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Letter to the Editor

Autochthonous ST405 NDM-5 producing *Escherichia coli* causing fatal sepsis in Northern Italy

Infections due to carbapenem-resistant *Enterobacterales* (CRE) are associated with high mortality rates and increasing incidence. In Italy, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) is by now endemic, whereas New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacterales* remain sporadic [1].

We describe a whole-genome sequencing (WGS) investigation of NDM-producing *Enterobacterales* at our centre after a case of fatal sepsis caused by sequence type (ST) 405 NDM-producing *Escherichia coli*.

A 78-year-old Italian cirrhotic man with unremarkable travel history was admitted to our hospital with spontaneous peritonitis and sepsis in November 2018 after a recent episode of septic shock treated with piperacillin-tazobactam. Meropenem was given promptly, but the patient rapidly developed septic shock. Blood cultures resulted positive for NDM-producing Gram-negative bacilli (GeneXpert), so ceftazidime/avibactam and aztreonam were commenced. Unfortunately, the patient's condition further worsened and he died 1 day after admission. NDM-producing *E. coli* strain Ec_sk73y73t was isolated from the patient's blood.

Given the severity of the case and the unexpected bacterial isolate, all NDM-producing strains detected in 2018 at the hospital were collected for WGS characterization. Two additional NDM-producing strains were found: one *E. coli* (Ec_sk74y74t) and one *K. pneumoniae* (Kp_sk75y75t).

Ec_sk74y74t was isolated in October 2018 from the urine of an Italian patient with no travel history admitted to the urology department for bladder and renal biopsies. Fosfomycin and colistin were administered as pre-operative prophylaxis for asymptomatic bacteriuria with no subsequent complications.

Kp_sk75y75t was isolated in August 2018 from the central line tip of a patient admitted to the neurosurgery ward after a long hospitalization in Greece due to subarachnoid haemorrhage. The patient was treated successfully with colistin and tigecycline, and the isolate was considered to have been imported.

Genomic DNA was sequenced using the Illumina MiSeq platform (250 × two runs) after library preparation (Nextera XT kit). Read quality was assessed using FastQC software, and reads were trimmed using Trimmomatic software and assembled with SPAdes. Genome assemblies were searched against the PATRIC database using Mash, and 50 similar genomes were retrieved and included in the coreSNP calling analysis [2]. Phylogenetic reconstruction was performed using RaxML with 100 pseudo-bootstraps. Resistance genes, virulence genes and plasmid incompatibility groups were assessed by BLAST searches of the ResFinder, VirFinder and PlasmidFinder databases, respectively.

WGS analyses revealed that the two *E. coli* strains belonged to two different STs: Ec_sk73y73t belonged to ST405 and Ec_sk74y74t belonged to ST2851, a single allele variant of the hyperepidemic ST410 (Fig. 1). Ec_sk73y73t harboured the following resistance genes: *bla*NDM-5, *bla*CMY-42, *aadA2, mdf(A), sul1, dfrA12, parE* S458A, *parC* S80I and *gyrA* S83L e D87N. Ec_sk74y74t harboured *bla*NDM-5, *bla*CMY-42, *bla*CTX-M-15, *rmtB, aadA2, bla*TEM-1B, *mph(A), mdf(A), sul1, tet(A)* and *dfrA12.* Kp_sk75y75t belonged to ST11 and harboured *bla*NDM-1, *aph(3'')-Ib, aac(3)-IIa, aac(6')-Ib-cr, aph(3')-Ia, aph(6)-Id, bla*CTX-M-15, *bla*TEM-*1B, bla*OXA-1, *oqxA, oqxB, fosA, mph(A), catB3, sul1, sul2* and *dfrA14.*

Ec_sk73y73t contained the *air* virulence gene, homologous of the yersinia invasive gene *eaeX* and its regulator *eilA* gene.

Both *E. coli* strains harboured an IncFII plasmid. Nonetheless, alignment of the contigs containing the IncFII incompatibility group in the two genome assemblies revealed large uncovered regions and nucleotide identity of ~95%.

The rapid fatal outcome observed in the study patient was likely influenced by his severe comorbidity as well as by presentation with septic shock. Nonetheless, it can also be attributed, at least in part, to the virulence genes (*eilA*/*air*) found in Ec_sk73y73t as well as to its susceptibility profile. Indeed, despite its rapid identification, the isolation of an NDM-producing isolate was totally unexpected based on our epidemiology, leading to a treatment delay of a few hours.

Data from the Italian national surveillance showed that bloodstream infections (BSIs) due to CRE are increasing, being mainly due to *K. pneumoniae* (98.1%). Among the carbapenemases identified, KPC was the most represented, while NDM resulted rarely detected (0.4% of *K. pneumoniae* and 1.4% of *E. coli*) [1].

A few other reports of infection and colonization due to NDM-producing *Enterobacterales* were reported in Italy before 2018 with most cases being imported [3] but with growing incidence of autochthonous cases [4], although rarely associated with severe clinical outcome.

More recent data show how the epidemiology of NDM-producing *Enterobacterales* in Italy is changing, as a large outbreak was reported in 2018–2019 in Tuscany with up to 350 cases, including 50 BSIs. The outbreak was considered likely to be clonal, although the potential source remains to be determined [5] and represents an example of how NDM-producing bacteria can spread easily.

We describe three genetically unrelated NDM-producing *Enterobacterales* strains, all belonging to emerging or hypervirulent clones. Both autochthonous *E. coli* strains harboured *bla*NDM-5, while the imported *K. pneumoniae* harboured *bla*NDM-1, supporting the hypothesis that a *bla*NDM-5 reservoir is present in Italy [4].

Therapeutic options for NDM-producing *Enterobacterales* are scarce and still in phase 3 (aztreonam/avibactam). Therefore, the spread of NDM-producing strains in a setting where KPCs are already endemic represents a serious clinical threat, and any effort

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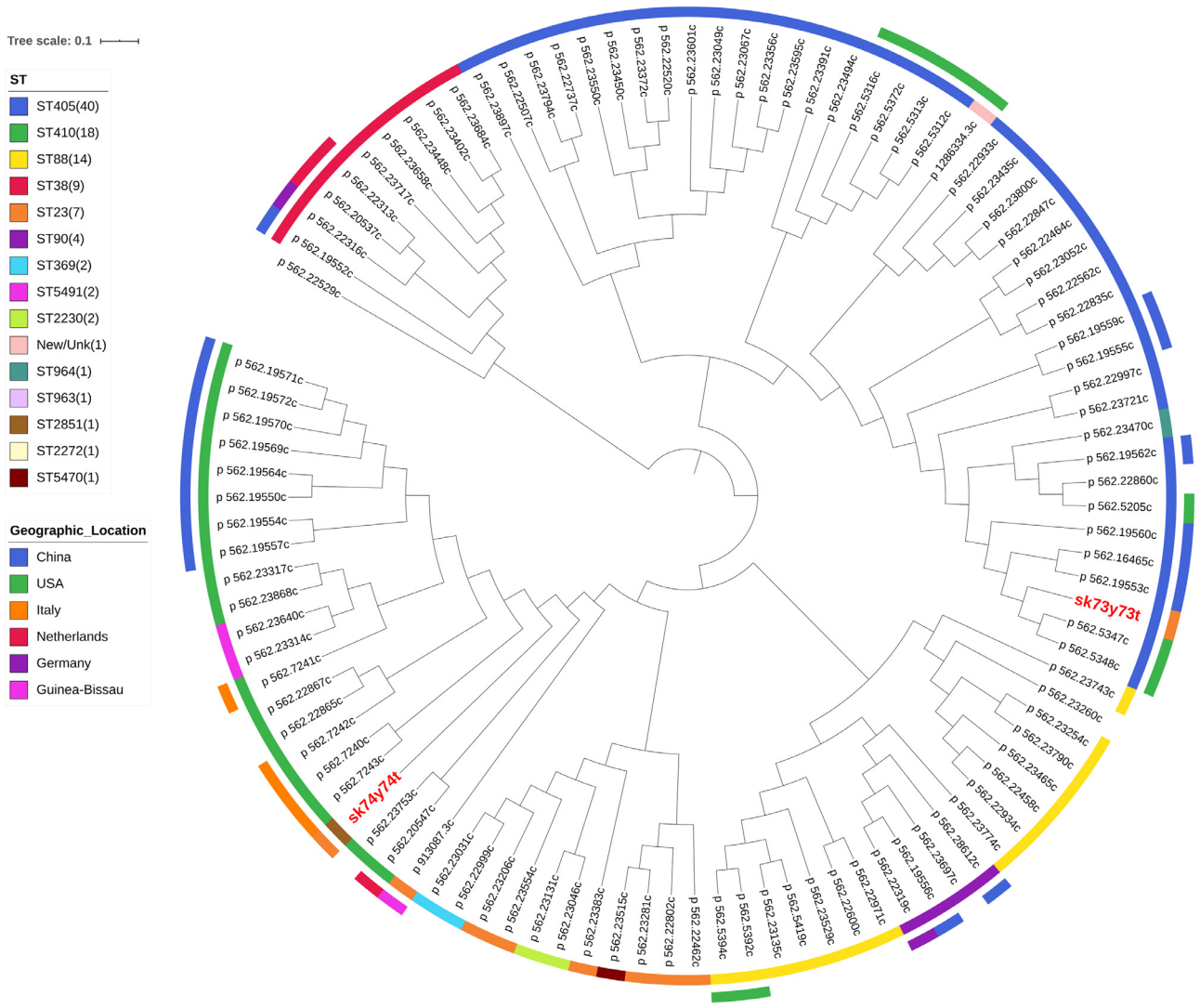

Fig. 1. Maximum likelihood phylogenetic tree including the two *Escherichia coli* isolates of the study (Ec_sk73y73t and Ec_sk74y74t) and 102 background strains retrieved from the PATRIC database. The sequence type of each strain (Achtman scheme) is shown on the coloured inner ring, and the geographic origin is shown on the outer ring.

should be undertaken to avoid this, including infection control and epidemiological surveillance.

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Competing interests

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Ethical approval

Not required.

Consent

Written informed consent was obtained from the two living patients or their legal representatives for publication of their clinical details.

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