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A genetic cluster of OXA-244 carbapenemase-producing *Escherichia coli* ST38 with putative uropathogenicity factors in the Netherlands

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Carbapenemase-producing Enterobacterales (CPE) represent an increasing global problem. Since 2016, the carbapenemase oxacillinase (OXA)-244, a single amino-acid variant of OXA-48 and present in *Escherichia coli* ST38, has been recognized as an emerging carbapenemase variant in several European countries. This prompted the ECDC to publish a rapid risk assessment in February 2021 with a follow-up in July.[1] The increase of OXA-244 was also observed in the Netherlands after analysis of the data from the Dutch national CPE surveillance. For the Dutch CPE surveillance, medical microbiology laboratories are requested to send isolates suspected of carbapenemase production, with a meropenem MIC of ≥0.25 mg/L and/or an imipenem MIC of ≥1 mg/L, or evidence of carbapenemase production, to the National Institute for Public Health and the Environment. Illumina next-generation sequencing (NGS) was performed on all isolates testing positive for carbapenemase production, from persons with a personal identifier code present to be able to detect multiple isolates from the same person.[2] Between January 2016 and June 2021, from 1203 unique CPE isolates (unique combination of species and carbapenemase gene per person), NGS results were available. Of these, 394 (33%; 394/1203) isolates were *E. coli* and NGS data were used for Clermont phylotyping,[3] classical MLST, whole-genome (wg)MLST analysis using an in-house *E. coli* wgMLST scheme, and core SNP analysis [Figure 1 and Figure S1(a and b), available as Supplementary data at *JAC* Online].[4] Among the 394 sequenced carbapenemase-producing *E. coli* isolates, 85 were MLST ST38, of which 30 contained $bla_{OXA-244}$. Seventeen of these 30 OXA-244-producing ST38 *E. coli* isolates formed a genetic cluster based on wgMLST and was termed EcoCluster-023. A genetic wgMLST cluster is defined as ≥2 isolates that differ by ≤25 wgMLST alleles.[4] The 17 EcoCluster-023 isolates differed by 1 to 21 wgMLST alleles from each other [Figure 1(b)]. In addition, core SNP analysis revealed 1 to 62 SNP differences among the 17 EcoCluster-023 isolates. [Figure S1(a and b)]. The wgMLST allelic variation and SNP differences may be explained since isolates were from cultures taken between August 2016 and May 2021, with 13 (76%) isolates from 2020 and 2021. The 17 isolates were derived from 17 different patients, and were submitted by 13 different laboratories from eight different provinces. The median age of the patients was 54 years (range 8 to 92) and 10 (59%) patients were women. Nine (53%) isolates were cultured from urine, and the other eight from rectal swabs. This percentage of isolates derived from urine cultures was considerably higher than the 80 (21.2%) among the other 377 carbapenemase-producing *E. coli* isolates from this study excluding this cluster. All EcoCluster-023 isolates produced carbapenemase, as tested with the carbapenem inactivation method.[5] The MIC of meropenem was below 1.5 mg/L for all isolates, as determined by Etest, therefore all isolates were susceptible according to EUCAST [Figure 1(c)]. Information on risk factors was available for 16 patients. There was no evidence for direct patient-to-patient transmission in this group. Eight (50%) persons had a known connection with Middle Eastern countries, Turkey in five patients and Syria in three. This was either as recent hospitalization in the previous 2 months ($n=2$) or up to a year ago ($n=3$), or as country of birth ($n=6$, including three with previous hospitalization there). Recent hospitalization abroad up to 2 months previous, is considered a risk factor in Dutch national guidelines. The prevalence of OXA-244 in Middle Eastern countries is unknown.

All EcoCluster-023 isolates were sequenced by Illumina NGS and 13 of these with Nanopore long-read sequencing. Genomic assemblies, resistome and replicome analyses were performed as described.[4] Sequence read data are available at the sequence read archive [Figure S1(b)], and circular assemblies at Genbank (PRJNA774636). The EcoCluster-023 ST38 isolates had the O:H-type O86:H18 and belonged to Clermont phylotype D as visualized in the wgMLST tree [Figure 1(a)]. Clermont typing enables determination of phylogroups, which are associated with distinct *E. coli* habitats.[3] The 394 *E. coli* isolates were divided into seven Clermont phylotypes (A, B1, B2, C, D, E, F) in the wgMLST tree, of which B2 (37.5%, $P=0.0084$) and D (30.3%, $P=0.0093$) were significantly associated with urine-retrieved isolates in relation to all other phylotypes, as determined using chi-squared and Fisher's exact tests [Figure 1(a), Table S1]. The association between the B2 and D phylogroups and uropathogenic *E. coli* (UPEC) has been reported before.[6] The $bla_{OXA-244}$ and *mdf*(A) genes of isolates belonging to EcoCluster-023 were located

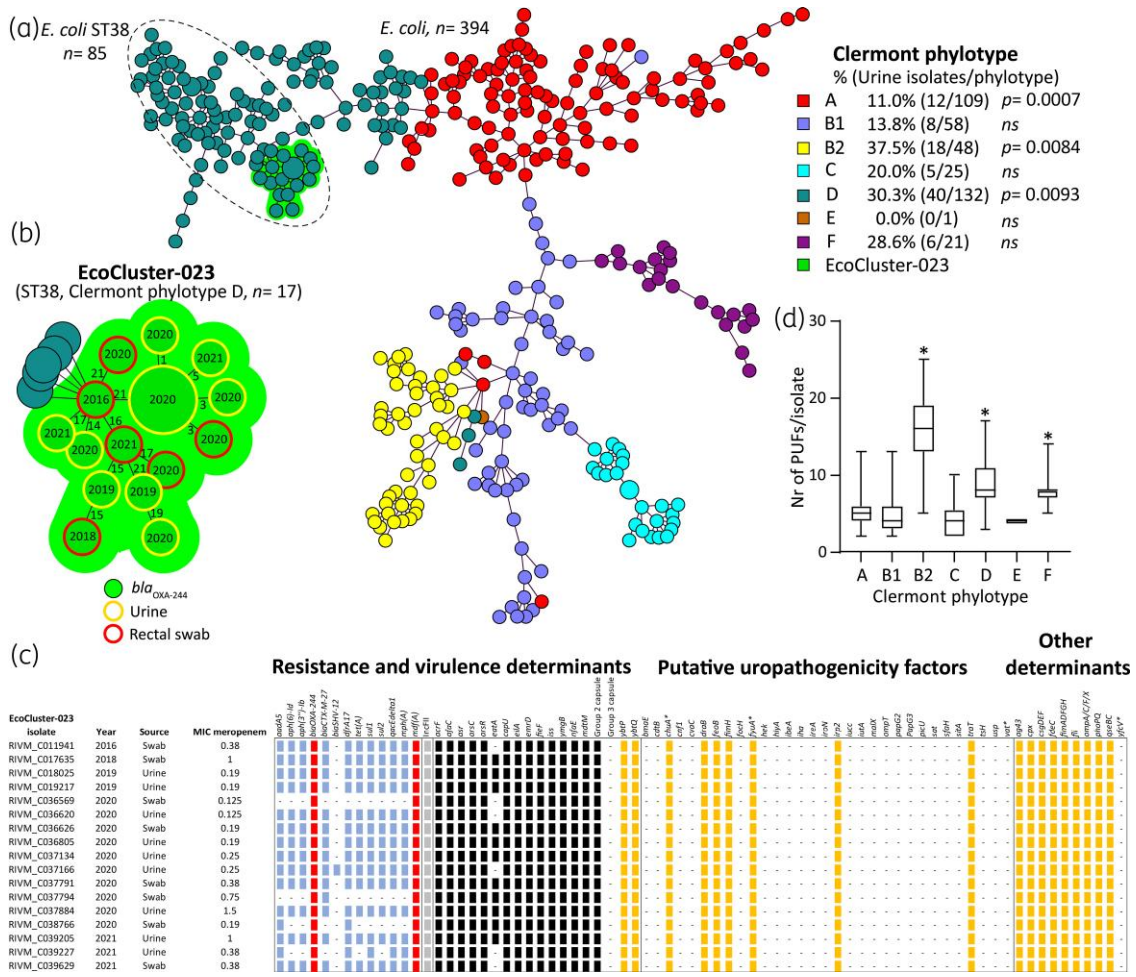

Figure 1. wgMLST tree of 394 carbapenemase-producing *E. coli* isolates showing a genetic cluster of OXA-244-producing *E. coli* ST38. (a) Minimum spanning tree of carbapenemase-producing *E. coli* from the Dutch national surveillance with EcoCluster-023 indicated with a green halo and Clermont phylotypes in multiple colours.[3] *E. coli* ST38 isolates are encircled. Inset: percentage of urine-retrieved isolates per Clermont phylotype. Statistical significance is indicated, *ns* = not significant. (b) Genetic relationship between EcoCluster-023 isolates (marked green) is indicated by allelic differences, each circle represents an isolate and a large circle represents >1 isolate. The year of isolation is marked in the circles. Isolation from urine is indicated in yellow, while rectal swabs are indicated in red. The minimum spanning tree was based on an in-house *E. coli* wgMLST as described previously.[4] The wgMLST cluster was defined as ≥2 isolates of which the genetic distance was ≤25 alleles (25/4503, ≤0.55% different). (c) Molecular characteristics of EcoCluster-023 isolates. Plasmid-localized antimicrobial resistance (AMR) genes are indicated in blue, chromosomal in red, uropathogenicity factors in yellow, other virulence and AMR determinants in black. Asterisks indicate genes implicated in uropathogenicity from Spurbeck *et al.*[11] (d) Number of PUFs per isolate plotted per Clermont phylogroup. Kruskal–Wallis non-parametric test was performed using GraphPad Prism 9.0 ($P < 0.0001$) and statistical significance indicated by an * and in Table S1.

on the chromosome. Genes encoding resistance for aminoglycosides [*aadA5, aph(3')-Ib, aph(6)-Id*], ESBL ($bla_{CTX\text{-}M\text{-}27}$), macrolides [*mph*(A)], quaternary ammonium compounds (*qacE*), sulphonamides (*sul1/2*), trimethoprim (*dfrA17*) and tetracycline [*tet*(A)] were localized on a plasmid of the IncFII family [Figure 1(c)]. There is no established signature set of putative uropathogenicity factors (PUFs) for UPEC that distinguishes them from non-UPEC strains.[6] However, genomic analysis of EcoCluster-023 revealed the presence of 7 of 31 recently described PUFs in the isolates.[6,7] The seven Clermont phylotypes were each characterized by different PUF signatures, of which Clermont phylotypes B2, D and F were significantly enriched in PUFs [Kruskal–Wallis statistic = 219.8, $P < 0.0001$, Figure 1(d), Table S1]. EcoCluster-023 isolates belonging to Clermont phylotype D had a distinct PUF signature when compared with PUF signatures from the other Clermont phylotypes, which may explain its association with urine-retrieved isolates. Other known uropathogenicity determinants were also present in EcoCluster-023, including pili (*fimADFGH*, *csgDEF*), flagella (*fli*), adhesin (*ag43*), two-component systems (*qseBC*, *cpx*, *phoPQ*), siderophores (*ybtQP*), outer membrane proteins (*ompA/C/F/X*) and Group 2 capsule (*kps*), while Group 3 capsule was absent [Figure 1(c)].[6,7] OXA-244-producing *E. coli* have been described as emerging in a number of European countries and the $bla_{OXA\text{-}244}$ gene has been found in different *E. coli* lineages, such as ST38 and ST131.[8–10] Since the EcoCluster-023 isolates do not harbour genetic resistance determinants for a

number of antibiotics commonly used for the treatment of urinary tract infections such as ciprofloxacin, nitrofurantoin and fosfomycin, its prevalence in the Netherlands is most likely underestimated, as GPs only request urinary cultures after first-line treatment failures. As OXA-244-producing *E. coli* generally have low MICs for carbapenems, detection by selective culture methods can be difficult and may lead to further underestimation.[10] As limited clinical information was collected in the Dutch CPE surveillance, the relationship between the PUFs and possible urinary tract infections could not be further assessed. In summary, we observed an increase in urine-associated *E. coli* ST38 producing OXA-244 carbapenemase with putative uropathogenicity factors in the Netherlands.

Ethics

Ethical approval was not needed for the study, since it is based on surveillance data only. Samples, from which the isolates were cultured, were all taken as part of routine healthcare.

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Transparency declarations

The authors have nothing to disclose.

Supplementary data

Table S1 and Figure S1 are available as Supplementary data at *JAC* online.

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