carattoli et al., 2005) revealed that the NDM-1-carrying plasmid in E. cloacae EC15 was of incompatibility group Inc I/M (Table 1), whereas the plasmid in K. pneumoniae KP12 carrying the NDM-1 determinant was not able to be typed. Once again, the study of these isolates showed that the gene coding for the widespread resistance determinant CTX-M-15 is not associated with the gene coding for NDM-1 on the same plasmid.

Multilocus sequence typing (MLST) was performed for K. pneumoniae KP12, as described earlier (Diancourt et al., 2005), which was found to be of the type ST14, which is one of the types observed in Indian isolates (Poirel et al., 2011c). In addition, the same ST14 type was identified in the first NDM-1-producing K. pneumoniae isolate from Sweden as well as other isolates with links to contamination in India (Poirel et al., 2011c). In both strains the surrounding genetic
environment was analysed for the presence of insertion sequences (IS) known to be associated with the \textit{bla}_{NDM-1} gene in \textit{Enterobacteriaceae} (Poirol et al., 2011c). Primers targeting the IS element \textit{Aba125} identified a remnant of IS\textit{Aba125} upstream of the \textit{bla}_{NDM-1} gene in the \textit{E. cloacae} isolate (EC15), whereas an entire \textit{ISAb125} element was identified upstream of the \textit{bla}_{NDM-1} gene in the \textit{K. pneumoniae} isolate (KP12). A bleomycin resistance gene \textit{bleMBL} was identified downstream of the \textit{bla}_{NDM-1} gene in both cases, which encodes a putative bleomycin (an antitumour drug)-resistance protein, as reported previously (Poirol et al., 2011c). These later results indicate that conserved structures surrounding the \textit{bla}_{NDM-1} gene are present in NDM-1-producing \textit{Enterobacteriaceae} isolated from the Indian subcontinent.

To the best of our knowledge, this is the first report of NDM-1-producing \textit{E. cloacae} and \textit{K. pneumoniae} isolates from community-acquired infections with diabetic foot ulcers. Although many reports have identified NDM-1-producing isolates worldwide (Nordmann et al., 2011a), clear identification of NDM-1 producers as a source of community-acquired infection is scarce (Nordmann et al., 2011b). The present data and earlier reports (Yong et al., 2009; Kumarasamy et al., 2010) indicate the need to control the dissemination of carbapenemase-producing \textit{Enterobacteriaceae} in the hospital setting, as well as in the community. This control will require the development of rapid, cheap and easy-to-handle diagnostic techniques for the identification of carbapenemase producers.

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