

Increase in OXA-244-producing *Escherichia coli* in the European Union/European Economic Area and the UK since 2013 – first update

20 July 2021

Summary

This update of a previous risk assessment on the same topic has been produced in response to the first evidence of healthcare-associated transmission of OXA-244-producing *E. coli* in the European Union/European Economic Area (EU/EEA), as well as a doubling of cases in the main cluster and three new countries having detected cases. These findings confirm the high risk for further spread of OXA-244-producing *E. coli* in the EU/EEA.

Following an urgent inquiry in ECDC's EPIS AMR-HAI posted by Norway regarding a healthcare-associated outbreak involving 12 cases of OXA-244-producing *Escherichia coli*, national public health reference laboratories in EU/EEA countries were invited to submit to ECDC whole genome sequencing (WGS) data collected since the previous rapid risk assessment (18 February 2020) for an update at European-level. The analysis included WGS data submitted to ECDC from 13 countries, completed with data from the public domain. Among 458 isolates of *E. coli* ST38, 370 carried the *bla*_{OXA-244} gene encoding for the OXA-244 carbapenemase. Several clusters were identified, including one large cluster (cluster A) with 225 closely-related OXA-244-producing *E. coli* ST38 isolates. Of these, 210 isolates were detected in 11 EU/EEA countries and the UK, and 15 isolates were from other countries. The cluster has 20 cgMLST allelic differences from root to tip of the cluster subtree and isolates generally carried both the *bla*_{OXA-244} and *bla*_{CTX-M-27} genes. Cases related to cluster A had a median age of 51 years, a high proportion of women, and the isolates were frequently isolated from urine samples.

The source and route of transmission for OXA-244-producing *E. coli* in the EU/EEA and the UK is currently unclear, and there is a need for further investigation to determine this so that adequate control measures can be implemented. The wide geographical dispersion of cases within countries, without cases being linked in place and time, indicates transmission in the community as the main mode of spread. The epidemiological data are incomplete for many cases. However, from available data, the high proportion of isolates from urine samples (as compared to screening samples and other sample types) and a considerable proportion of outpatients at the time of sampling would also seem to indicate transmission in the community.

The low genetic diversity of isolates in cluster A could have several explanations, including a recently emerging clone, but may also point to a common source, place or region of acquisition for OXA-244-producing *E. coli*. Information on travel was available for only a few cases, limiting the validity of conclusions. Nevertheless, these scarce travel data point to North Africa and the Middle East as potential regions of origin for OXA-244-producing *E. coli* ST38, which is in line with the known distribution of OXA-48-like carbapenemases and previous environmental detection of OXA-244-producing *E. coli* ST38. However, it is unclear whether travel alone could sufficiently explain the widespread and rapid increase in cases in 12 different EU/EEA countries and the UK. Another possibility could be that a source product, potentially originating from the above-mentioned regions, has been distributed to the European countries involved or that there is transmission in the community within the EU/EEA and the UK.

Transmission via food (from animal or non-animal origin), by contact with the environment or direct contact with animals, is hypothetically possible. However, no OXA-244-producing *E. coli* isolates from food or animal sources have so far been identified in the EU/EEA. Imported food or animal products from regions where OXA-48-like carbapenemases are endemic, or even food from domestic sources can therefore neither be excluded, nor confirmed as a possible contributing factor. However, given the potential public health implications, the possibility of food and animal products contaminated with OXA-244 producing *E. coli* being distributed over a large geographical area requires investigation. Therefore, further studies need to be considered to determine the likelihood of the environment, crops, animals, and foods contaminated with OXA-244-producing *E. coli* having contributed to this outbreak.

The observed increase in the number of cases of a difficult-to-detect carbapenemase (OXA-244) in a species (*E. coli*) that causes community-acquired infections is of concern. Data are missing on the clinical consequences of carbapenems being used for treatment of OXA-244-producing *E. coli* infections. However, other OXA-48-like carbapenemases have been associated with treatment failures despite *in vitro* susceptibility. The risk for further spread of OXA-244-producing *E. coli* in the EU/EEA is high, given the rapid and simultaneous increase in various EU/EEA countries. A recent healthcare-associated outbreak in Norway has shown that OXA-244-producing *E. coli* ST38 has the potential to spread in healthcare settings. The difficulties with laboratory detection related to the relatively low level of carbapenem resistance conferred by the OXA-244 carbapenemase are probably resulting in considerable under-detection. Without adaptation of microbiological methods and surveillance, OXA-244-producing *E. coli* may therefore continue to spread unnoticed.

If awareness, sampling frequency and capacity to detect OXA-244-producing *E. coli* is improved in clinical microbiology laboratories, and appropriate infection prevention and control measures are implemented on a timely basis, the risk for transmission within healthcare settings can be reduced. Options for achieving such a reduction include national alerts to clinical microbiology laboratories, laboratory guidance to improve detection of OXA-244-producing *E. coli* and submission of all suspected isolates to national reference laboratories for further analysis. This should be combined with the collection of epidemiological data on cases and associated risk factors including travel and healthcare history, nutritional habits and contact with animals. A questionnaire has been developed by ECDC for further investigation of cases and can be made available upon request. Prevention measures for OXA-244-producing *E. coli* in healthcare settings should be implemented as recommended for other carbapenem-resistant Enterobacterales. For further details, please refer to the 'Options for response' section below.

Event background

Urgent inquiry from Norway

In November 2020, Norway reported to the EPIS AMR-HAI platform the detection of five cases of OXA-244-producing *E. coli* ST38 in three hospitals in the county of Vestland, Norway. The respective isolates originated from clinical samples of patients who were admitted to different wards without a clear epidemiological link. Extensive screening was initiated in the affected wards which led to the detection of four additional cases, three of which had shared a room with one of the previously-identified cases. No further common exposures were identified. Whole genome sequencing (WGS) analysis of six of the OXA-244-producing *E. coli* ST38 isolates involved showed that these isolates were closely related by core-genome multilocus sequence typing (cgMLST) with 0-3 single nucleotide polymorphism (SNPs). Comparison with other OXA-244-producing *E. coli* ST38 isolates from different parts of Norway did not show any relevant genetic or epidemiological link to the above-described cluster in the county of Vestland.

The six typed isolates from the county of Vestland cluster carried an additional extended-spectrum beta-lactamase (ESBL) gene, *bla*_{CTX-M-27}, and were detectable on chromogenic ESBL-screening agar plates. The isolates were resistant to trimethoprim-sulfamethoxazole, but susceptible to ciprofloxacin and aminoglycosides. Analysis of the six outbreak isolates and 11 unrelated OXA-244-producing *E. coli* ST38 isolates showed that all isolates were susceptible to meropenem, in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints, but were identified as suspect carbapenemase producers by the recommended EUCAST carbapenemase screening breakpoints.

The Norwegian National Reference Laboratory (NRL) informed local clinical microbiology laboratories in Norway of the characteristics of the outbreak isolates and the challenges regarding their detection. Local hospitals and clinical microbiology laboratories have therefore been on high alert to identify further cases. In total, 12 cases (six clinical isolates and six screening isolates) were identified as belonging to this cluster, with the last case detected in November 2020. No further cases have occurred since this date and the outbreak is considered to have been controlled.

Data collection

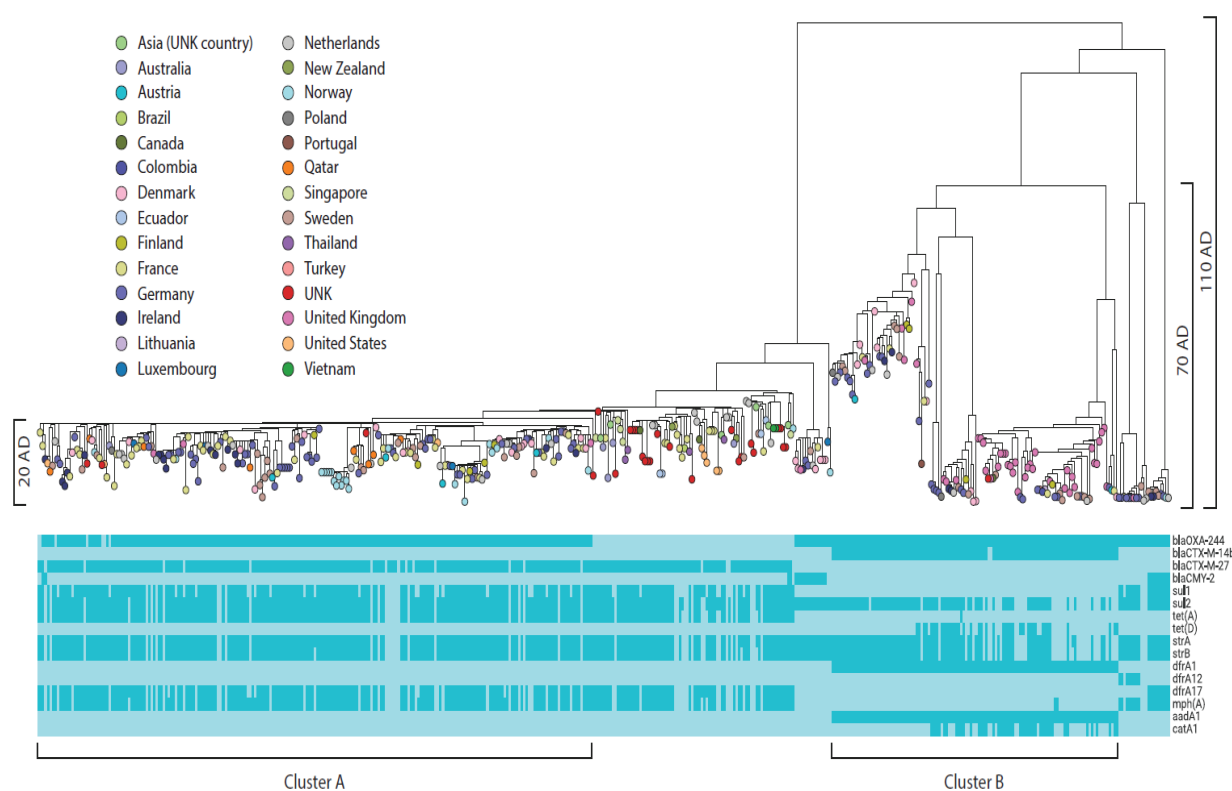
Following the urgent inquiry from Norway, ECDC asked NRLs participating in the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) for additional WGS data on OXA-244-producing *E. coli* ST38 isolates collected since the publication of the previous ECDC rapid risk assessment [1]. WGS data were submitted by 11 EU/EEA countries between November 2020 and January 2021. A total of 620 sequences were available for further analysis, including the data from the previous assessment and additional data identified in the public domain. After removal of duplicates (n=68), sequences that failed quality control (n=25) and outlier isolates (n=69, mainly of sequence types other than ST38), data from 458 isolates were included in the analysis.

Epidemiological analyses mainly focused on OXA-244-producing *E. coli* ST38 isolates to homogenise the dataset, resulting in a total of 370 remaining sequences. Of these, 310 sequences were from EU/EEA countries, either submitted directly to ECDC (n=285) or from the public domain (n=25), including sequences from Austria (n=6), Denmark (n=32), Finland (n=9), France (n=49), Germany (n=91), Ireland (n=24), Lithuania (n=1), Luxembourg (n=4), the Netherlands (n=22), Norway (n=24), Poland (n=3), Portugal (n=1), Sweden (n=44), and the United Kingdom (n=45). In open-access databases, additional sequences of *E. coli* ST38 isolates with the *bla*_{OXA-244} gene were available from Canada (n=1), Colombia (n=1), Qatar (n=8), Turkey (n=1), United States (n=2) and other unknown countries of origin (n=2).

WGS analysis

Ten European countries (EU/EEA and the United Kingdom (UK)) submitted raw sequence data for analysis, while the three remaining countries (Ireland, Norway, and Poland) contributed data already assembled using SPAdes. Closely-related sequences from the public domain (n=143) were found in NCBI pathogen detection using the SNP cluster code and downloaded through the Short Read Archive. The combined dataset was analysed in Bionumerics, using the Enterobase [2] core genome MLST scheme (2500 loci) and the Bionumerics *E. coli* plugin for genotypic resistance and virulence determination. All sequences with >2000 core genome loci determined were included in the analysis (n=458). The allelic profiles were clustered using neighbour joining and the results were visualised using MicroReact [3]. Annotated long-read closed complete genome sequences, including plasmids provided by the Netherlands (n=6) and from the public domain (n=1), were also analysed to determine the genomic location of resistance and virulence genes.

Figure 1. Neighbour joining tree of *E. coli* ST38 non-outlier sequences collected from EU/EEA countries and the UK and from the open-access databases (n=458)



The tree was constructed using Bionumerics, with the Enterobase *E. coli* core genome MLST scheme. The bottom part of the figure shows the presence (dark blue), or absence (light blue) of resistance genes that commonly occurred in the dataset. Cluster A spanned 20 cgMLST allelic differences (AD) from root to tip, while the entire tree spanned about 110 AD from root to tip.

Several *E. coli* ST38 clusters were identified as shown in Figure 1, including cluster A, with 20 cgMLST allelic differences (AD) from root to tip of the cluster subtree. Cluster A contained 225 isolates from 12 European countries (11 EU/EEA countries and the UK) that had contributed sequence data, plus isolates from Australia, Qatar, and the United States. These isolates were detected between 2016 and 2020. Isolates in cluster A generally carried both the *bla*_{OXA-244} and *bla*_{CTX-M-27} genes. In addition, most of the isolates in this cluster carried genes encoding resistance to tetracycline, sulfonamides, trimethoprim, aminoglycosides, and macrolides (Figure 1). One long-read sequenced genome provided by the Netherlands matched cluster A. This genome carried *bla*_{OXA-244} on the chromosome and included a 147 kbp resistance plasmid containing *sul1*, *sul2*, *strA*, *strB*, *tet(A)*, *aadA5*, *bla*_{CTX-M-27}, *mph(A)*, and *dfrA17* and a 211 kbp plasmid without any resistance genes. The *bla*_{OXA-244} gene generally appeared on very short contigs (consensus sequences originating from the assembly of short, overlapping DNA fragments) in the short-read data assemblies, so it is not possible to determine if it was located on the chromosome for all isolates in cluster A. However, this is likely, given the close genetic relationships within the cluster.

Another cluster (cluster B) of 116 isolates was detected (Figure 1). The isolates in cluster B carried both the *bla*_{CTX-M-14b} and *bla*_{OXA-244} genes, both on the chromosome, as indicated by three different long read sequenced genomes matching this cluster, plus a variable set of plasmid-borne resistance genes and virulence genes. Cluster B contained several historical isolates from the 2013–2015 EuSCAPE study [4] and had an overrepresentation of isolates from the UK (40 of 55 isolates from the UK belonged to this cluster).

A large set of outlier isolates (n=69) from Austria, Denmark, Ireland, the Netherlands, Norway, the UK and the United States was also detected (not shown in Figure 1). These outlier isolates carried *bla*_{OXA-244} plus a variable set of additional resistance genes. Three long-read sequenced genomes were among these outliers, all carrying *bla*_{OXA-244} on the chromosome. An interactive phylogenetic tree (including genotypic resistance, virulence gene characterisation, year and country of isolation where available) of all included isolates can be found at: <https://microreact.org/project/kicD5FD8jrocYNvxjoR9GG/69aae051>.

Epidemiological analysis

Case definitions

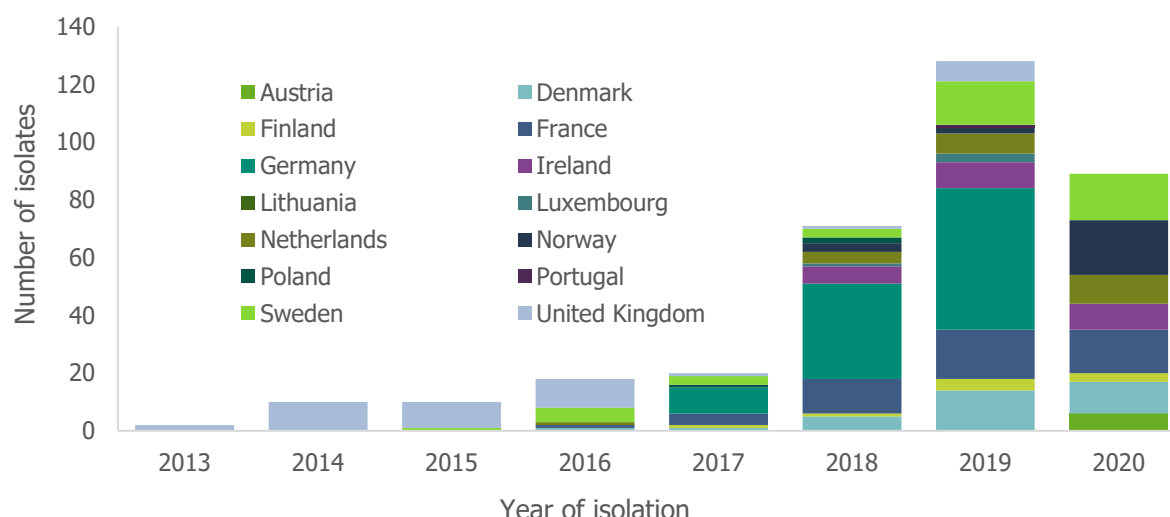
Based on the WGS analysis above, cases were defined as:

1. Cases of *E. coli* ST38: all cases detected with *E. coli* ST38 isolates included in Figure 1 (n=458);
2. Cases of OXA-244-producing *E. coli* ST38: subgroup of group defined under 1. with isolates carrying the *bla*_{OXA-244} gene (n=370) as shown in Figure 1 - first row below the tree (n=370);
3. Cases in cluster A: subgroup of group defined under 1. with isolates belonging to the closely-related cluster within a 20 AD range displayed in Figure 1 and, in general, identifiable by co-carriage of *bla*_{OXA-244} and *bla*_{CTX-M-27} (n=225);
4. Cases in cluster B: subgroup of group defined under 1. with isolates belonging to the less closely-related cluster with ~70 AD as marked in Figure 1 and in general identifiable by co-carriage of *bla*_{OXA-244} and *bla*_{CTX-M-14b} (n=116).

OXA-244-producing *E. coli* ST38 isolates

The 458 *E. coli* ST38 non-outlier isolates displayed in Figure 1 have a global distribution and have been detected in Asia, North America, South America, Oceania, and Europe. However, the detection in specific countries might reflect capacity for detection and reporting of AMR bacteria and WGS capacity rather than the geographical distribution of *E. coli* ST38. When focusing on OXA-244-producing *E. coli* isolates (n=370), most of the cases have been detected in EU/EEA countries and the UK; 355 (95.9%) of the 370 isolates. Only a few of the cases from the EU/EEA (n=22) were detected in the three years before 2016, followed by a moderate increase in 2016 (n=18) and 2017 (n=20) (Figure 2). A strong increase in the number of cases was observed in 2018 (n=71) and 2019 (n=128). Data for 2020 are not yet complete (n=89), and for seven isolates no date of isolation was available.

Information on the type of sample was available for 338 (91.4%) of 370 cases of OXA-244-producing *E. coli*. Of these, 171 (50.6%) isolates originated from urine samples and 121 (35.8%) from screening samples. Isolation from blood (n=12, 3.6%) and various other sites (n= 34, 10.1%) was rare. Most of the 208 cases for which information on gender was available were women (n=145, 69.7%) and the median age was 54 years (Table 1). Information on outpatient/inpatient status was only available for 108 (29.2%) cases. While many of these cases were admitted to hospital at the time of detection of OXA-244-producing *E. coli* ST38 (n=64, 59.3%), a considerable proportion were documented as outpatients (n=44, 40.7%) (see table in Annex 1). However, the epidemiological information presented may be biased by different sampling approaches in the countries in relation to screening and clinical sampling.

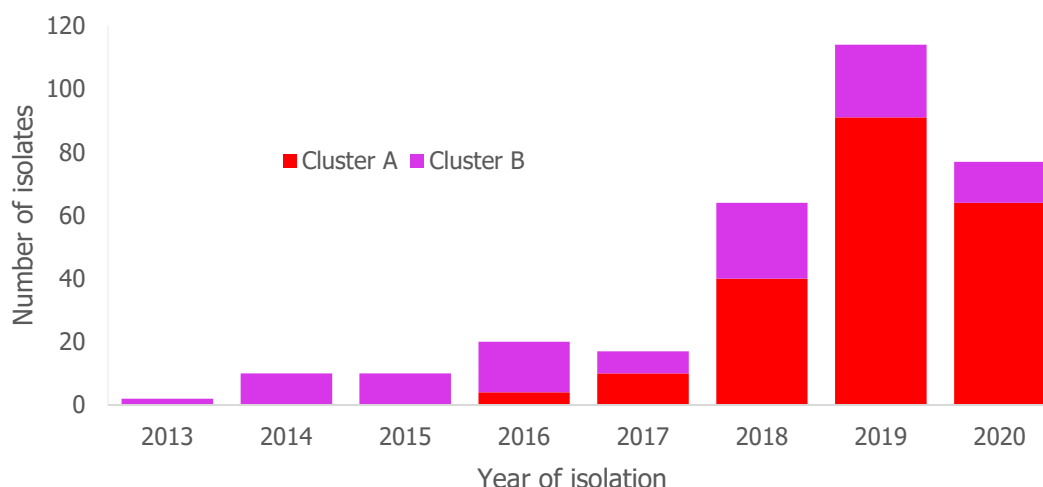
Figure 2. Number of OXA-244-producing *E. coli*/ST38 isolates per year, EU/EEA and the UK, 2013–2020 (n=348)*

* The figure only includes cases for which WGS data and year of isolation are available (n=348).

Cases related to clusters A and B

Ten of the 13 EU/EEA countries submitting data for this assessment detected isolates from clusters A and B. Of the three remaining countries, Luxembourg and Norway only detected cases in cluster A, while Portugal only reported one case in cluster B. Cluster A (previously 'main cluster') has increased considerably in size and now involves 225 cases, compared to 114 cases presented in the previous rapid risk assessment. All these cases were detected in the EU/EEA and the UK, with the exception of 15. While cases in cluster B occurred earlier and also continue to be detected at a lower and more stable level, it is cluster A that is chiefly responsible for the large increase in OXA-244-producing *E. coli* ST38 cases in EU/EEA countries since 2017 (Figure 3). Even if the data must be interpreted with caution due to many missing values, there seems to be a tendency towards a higher proportion of women (75% versus 65%, for isolates with available information), a higher proportion of isolates originating from urine samples (50.9% versus 43%, for isolates with available information) and a lower median age (51 versus 57 years) in cases linked to cluster A compared to cluster B. It should be noted that 25 cases in these clusters were children under ten years, with 20 and five cases in clusters A and B, respectively.

An attempt was also made to determine closely-related clusters with possible recent transmission in the dataset, with a threshold of seven AD, based on the phylogenetic distance of isolates in the Norwegian hospital outbreak cluster representing cases that occurred within a defined time period of five months and in a limited geographical area. However, this approach resulted in the detection of 62 closely-related clusters within seven AD among the 458 *E. coli* ST38 isolates in this dataset, including clusters with isolates from up to 16 countries and isolates occurring over a period of up to six years. This approach was not used for further epidemiological analysis.

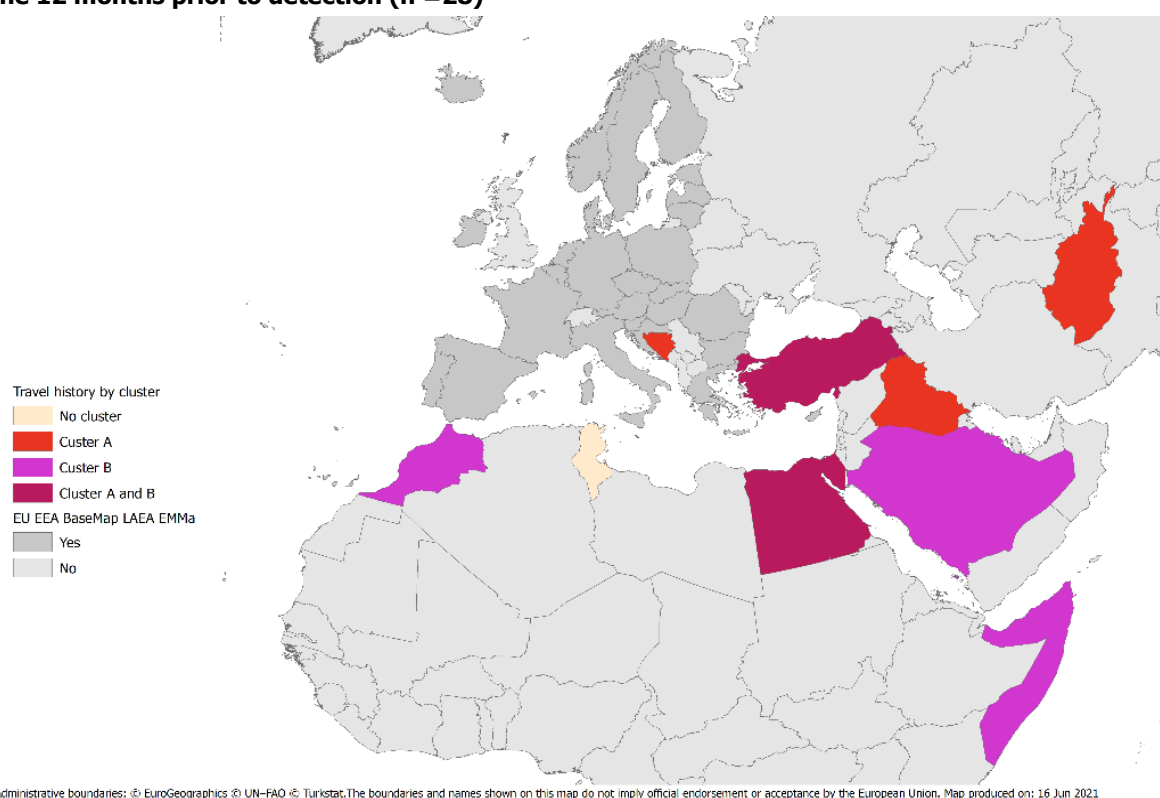
Figure 3. Number of OXA-244-producing *E. coli* ST38 isolates in clusters A and B per year, EU/EEA and the UK, 2013–2020 (n=314 isolates)*

* Only cases with available year of isolation are shown.

Travel history

Data on the travel history outside of the EU/EEA in the 12 months prior to isolation of *E. coli* ST38 were only available for 44 (9.6%) of the 458 cases, with high variability of data availability between countries, ranging from missing data for all national cases to relatively complete documentation. The most frequent travel destinations for cases were Egypt (n = 15), and Turkey (n=6). All other countries mentioned (Afghanistan, Bosnia and Herzegovina, Iraq, Morocco, Saudi Arabia, Somalia, and Tunisia) were only visited by one case each (Figure 4, table in Annex 1). The data are difficult to interpret due to the many missing values. In addition, the high frequency of documented travel to Egypt and Turkey may simply reflect the preferred destinations of citizens with available travel information. Nevertheless, the scarce travel information may suggest a link to North Africa and the Middle East as potential regions of origin for OXA-244-producing *E. coli* ST38, which is in line with the known distribution of OXA-48-like carbapenemases and previous environmental detection of OXA-244-producing *E. coli* ST38. It should be noted that 14 cases were explicitly documented as not having travelled in the 12 months before detection of OXA-244-producing *E. coli* ST38 which raises the question as to whether there may be other routes of introduction or community transmission within the EU/EEA.

Figure 4. Countries of travel outside of the EU/EEA for cases of OXA-244-producing *E. coli* ST38, in the 12 months prior to detection (n =28)



Disease background

Disease spectrum

E. coli is the most common pathogen responsible for urinary tract infections in the community. At the same time, *E. coli* is also the most common cause of bacteraemia in high-income countries with an estimated case fatality of 12% in a recent review [5]. Approximately 44% of the bacteraemia episodes analysed in this review were classified as community-acquired, 27% as community-onset healthcare-associated, and 27% as hospital-acquired, and the primary source was most frequently a urogenital infection, followed by biliary and other intra-abdominal infections [5]. The incidence of *E. coli* bacteraemia increased considerably with age [5]. According to the 2017 report of the Healthcare-associated Infections Surveillance Network (HAI-Net), for healthcare-associated infections in intensive care units in the EU/EEA, *E. coli* was associated with 32.1% of urinary tract infections, 13.5% of pneumonia episodes and 9.2% of bloodstream infections [6]. However, not all *E. coli* sequence types are equally likely to cause extraintestinal infections. Only a limited set of extraintestinal pathogenic *E. coli* (ExPEC) lineages has been described as being responsible for most *E. coli* infections [7]. *E. coli* ST38 has been identified as one of the 20 main ExPEC sequence types responsible for the majority of extraintestinal infections caused by *E. coli* [7]. Moreover, *E. coli* ST38 occurs globally and often carries genes encoding for extended-spectrum-beta-lactamases (ESBLs) [8] and has been responsible for urinary tract infections [8,9] and bloodstream infections in human patients [10].

Carbapenem resistance in *E. coli*

According to 2019 surveillance data from the European Antimicrobial Resistance Surveillance System (EARS-Net), carbapenem resistance is rarely reported among invasive (bloodstream and cerebrospinal fluid) *E. coli* isolates, with only Greece and Spain reporting 1% to <5% of *E. coli* isolates as carbapenem-resistant [11]. In all other EU/EEA countries, this proportion was below 1% [11]. Although carbapenem-resistant isolates remained rare among the invasive *E. coli* isolates included in EARS-Net, there was a small but significant increase in the EU/EEA population-weighted mean between 2015 and 2019 [12]. In addition, it should be noted that carbapenem resistance proportions reported by EARS-Net are based on EUCAST clinical breakpoints for meropenem and/or imipenem and do not include ertapenem [12]. Therefore, many of the isolates in the data set analysed for this rapid risk assessment would probably be recorded as carbapenem-susceptible if reported to EARS-Net.

There is no European surveillance system for monitoring carbapenem resistance in non-invasive isolates (i.e. from infections other than those of the bloodstream or cerebrospinal fluid) or isolates from screening samples. A multinational study including Finland, Germany, Latvia, Poland, Sweden and Russia did not find resistance to meropenem in 775 *E. coli* urinary isolates collected between October 2015 and January 2017 [13], whereas in the 2013–2014 EuSCAPE survey most participating countries submitted at least one carbapenem-resistant *E. coli* isolate [4]. Urine was the most common site of isolation [4].

Epidemiology of OXA-244 producing *E. coli*

OXA-244, an Ambler class D carbapenemase, is a variant of the OXA-48 carbapenemase, which is endemic in North Africa, Turkey and the Middle East [14]. OXA-244 is characterised by a single Arg214Gln mutation compared to OXA-48 [15] and was first described in 2012, in Malaga, Spain, in an isolate of *Klebsiella pneumoniae*. The *bla*_{OXA-244} gene was found on a plasmid and the isolate was phenotypically resistant to ertapenem, but not imipenem and meropenem, according to EUCAST clinical breakpoints [16]. The *bla*_{OXA-244} gene has since been found on both plasmids and integrated into the chromosome in various genetic environments [17,18]. With the exception of the first description in *K. pneumoniae*, the OXA-244 carbapenemase has mainly been identified in *E. coli* and not in other species of Enterobacterales. In *E. coli* it has been found in various sequence types (see below) and most frequently in ST38.

In the EU/EEA, the first OXA-244-producing *E. coli* isolate was reported in Germany in 2013 [19], followed by further reports of increasing regional and national dissemination of OXA-244-producing *E. coli* ST38 [20,21]. Apart from the data presented in the previous ECDC rapid risk assessment from February 2020, national reports including data on OXA-244-producing *E. coli* ST38 have also been published from France [15,18], the Netherlands [22,23] and Denmark [24]. Detection of *bla*_{OXA-244} has also been reported for *E. coli* sequence types other than ST38 in Germany (ST10, ST46, ST69, ST131, ST167, ST224, ST227, ST295, ST361, ST648, ST1722, ST2206, ST3268, ST3541, ST5051) [21,25], France (ST10, ST69, ST131, ST167, ST224, ST361, ST617, ST648, ST1193, ST1722, ST3541) [18], Denmark (ST10, ST69, ST167, ST361 and ST3268) [24], the Netherlands (ST69, ST99, ST349, ST361, ST1722) [23], Ireland (ST1722) [26], Belgium (ST not reported) [27], and Czechia (ST167) in a case with prior hospitalisation history in North Africa [28]. Outside of the EU/EEA, OXA-244-producing *E. coli* clinical isolates have been reported in the UK [29], Switzerland [30], Russia [31], Turkey [32], Egypt [33,34] and Colombia [35].

Laboratory detection of OXA-244-producing *E. coli*

The OXA-244 carbapenemase is expressed at low levels due to a limited copy number of the gene on the chromosome and also only has low-level hydrolytic activity against carbapenems [36]. OXA-244-producing isolates are therefore frequently susceptible to meropenem and/or imipenem based on clinical breakpoints which makes them difficult to detect using clinical routine testing in microbiology laboratories. For OXA-244-producing *E. coli* isolates in the dataset analysed for this rapid risk assessment for which meropenem minimum inhibitory concentrations (MICs) were available (n=124), many (n=113, 91%) were fully susceptible according to the EUCAST clinical breakpoint for meropenem. Similarly, 67 (99%) of 68 isolates with available MIC data were susceptible to imipenem according to the clinical breakpoint. However, 101 (83%) of 122 isolates had an MIC above the EUCAST screening breakpoint of >0.125 mg/L for meropenem requiring further testing for carbapenemase production [37]. OXA-244-producing *E. coli* isolates have also been reported to be less frequently susceptible to ertapenem [17]. Ertapenem MICs were available for only 54 isolates in the dataset analysed for this rapid risk assessment. Of these, 52 (96%) isolates were resistant according to the EUCAST clinical breakpoint and all were detected by the EUCAST screening cut-off. However, this preliminary information would need to be validated by more complete and systematic antimicrobial susceptibility testing (AST).

Due to the low level of carbapenem resistance, OXA-244-producing *E. coli* often do not grow on media used to screen for Enterobacterales producing other carbapenemases such as *K. pneumoniae* carbapenemase (KPC) or New Delhi metallo-beta-lactamase (NDM) [17,38]. In addition, a reduction in the hydrolysis of temocillin caused by the Arg214Gln mutation has also been shown in biochemical assays and in silico studies [36]. This affects the performance of screening media using temocillin [38] and algorithms using high-level temocillin resistance for confirmation of OXA-48 production. Moreover, in the analysis performed for this rapid risk assessment, OXA-244-producing *E. coli* frequently also carried ESBL genes, such as *bla*_{CTX-M-27} or *bla*_{CTX-M-14b} (Figure 1) and has been reported to grow on screening media for detection of ESBL-producing Enterobacterales [17]. Therefore, these isolates may be reported as ESBL-producing *E. coli* based on phenotypic testing results, unless other methods are used to screen for the presence of OXA-48-like carbapenemases.

Commercial and in-house rapid diagnostic tests have been developed for the detection of the five most common carbapenemases, including OXA-48-like carbapenemases. These include immunochromatographic assays [39,40], multiplex nucleic acid-based methods [41,42], colorimetric methods [17,41,43], and various in-house culture-based techniques [41,44]. Some of these methods have shown good performance, not only when tested on isolated bacterial colonies, but also on positive blood cultures [27,45–48] or rectal swabs [49,50], which if applied could reduce the turnaround time. Nevertheless, none of these methods can specifically identify which of the OXA-48-like variants is present and very few OXA-244-producing isolates have been included in the respective validation studies for these tests.

An in-house PCR protocol has been reported to rapidly detect the OXA-244-producing *E. coli* ST38 isolates of the main cluster in Germany [21] (cluster A in this assessment), but this PCR protocol does not allow for the detection of OXA-244-producing *E. coli* ST38 isolates outside of this cluster or of *E. coli* isolates of other sequence types. Nevertheless, given that the increase in the EU/EEA is predominantly driven by *E. coli* ST38 cluster A isolates, this PCR protocol might be an alternative in situations where WGS capacity is limited. At the reference laboratory level, however, WGS remains the best option for confirmation of *bla*_{OXA-244} and determination of the sequence type of *E. coli* isolates. In previous investigations the delay in availability of WGS results has hampered further investigation of cases and many EU/EEA countries still lack sufficient WGS capacity for surveillance of antimicrobial resistance at national level. The various difficulties with laboratory detection of OXA-244-producing *E. coli* isolates described above, including susceptibility of isolates to the most frequently tested carbapenems according to clinical breakpoints, lack of growth on classical screening media for carbapenemase-producing Enterobacterales, and lack of reference capacity for WGS are probably resulting in considerable under-detection of OXA-244-producing *E. coli* in the EU/EEA.

Monitoring of AMR in zoonotic and commensal bacteria

Since 2014, the monitoring of antimicrobial resistance in zoonotic and commensal bacteria has been fully harmonised across the European Union (Commission Decision 2013/652/EU) [51] and is performed by the national competent authorities and national reference laboratories on AMR from the veterinary and food sectors. *Salmonella* spp. and indicator commensal *E. coli* isolates collected for AMR monitoring are tested for susceptibility to meropenem. Since 2015, the AMR monitoring has also included mandatory specific monitoring ESBL-/AmpC-/carbapenemase-producing *E. coli* from food-producing animal matrices and their meat, using selective media containing cefotaxime for all EU Member States. In addition, voluntary carbapenemase-specific monitoring is also recommended, using selective pre-enrichment and subsequent plating on selective carbapenem-containing media that allow for the detection of OXA-producing isolates.

If meropenem resistance is detected (MIC > 0.125 mg/L), further testing is performed for a combination of beta-lactams, including temocillin, imipenem and ertapenem. All presumptive carbapenemase-producing isolates are subjected to WGS at the EURL-AR (DTU, Copenhagen) or in the EU/EEA countries. As reported in the last EFSA and ECDC report on this issue, published on 24 February 2021, no OXA-244-producing isolate was detected until 2019 [52]. However, OXA-48-producing *E. coli* was isolated from a fattening pig in Germany in 2018 [52]. OXA-162-producing *E. coli* was also previously detected in poultry and poultry meat in Romania as a result of the AMR monitoring [53]. Data from the monitoring are reported to EFSA which prepares the EUSR-AR reports in collaboration with ECDC [53].

Risk assessment questions

What is the risk for further spread of OXA-244-producing *E. coli* in the EU/EEA?

ECDC risk assessment for the EU/EEA

Frequency of occurrence

The status of spread of OXA-244-producing *E. coli* in the EU/EEA is unclear. Carbapenem resistance in *E. coli* has so far been rare in Europe [54], with most countries reporting less than 1% of invasive *E. coli* isolates as carbapenem-resistant. However, due to the low level of carbapenem resistance conferred by the OXA-244 carbapenemase, OXA-244-producing *E. coli* might not be detected and reported as carbapenem-resistant in surveillance systems such as EARS-Net. As described in the section on laboratory detection, under-detection is likely, unless diagnostic procedures are adapted. An EU/EEA-wide analysis of spread is not possible due to the lack of reference WGS capacity in many EU/EEA countries. Nevertheless, 12 of 13 countries that could contribute data to this analysis had isolates in cluster A and 11 countries had isolates in cluster B, indicating widespread dissemination in the EU/EEA.

Treatment options

Due to scarcity of clinical data, there is general uncertainty as to the best treatment options and strategies for OXA-48-producing Enterobacterales [55]. This also applies to Enterobacterales producing OXA-48-like carbapenemases such as OXA-244. While it might still be possible to treat infections with OXA-244-producing *E. coli* using meropenem, data are missing on the clinical consequences of carbapenems being used for treatment of OXA-244-producing *E. coli*.

infections. Despite the low hydrolytic activity of OXA-48-like enzymes, there has been a discordance between in vitro susceptibility test results and in vivo outcomes of infections with isolates producing OXA-48-like enzymes, and carbapenems have shown poor bactericidal activity in animal models despite low MICs [56]. It should be noted that most of the OXA-244-producing *E. coli* isolates in this analysis also produced ESBLs. The isolates may be susceptible to other classes of antibiotics and treatment decisions should be made on an individual basis based on AST results, in consultation with an infectious disease specialist or a clinical microbiologist.

Potential routes of introduction and spread

Transmission in healthcare settings

Healthcare settings provide an environment with a high selection pressure due to antimicrobial use favouring multidrug-resistant organisms. Due to the difficulties in detecting OXA-244-producing isolates in clinical microbiology laboratories, appropriate infection prevention and control measures for containing highly-resistant pathogens might not be implemented, which increases the likelihood of transmission in hospitals. The outbreak in Norway involving 12 cases highlights the potential for healthcare-associated transmission of OXA-244-producing *E. coli* in EU/EEA countries when introduced into healthcare settings. A recent genomic study also demonstrated that transmission events, also of antimicrobial-susceptible *E. coli*, occur frequently in hospital settings [57].

Travel

Disturbances in the gut microbiota, for example during international travel, might facilitate the acquisition of ExPEC lineages [7] such as *E. coli* ST38, and there are reports on carriage of carbapenemase-producing *E. coli* ST38 following travel. In a Swiss study, a healthy individual who reported annual visits to Turkey was found to carry an OXA-48-producing *E. coli* ST38 ExPEC lineage D [58]. In another study in the Netherlands, one traveller persistently carried an OXA-244-producing *E. coli* ST38 for six months after visiting Indonesia, and the co-travelling spouse also became positive three months after travel [22]. In both the studies mentioned, the cases described had no exposure to local healthcare during travel [22,58]. In this analysis, epidemiological data on travel or previous hospitalisation were only available for a few patients, limiting the assessment of travel as a risk factor. Nevertheless, whenever such information was available, Turkey and Egypt were relatively common in being reported as previous travel destinations or countries of hospitalisation. OXA-48-like carbapenemases are endemic in both countries [14]. On the other hand, both countries are also popular tourist destinations for people in the EU/EEA, and therefore travel history may reflect the most frequent travel destinations. Nevertheless, it is likely that acquisition during travel contributes to the introduction of OXA-244-producing *E. coli* into the EU/EEA.

Environment

E. coli is an indicator for faecal contamination in the environment originating from both humans and animals. *E. coli* ST38 has been shown to be present in wastewater, and has been isolated from different types of food, including vegetables and chicken meat [59,60]. WGS data on several related isolates originating from environmental sources were retrieved from open-access databases - e.g. an isolate from a wastewater in-flow in the United States. OXA-244-producing *E. coli* ST3541 have been found in wastewater in Algeria [61] and *E. coli* ST38 in an estuary in Lebanon [62]. The authors of the study in Lebanon stated that 'even though water from estuaries is not intended for human consumption, it is used to water animals and irrigate crops' [62]. OXA-48-producing *E. coli* ST38 have also been detected in wastewater in Basel, Switzerland [63]. Related *E. coli* ST38 isolates from both water and humans have previously been described [64]. Furthermore, *E. coli* ST38 has been documented in pets, livestock and wild animals [65-69]. Flies on a cattle farm in Japan have been shown to carry ESBL-producing *E. coli* ST38 [70]. Recently, an OXA-244-producing *E. coli* ST361 was isolated from the nasal swab of a dog in Egypt [71].

Food and animal products

Transmission via food (from animal or non-animal origin), as a result of contact with the environment or direct contact with animals, is hypothetically possible. However, no OXA-244-producing *E. coli* isolates from food or animal sources have so far been identified in Europe. Therefore, imported food or animal products from regions where OXA-48-like carbapenemases are endemic, or even food from domestic sources cannot be either excluded or confirmed as a possible contributing factor. *E. coli* ST38 isolates that did not carry the *bla*_{OXA-244} gene have previously been found in meat, fowl and humans in Germany [60], and in 'retail chicken' in Norway, with related isolates also found in humans [67]. In a Swiss study investigating imported vegetables for the presence of ESBL-producing Enterobacteriales, *E. coli* ST38 was isolated from okra originating from India and curry leaves from the Dominican Republic. Both isolates also carried *bla*_{CTX-M-14}, but were not investigated for the presence of carbapenemase genes encoding for carbapenemases [59]. Clonal *E. coli* ST38 isolates have also been found in Swiss 'retail meat' [72]. In Algeria and Lebanon, OXA-48-producing *E. coli* ST38 has been found in fowl [73,74]. In a prospective study in the United States, 'retail meat' was shown to be a possible source of *E. coli* responsible for community-acquired urinary tract infections, and ST38 was a common sequence type for both humans and 'retail meat' isolates [75]. Further investigation of crops, animals and foods as a possible source of OXA-244-producing *E. coli* should therefore be considered, although at present there are no reports on the detection of OXA-244-producing *E. coli* from these sources in the EU/EEA in scientific literature or from the ongoing harmonised monitoring in the EU.

Risk of further spread

The observed increase in the number of cases of a difficult-to-detect carbapenemase (OXA-244) in a species (*E. coli*) that causes community-acquired infections is of concern. The risk of OXA-244-producing *E. coli* spreading further in the EU/EEA is high, given the rapid and simultaneous increase in multiple countries between 2016 and 2019 despite the difficulties with laboratory detection. *E. coli* ST38 is a high-risk sequence type of *E. coli* due to its ability to cause extra-intestinal infections, its global distribution, and its association with antimicrobial resistance. There is a need to determine the extent to which OXA-244-producing *E. coli* is already established in the community and the environment. However, the increase in cases over the last few years may also partially be attributable to increased awareness, changes in laboratory testing methodology and the availability of molecular methods, including WGS.

The outbreak in Norway has shown that OXA-244-producing *E. coli* has the potential to spread in healthcare settings. If awareness, sampling frequency and capacity to detect OXA-244-producing *E. coli* is improved in clinical microbiology laboratories, and appropriate infection prevention and control measures are implemented when detected, the risk of transmission within healthcare settings can be reduced. However, without adaptation of microbiological methods and surveillance, OXA-244-producing *E. coli* may continue to spread unnoticed. Community-associated spread of OXA-244-producing *E. coli* could potentially reach a larger and healthier population than that normally linked to healthcare-associated spread. Standard control approaches to reduce healthcare-associated spread, such as infection prevention and control measures and antimicrobial stewardship, would probably be ineffective if acquisition of OXA-244-producing *E. coli* is independent of healthcare and prior antimicrobial use (for example, due to acquisition during travel or from a food source.) There is therefore a need for further investigation to determine the sources and routes of transmission of OXA-244-producing *E. coli* in the EU/EEA, so that adequate control measures can be implemented.

Options for response

For carbapenem-resistant Enterobacterales (CRE) control measures in general, please refer to the ECDC Rapid Risk Assessment on Carbapenem-resistant Enterobacteriaceae – second update, 26 September 2019 [76].

Awareness and laboratory capacity to identify OXA-244 carbapenemase

There is a need to increase clinical and public health awareness and the capacity for detection of OXA-244 carbapenemase-producing *E. coli* in clinical microbiology laboratories throughout the EU/EEA. Use of the EUCAST screening breakpoints for carbapenemase-producing Enterobacterales [37] is recommended, since many isolates for which data were available would have been detected using these breakpoints, whereas clinical breakpoints, at least for meropenem and imipenem, are not suitable for detection of OXA-244 producing *E. coli*. However, as data were missing for most isolates, this needs to be confirmed with additional AST results. Strategies and protocols to screen for carriage of OXA-244-producing *E. coli* need to be developed as there is currently limited evidence regarding the optimal strategy for detecting OXA-244. A systematic collection of OXA-244-producing *E. coli* isolates at the national reference laboratories would improve understanding of the extent of their spread. Given the various clusters of *E. coli* ST38 isolates in this dataset and the association of *bla*_{OXA-244} with other sequence types of *E. coli*, WGS is the most appropriate method for identifying *bla*_{OXA-244} and sequence types of *E. coli*. However, WGS results would need to be available in a timely manner for the purposes of surveillance and further investigation.

Prospective data collection

Despite the collection of WGS and epidemiological data from 12 EU/EEA countries and the UK, there is no strong evidence on common exposures and routes of transmission that could explain the observed increase in OXA-244-producing *E. coli*. The lack of precise information on the exposure of cases to risk factors, together with the lack of clarity in relation to time of exposure, have been major obstacles in this investigation. In the absence of real-time WGS-based surveillance, specific PCR tests could be beneficial for reducing the time between sample collection and phylogenetic cluster identification. This would enable the timely collection of epidemiological information from patients to identify possible exposures. To test and further elaborate on the hypotheses discussed in this rapid risk assessment, the prospective and comprehensive collection of national data on cases of OXA-244-producing *E. coli* would be required. This would involve the systematic collection of data on travel and healthcare history, nutritional habits and contact with animals. A questionnaire has been developed by ECDC for further investigation of cases and can be made available upon request. ECDC encourages EU/EEA countries to collect further information and perform analytical studies to identify the source and mode of transmission of OXA-244-producing *E. coli*.

Preventing spread in healthcare settings

Prevention measures for OXA-244-producing *E. coli* in healthcare settings should be implemented as recommended for other carbapenem-resistant Enterobacterales. For details please refer to the measures outlined in ECDC's Rapid Risk Assessment 'Carbapenem-resistant Enterobacteriaceae, second update' [76].

Consulted experts

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Public health experts from EU/EEA countries that have provided WGS and epidemiological data: Austria: Petra Apfalter; Denmark: Anette M. Hammerum, Henrik Hasman, Louise Roer – Statens Serum Institut; Finland: Jari Jalava, Kati Räisänen – Finnish Institute for Health and Welfare; France: Laurent Dortet, Cécile Emeraud – National Reference Centre for carbapenemase-producing Enterobacterales; Germany: Jörg B. Hans, Niels Pfennigwerth – German National Reference Centre for multidrug-resistant Gram-negative bacteria, Ruhr-University Bochum; Ireland: Niall Delappe, Martin Cormican – National Carbapenemase Producing Enterobacterales Reference Laboratory; Luxembourg: Alexandre Mzabi, Monique Perrin – Laboratoire National de Santé; the Netherlands: Daan Notermans – National Institute for Public Health and the Environment (RIVM), Norway: Ørjan Samuelsen – University Hospital of North Norway, Oliver Kacelnik – Norwegian Institute of Public Health; Poland: Elżbieta Literacka – National Reference Centre for Antimicrobial Susceptibility; Portugal: Manuela Caniça – National Reference Laboratory for Antimicrobial Resistance, National Institute of Health Dr Ricardo Jorge; Sweden: Barbro Mäkitalo, Karin Westmo – Public Health Agency of Sweden.

External experts: Katie Hopkins, Public Health England, UK.

The European Food Safety Authority was also consulted on this report.

All experts have submitted declarations of interest, and a review of these did not reveal any conflict of interest.

Disclaimer

ECDC issues this risk assessment document based on an internal decision and in accordance with Article 10 of Decision No 1082/13/EC and Article 7(1) of Regulation (EC) No 853/2004 establishing a European centre for disease prevention and control (ECDC). In the framework of ECDC's mandate, the specific purpose of an ECDC risk assessment is to present different options on a certain matter. The responsibility on the choice of which option to pursue and which actions to take, including the adoption of mandatory rules or guidelines, lies exclusively with the EU/EEA Member States. In its activities, ECDC strives to ensure its independence, high scientific quality, transparency and efficiency.

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Annex 1

Epidemiological characteristics of all non-outlier *E. coli* ST38 cases, OXA-244-producing *E. coli* cases, as well as cases in clusters A and B

Characteristic	All <i>E. coli</i> ST 38 non-outlier cases (n=458)	OXA-244-producing <i>E. coli</i> ST38 cases (n =370)	Cluster A cases* (n = 225)	Cluster B cases (n=116)
Affected countries	Australia (n=5), Austria (n=6), Brazil (n=1), Canada (n=3), Colombia (n=1), Denmark (n=32), Ecuador (n=4), Finland (n=9), France (n=62), Germany (n=91), Ireland (n=24), Lithuania (n=1), Luxembourg (n=4), Netherlands (n=32), New Zealand (n=2), Norway (n=25), Poland (n=3), Portugal (n=1), Qatar (n=8), Singapore (n=5), Sweden (n=46), Thailand (n=4), Turkey (n=1), United Kingdom (n=45), United States (n=8), Vietnam (n=2), Asia, unknown (n=6), Missing/unknown (n=27)	Austria (n=6), Canada (n=1), Colombia (n=1), Denmark (n=32), Finland (n=9), France (n=49), Germany (n=91), Ireland (n=24), Lithuania (n=1), Luxembourg (n=4), Netherlands (n=22), Norway (n=24), Poland (n=3), Portugal (n=1), Qatar (n=8), Sweden (n=44), Turkey (n=1), United Kingdom (n=45), United States (n=2), Missing/unknown (n=2)	Australia (n=1), Austria (n=4), Denmark (n=16), Finland (n=5), France (n=43), Germany (n=56), Ireland (n=17), Luxembourg (n=3), Netherlands (n=14), Norway (n=22), Poland (n=1), Qatar (n=8), Sweden (n=25), United Kingdom (n=3), United States (n=3), Missing/unknown (n=3)	Austria (n=2), Canada (n=1), Colombia (n=1), Denmark (n=10), Finland (n=4), France (n=5), Germany (n=24), Ireland (n=4), Lithuania (n=1), Netherlands (n=5), Poland (n=2), Portugal (n=1), Sweden (n=14), Turkey (n=1), United Kingdom (n=40), Missing/unknown (n=1)
Year of isolation	2012 (n=1), 2013 (n=11), 2014 (n=17), 2015 (n=18), 2016 (n=27), 2017 (n=31), 2018 (n=82), 2019 (n=133), 2020 (n=90), Missing/unknown (n=48)	2013 (n=3), 2014 (n=10), 2015 (n=10), 2016 (n=18), 2017 (n=20), 2018 (n=75), 2019 (n=132), 2020 (n=90), Missing/unknown (n=12)	2016 (n=4), 2017 (n=12), 2018 (n=44), 2019 (n=95), 2020 (n=65), Missing/unknown (n=4)	2013 (n=3), 2014 (n=10), 2015 (n=10), 2016 (n=16), 2017 (n=7), 2018 (n=24), 2019 (n=23), 2020 (n=13), Missing/unknown (n=10)
Type of sample	Blood (n=20), Urine (n=184), Screening (n=122), Other (n=57), Missing/unknown (n=75)	Blood (n=12), Urine (n=171), Screening (n=121), Other (n=34), Missing/unknown (n=32)	Blood (n=8), Urine (n=109), Screening (n=82), Other (n=15), Missing/unknown (n=11)	Blood (n=4), Urine (n=45), Screening (n=30), Other (n=13), Missing/unknown (n=12)
Median age (25th-75th percentile)	54 years (33-71)	54 years (33-71)	51 years (25.5-70.5)	57 years (39.5-70.5)
Sex	Female (n=147), Male (n=63), Missing/unknown (n=171)	Female (n=145), Male (n=63), Missing/unknown (n=162)	Female (n=78), Male (n=26), Missing/unknown (n=121)	Female (n=51), Male (n=28), Missing/unknown (n=37)
Prior travel or hospitalisation outside of the EU/EEA (within 12 months)	Afghanistan (n=1), Bosnia and Herzegovina (n=1), Egypt (n=15), Iraq (n=1), Morocco (n=1), Saudi Arabia (n=1), Somalia (n=1), Tunisia (n=1), Turkey (n=6), Many countries (n=2), None (n=14), Missing/unknown (n=414)	Afghanistan (n=1), Bosnia and Herzegovina (n=1), Egypt (n=15), Iraq (n=1), Morocco (n=1), Saudi Arabia (n=1), Somalia (n=1), Tunisia (n=1), Turkey (n=6), Many countries (n=2), None (n=14), Missing/unknown (n=326)	Afghanistan (n=1), Bosnia and Herzegovina (n=1), Egypt (n=7), Iraq (n=1), Turkey (n=4), Many countries (n=1), None (=5), Missing/unknown (n=205)	Egypt (n=7), Morocco (n=1), Saudi Arabia (n=1), Somalia (n=1), Turkey (n=2), Many countries (n=1), None (n= 7), Missing/unknown (n=96)
Inpatient/ outpatient status	Inpatient (n=64), Outpatient (n=44), Missing/unknown (n=350)	Inpatient (n=64), Outpatient (n=44), Missing/unknown (n=262)	Inpatient (n=36), Outpatient (n=19), Missing/unknown (n=170)	Inpatient (n=23), Outpatient (n=18), Missing/unknown (n=75)

*Cluster A was called 'main cluster' in the previous rapid risk assessment.