Phylogenetic diversity of \textit{Escherichia coli} strains producing NDM-type carbapenemases

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\textbf{Background:} The global accumulation of \textit{Escherichia coli} with CTX-M extended-spectrum $\beta$-lactamases partly reflects the dissemination of clonal lineages, notably ST131 and ST405. More recently, \textit{E. coli} have emerged that produce NDM carbapenemase. We sought to determine the clonal diversity of \textit{E. coli} with this enzyme from English hospitals, and to compare them with isolates from Pakistan and India.

\textbf{Methods:} The 18 NDM-positive \textit{E. coli} were from hospitals in England ($n=10$), Pakistan ($n=7$) and India ($n=1$). Isolates were compared by phylogenetic grouping, multilocus sequence typing and PFGE of XbaI-digested DNA. Isolates were screened by PCR for acquired AmpC genes, \textit{bla}_{CTX-M}, and the 16S rRNA methylase genes \textit{armA} and \textit{rmtC}.

\textbf{Results:} Most of the isolates belonged to phylogenetic groups B1 ($n=9$) or D ($n=7$); two were group A and none was group B2. Nine isolates from England and Pakistan belonged to the B1 lineage ST101, with seven of these clustering at $>77\%$ similarity by PFGE. Other lineages included ST405 ($n=3$, group D), ST648 ($n=3$, group D), the ST23 complex (one each of ST90 and ST410, both group A) and ST156 ($n=1$, group D). Sixteen of 18 isolates had a group 1 CTX-M gene, 13 had a CIT-type acquired AmpC, and 16 had either or both of \textit{armA} and \textit{rmtC}.

\textbf{Conclusions:} The \textit{E. coli} isolates producing NDM-1 carbapenemase belonged to six sequence types and included diverse clonal lineages. Nevertheless, isolates of B1-ST101 accounted for half the collection, and included isolates from both England and Pakistan. None of the isolates belonged to ST131 or to phylogroup B2.

\textbf{Keywords:} NDM $\beta$-lactamase, carbapenemase, \textit{E. coli}, MLST

\section*{Introduction}

\textit{Escherichia coli} is the most frequent cause of urinary tract infections and bacteraemia in the UK. Antibiotic resistance has increased globally in this species since 2000 and it is now the major host of CTX-M-type extended-spectrum $\beta$-lactamases (ESBLs). Multilocus sequence types (MLSTs) ST131 and ST405, often producing ESBL enzymes, have become successful resistant clones worldwide.\textsuperscript{1} Most ESBL-producing strains remain susceptible to carbapenem and their spread has resulted in the increased use of carbapenem for therapy.

Carbapenemase-producing \textit{E. coli} are rare, but increasing, in the UK. The HPA's Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) confirmed its first producer in 2006, another in 2008, 6 more in 2009 and 27 in 2010. Nine of the 35 produced KPC carbapenemases, 7 produced OXA-48, 2 produced IMP metalloenzymes and 1 a VIM-type metalloenzyme (N. Woodford and D. M. Livermore, unpublished data). However, the carbapenemase most often detected among these \textit{E. coli} was NDM-1, which is often associated with travel and hospitalization in the Indian subcontinent.\textsuperscript{2} During 2008–10, ARMRL confirmed 16 \textit{E. coli} isolates with NDM enzymes from 11 patients in 10 English hospitals.\textsuperscript{2}

Elsewhere, from countries with fewer cases than the UK, diverse sequence types have been reported among \textit{E. coli} with NDM carbapenemase, including single representatives of ST405, ST410 and ST131 in Canada, Norway and France, respectively.\textsuperscript{3–5} However, single representatives of ST101 with NDM-1 enzyme have been reported in each of Australia, Germany and Canada, suggesting a possible association.\textsuperscript{5–8}

We sought to determine the clonal diversity of the substantially larger group of \textit{bla}_{NDM}-positive \textit{E. coli} from England,
and to compare them with isolates obtained in Pakistan and India.

**Materials and methods**

**Bacterial isolates**

Ten *E. coli* with NDM-1 carbapenemase from 10 different English hospitals were studied, representing one isolate from each of the first 10 patients known to be affected. Five of these 10 patients had confirmed recent travel history to India or Pakistan. In one of these five, travel to India was healthcare associated and, in another, the patient had been transferred to England from a hospital in Pakistan. The travel history of the remaining five patients from England was unknown. These 10 *E. coli* had been isolated from blood (n = 4), urine (n = 3), faeces (n = 1), a post-operation abdominal swab (n = 1), and a burn site (n = 1).

The seven NDM-positive *E. coli* from Pakistan were isolated from six patients in Karachi. Four were isolated from three patients hospitalized at the site with the laboratory and three were isolated from patients in the community or were referred by other hospitals in Karachi. Clinical data for these isolates were unavailable. The isolate from Assam, India, was from an intravenous access line infection.

**Susceptibility testing and PCR**

MICs were determined by agar dilution using BSAC methodology.9 The *blaNDM* gene was identified by PCR using primers NDM-F (5′-GGG CAG TCG CTT CCA ACG GT-3′) and NDM-R (5′-GTG CTC AGT GTC GGC AT-3′) to amplify an internal fragment of 475 bp. The conditions included an initial denaturation step of 5 min at 95°C, followed by 30 cycles of 40 s at 95°C, 30 s at 58°C and 30 s at 72°C, and then a final extension step of 5 min at 72°C. Isolates were screened for acquired AmpC genes (MOX, CIT, DHA, ACC, ENT and FOX) and for *blaCTX-M* by PCR using primers and conditions described previously.10,11 Primer pairs ArmA-F (5′-CCC TTC TCC TTT CC-3′) and ArmA-R (5′-TGC ATC AAA TAT GGG GGT-3′) and RmtC-R (5′-TTC CAT CCC AAC ATC TCT CC-3′) were used to amplify fragments of the 16S rRNA methylase genes *armA* and *rmtC*, respectively. The PCR conditions were an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C for 25 s, 54°C for 40 s and 72°C 50 s, and then a final cycle of 72°C for 6 min.

**Strain typing**

The isolates were assigned to the major *E. coli* phylogenetic groups (A, B1, B2 and D) by multiplex PCR.12 MLST, as described by the Environmental Research Institute (ERI), University College Cork (UCC) (http://mlst.ucc.ie/mlst/dbs/Ecoli), was used to determine the sequence types of all isolates; fragments of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) were amplified by PCR and sequenced using an ABI Genetic Analyser capillary platform 3130XL (Applied Biosystems, CA, USA). Sequence types were assigned using the *E. coli* MLST database at the ERI UCC web site and were compared with the species’ population structure using eBURST (http://eburst.mlst.net/).

PFGE of XbaI-digested genomic DNA was used to compare the isolates. Samples were electrophoresed on an agarose gel (1.2% w/v in 0.5% Tris/EDTA buffer) using a CHEF DRiI apparatus (Bio-Rad, Hemel Hempstead, UK) with a 5–35 s linear ramp for 30 h at 6 V/cm at 12°C. BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) was used to analyse the banding patterns obtained and profiles with ≥85% similarity (UPGMA, Dice coefficient) were considered to be highly related.

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**Figure 1.** Dendrogram of PFGE banding patterns showing the genetic relatedness of *E. coli* producing NDM-1 enzyme, their phylogenetic characteristics and selected resistance genes.
Results and discussion

Resistance genes and antibiotic susceptibilities

In addition to \( \text{bla}_{\text{NDM-1}} \), 11/18 isolates harboured genes encoding both group 1 CTX-M-type ESBLs and CIT-type acquired AmpC enzymes, which are ancestrally derived from Citrobacter freundii (Figure 1); 5 had group 1 CTX-M-type ESBLs alone and 2 had only CIT-type AmpC enzymes. None of the isolates had \( \text{bla}_{\text{NDM-1}} \) without an accompanying ESBL or CIT AmpC enzyme. Sixteen isolates had 16S rRNA methylase genes: 9 had \text{rmtC} only; 4 had \text{armA} only; and 3 isolates, all from Pakistan, had both.

Reflecting these resistance mechanisms, 17 isolates were resistant by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria to all \( \beta \)-lactams, and 1 isolate with \( \text{bla}_{\text{NDM-1}} \) and CIT was resistant to all except aztreonam, to which it showed intermediate susceptibility (MIC, 8 mg/L). There was near-universal resistance to aminoglycosides (amikacin, gentamicin and tobramycin), although the two isolates that lacked \text{armA} or \text{rmtC} remained susceptible to amikacin, with one of these susceptible also to tobramycin and gentamicin. A single isolate remained susceptible to ciprofloxacin. All 18 isolates were susceptible to colistin (MICs \( \leq \) 1 mg/L) and tigecycline (MICs \( \leq \) 1 mg/L); fosfomycin was not tested.

Strain typing

Most of the isolates belonged to phylogenetic groups B1 (\( n = 9 \)) or D (\( n = 7 \)), only two to group A and none to group B2, the main \( E. coli \) group associated with extraintestinal infections (Figure 1). By MLST they belonged to six sequence types, scattered through the \( E. coli \) population structure (Figure 2). The most frequent was ST101 (\( n = 9 \)), which included 4 of 10 isolates from England and 5 of 7 from Pakistan. These nine ST101 isolates corresponded to the nine phylogenetic group B1 organisms. Single isolates of ST101 \( E. coli \) with NDM-1 enzyme have also been reported previously in Australia, Germany and Canada,\(^5\)–\(^8\) with the German and Canadian isolates also confirmed to be phylogroup B1, and were from patients who had been hospitalized in the Indian subcontinent (two in India and one in Bangladesh).

The clonal spread of B1-ST101 \( E. coli \) has also been associated with group 9 CTX-M ESBLs in Spain, where the successful dissemination of this sequence type may, in part, be explained by the accumulation of a large number of virulence genes.\(^{13}\) ST101 is the predicted founder of a clonal complex that includes 11 single locus variants and 2 double locus variants (http://eburst.mlst.net/).

Three further English isolates belonged to phylogenetic group D-ST405, a sequence type that has been associated internationally with the carriage of ESBLs,\(^1\) though with NDM-1 \( \beta \)-lactamase only in one isolate to date.\(^6\) One isolate from England and two from Pakistan belonged to phylogenetic group D-ST648. Two isolates [one from Assam, India (ST90) and the other from England (ST410)] belonged to clonal complex 23; both belonged to phylogenetic group A. An ST410 \( E. coli \) with NDM-1 enzyme has been reported.

Figure 2. ‘Population Snapshot’ determined by eBURST analysis (http://eburst.mlst.net/), showing the clusters of linked and unlinked sequence types in the \( E. coli \) MLST database (‘Achtman’ scheme; 1899 sequence types; http://mlst.ucc.ie/mlst/dbs/Ecoli). Sequence type labels have been removed. The phylogenetic groups and sequence types found to host \( \text{bla}_{\text{NDM-1}} \) in this study are indicated.
Phylogenetic diversity of NDM-positive E. coli

previously in Norway in a patient previously hospitalized in India. The remaining isolate, from England, belonged to ST156 and phylogenetic group D.

PFGE clustered seven of the nine ST101 isolates at >77% banding pattern similarity (Figure 1). Within this cluster, two sets of more closely related isolates were identified; four isolates from Pakistan, two of them from the same patient, were ≥87.5% similar to each other, and two English isolates, from patients in different London hospitals, were related at 88%. More generally, the ST101 isolates had diverse PFGE banding patterns, with all nine clustering together only at 66% similarity, a level that also included isolates of ST90 (phylogenetic group A) and ST156 (phylogenetic group D). The three ST405 isolates also were diverse by PFGE, clustering only at 64% similarity, together with isolates of ST410 and ST648. This ‘cluster’ included isolates of phylogenetic groups A and D. The two ST648 isolates from Pakistan were closely related with a similarity of 85%, but were distinct from an isolate from England of the same sequence type. In view of the overall diversity of the B1-ST101 isolates by PFGE, it seems more likely that this sequence type has acquired plasmids encoding NDM-1 enzyme on several occasions rather than that a single B1-ST101 variant with NDM-1 β-lactamase has disseminated. Nevertheless, some of the more closely related subgroups may represent clonal expansion and this seems especially likely in Karachi where four isolates had very similar PFGE profiles.

Clinical data were not available for the source patients of the seven isolates from Pakistan. For the remaining isolates there was no clear association between the phylogroup and the infection or colonization status of the patient, e.g. isolates belonging to the ‘commensal’ phylogroups B1 and A were recovered from urine (n=3), blood (n=1), an intravenous access line (n=1) and faeces (n=1).

In summary, we have shown that the blaNDM-1 gene occurs in E. coli belonging to diverse phylogenetic lineages, although ST101 (phylogenetic group B1) was the most frequent host lineage among these isolates from England and Pakistan. The second most frequent lineage was ST405 (phylogenetic group D), which is disseminated globally and often associated with CTX-M-type ESBLs. None of our NDM-1 isolates belonged to ST131, another international clone associated with ESBL production, or to phylogroup B2, the main uropathogenic group. Our findings, along with previous reports from Australia, Germany, and Canada, highlight the potential for the ST101 and ST405 clones of E. coli to disseminate NDM-type enzymes.

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References

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