RAPID RISK ASSESSMENT

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

18 February 2020

Summary

Following an urgent inquiry in ECDC's EPIS AMR-HAI regarding an increasing number of cases of OXA-244-producing *Escherichia coli* and identification of a cluster of *E. coli* sequence type (ST) 38 in Germany, national public health reference laboratories in European Union/European Economic Area countries were invited to submit whole genome sequencing (WGS) data to ECDC for a European-level analysis. The pooled European data showed that 33 cases of OXA-244-producing *E. coli* were detected between 2013 and 2015, followed by 31 cases in 2016, 21 cases in 2017, 83 cases in 2018 and 116 cases in 2019. Increased use of WGS may have enabled more discriminatory identification of cases from sample collections and contributed to the observed increase in cases. The WGS data identified one main, geographically dispersed cluster of *E. coli* ST38 with chromosomally encoded bla_{OXA244} present in all countries that had submitted data (Denmark, Finland, France, Germany, Ireland, Luxembourg, the Netherlands, Norway, Sweden and the United Kingdom). Most of the cases in this cluster were detected in 2018 and 2019.

The source and the route of transmission of OXA-244-producing *E. coli* in the EU/EEA and the UK is currently unclear. An overlap in time and place was identified for a few cases in Germany, indicating possible person-to-person transmission. Healthcare-associated clusters were not observed and transmission in healthcare settings cannot explain the dispersed distribution in ten countries and different regions within countries without linked hospital referral networks.

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 further spread of OXA-244-producing *E. coli* in the EU/EEA is probably high, given the rapid and simultaneous increase in multiple countries. If awareness, sampling frequency and capacity to detect OXA-244 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 will be low. However, without adaptation of microbiological methods and surveillance, OXA-244-producing *E. coli* may continue to spread unnoticed. There is a risk that transmission of OXA-244-producing *E. coli* in the community may contribute to the loss of carbapenems as options for treatment of serious *E. coli* infections in the EU/EEA. Therefore there is an urgent need for further investigation to determine the source and routes of transmission of OXA-244-producing *E. coli* in the EU/EEA, and implement adequate control measures.

The low genetic diversity of the main cluster could have a number of explanations, including a recently emerging clone, but may also point to a source of OXA-244-producing *E. coli* that could have been distributed to all ten of the countries involved. Information about travel was available for only a few cases (12%), meaning that it has been impossible to draw conclusions on the importance of travel as a risk factor. Transmission via food (animal or non-animal origin), by contact with the environment or direct contact with

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animals, is hypothetically possible. However, no OXA-244-producing *E. coli* isolates from food or animal sources have so far been identified in Europe. 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 would require investigation. Further studies therefore 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.

Options for response 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, combined with prospective collection of epidemiological data on cases and associated risk factors. For further details, please refer to the 'Options for response' section below.

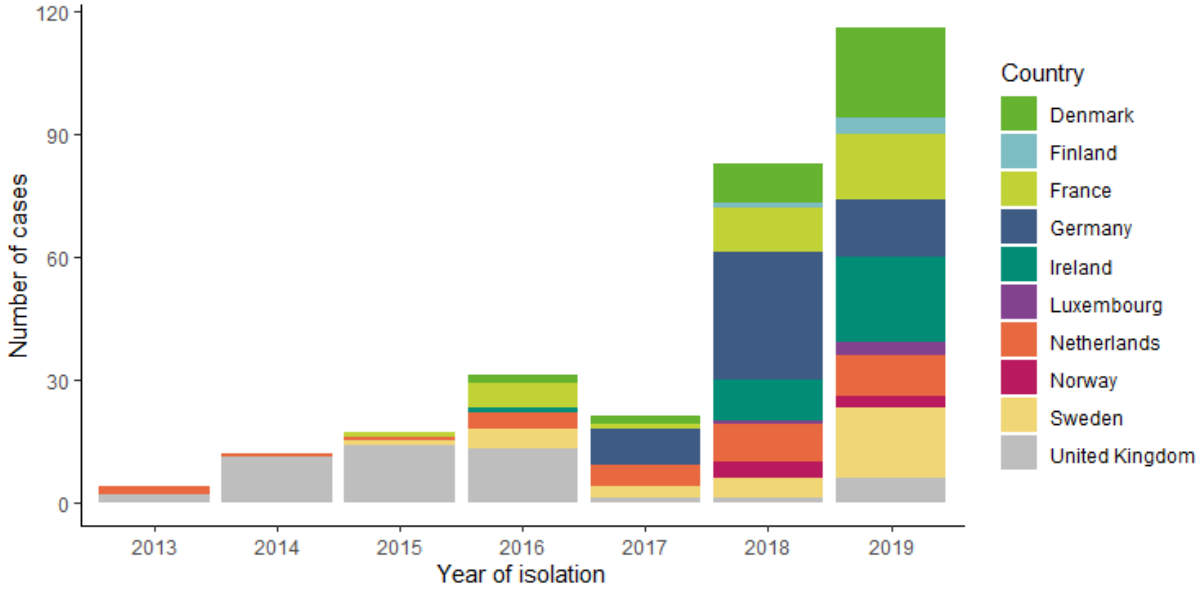
Event background

Between 2017 and 2019, the National Reference Centre for multidrug-resistant gram-negative bacteria and the German public health service detected an increase in oxacillinase-244 (OXA-244)-producing *E. coli* [1,2]. In Germany between January and October 2019, 134 cases were confirmed, compared to 29 cases in 2017 and 87 cases in 2018. The isolates originated from hospitals in nine different German federal states. Whole genome sequencing (WGS) followed by core genome multi-locus sequence typing (cgMLST) and single-nucleotide polymorphism (SNP) analyses showed that 14 sequence types contained the $bla_{\text{OXA-244}}$ gene. Among the 14 sequence types, one cluster of 63 isolates (2017, n=6; 2018, n=19; 2019, n=38) belonging to *E. coli* ST38 was predominant [2]. The Robert Koch Institute (RKI) conducted epidemiological investigations of cases of this cluster in Germany. Epidemiological investigation, based on the questioning of patients, was hampered by a lack of clarity relating to the time of exposure and the current relatively long period of time between pathogen detection and availability of WGS results. Consequently, a clear pattern of common exposures or route of transmission could not be identified, and so a European-level investigation of epidemiological information and WGS data was proposed, coordinated by ECDC.

For the collaborative data collection, a case was defined as any isolate of *E. coli* carrying the $bla_{\text{OXA-244}}$ gene. In total, 326 non-duplicate sequences were considered for further analysis. A total of 11 of these were excluded as WGS data failed quality control. WGS data were available for isolates from Denmark (n=36), Finland (n=5), France (n=35), Germany (n=54), Ireland (n=32), Luxembourg (n=4), the Netherlands (n=34), Norway (n=7), Sweden (n=33; one of which was available from an open-access database) and the United Kingdom (n=53; five of which were available from an open-access database). With the open-access databases, additional sequences of *E.coli* isolates with the $bla_{\text{OXA-244}}$ gene were available from Asia (unknown country) (n=6), Australia (n=2), Canada (n=2), Colombia (n=1), Lithuania (n=1), Singapore (n=3), Thailand (n=2), Turkey (n=1), United States (n=3) and one sequence of unknown origin. Additionally, the European Food Safety Authority (EFSA) was asked to liaise with the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR) to collect data on OXA-244-producing *E. coli* from the veterinary and food sector.

Analysis of the data submitted showed that the increase in cases of OXA-244-producing *E. coli* had not only occurred in Germany, but also in the cumulative data for the EU/EEA and the UK, as shown in Figure 1. The first isolates detected in the Netherlands and the United Kingdom were from 2013. Overall between 2013 and 2015, 33 cases were found, followed by 31 cases in 2016, 21 cases in 2017, 83 cases in 2018 and 116 cases in 2019. One isolate was from 2020 and eight isolates had no sampling date available. An increase in OXA-244-producing *E. coli* ST38 has also been observed in Switzerland where a related warning has been published by the national reference laboratory [3]. For the isolates analysed in this risk assessment for which antimicrobial susceptibility testing results were available, minimum-inhibitory concentrations (MIC) using screening cut-offs recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [4] enabled the detection of 88.2% of OXA-244-producing *E. coli* isolates using meropenem and 100% using ertapenem, whereas clinical breakpoints were not suitable for this purpose.

Figure 1. Number of cases of OXA-244-producing *E. coli* per year between 2013 and 2019 in the EU/EEA and the UK

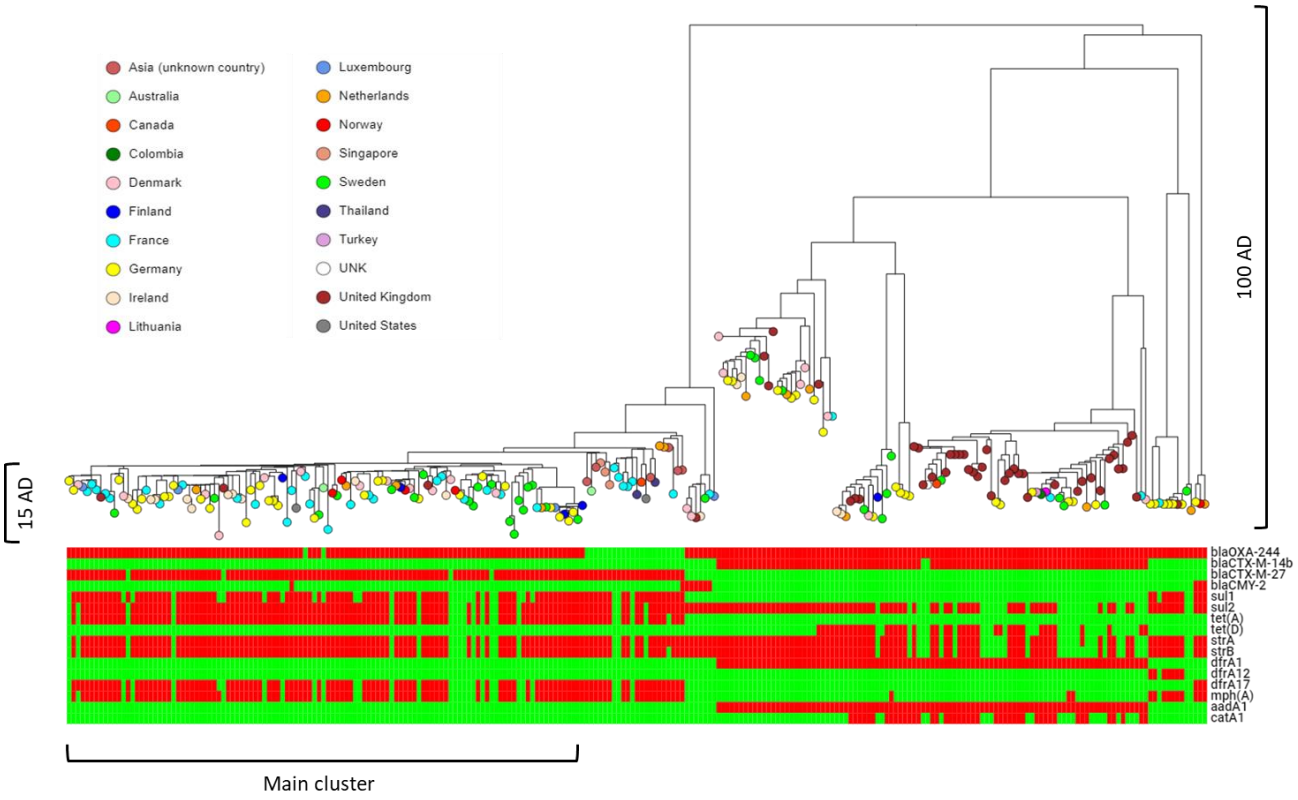


Figures include only cases for which WGS data were available. Only isolates with available date of isolation are shown. Note that not all countries have completed WGS of isolates from 2019.

WGS analysis

Eight countries from the EU/EEA and the UK submitted raw sequence data analysis, while the two remaining countries (Ireland and Norway) contributed data already assembled using SPAdes. Closely related sequences from the public domain (n=37) 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 [5] core genome MLST scheme (2500 loci) and the Bionumerics *E. coli* plugin for genotypic resistance and virulence determination. ResFinder v3.2 [6] was used for verification of the genotypic resistance results. All sequences with >2000 core genome loci determined were included in the analysis (n=315). The allelic profiles were clustered using neighbour joining and the results were visualised using MicroReact [7]. 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 2. Neighbour joining tree of OXA-244-producing *E. coli* ST38 non-outlier sequences collected from EU/EEA countries, the UK and from the open-access databases (n=251)



The tree was constructed using Bionumerics, with the Enterobase E. coli core genome MLST scheme. The bottom part of the figure also shows the presence (red), or absence (green) of resistance genes that commonly occurred in the dataset. The main cluster spanned 15 cgMLST allelic differences (AD) from root to tip, while the entire tree spanned about 100 AD from root to tip.

Several clusters belonging to *E. coli* ST38 were identified as shown in Figure 2, including one main cluster, with 15 cgMLST allelic differences (AD) from root to tip of the cluster subtree, that contained 113 isolates from all ten EU/EEA countries and the UK that had contributed sequence data, plus one isolate from the United States. These isolates were mainly detected in 2018 and 2019. Two other clusters of more than 20 isolates were detected (Figure 2), as well as a large set of outlier isolates (n=64) from Denmark, Ireland, the Netherlands, Norway, the United Kingdom and the United States (not shown in Figure 2). Available epidemiological information for the main cluster, the other isolates and the outliers are shown in Table 1.

Isolates in the main cluster generally carried both the $bla_{\text{CTX-M-27}}$ and $bla_{\text{OXA-244}}$ genes. In addition, most of the isolates in this main cluster carried genes encoding resistance to tetracyline, sulfonamides, trimethoprim, aminoglycosides and macrolides (Figure 2). One long-read sequenced genome provided by the Netherlands matched the main cluster. This genome carried $bla_{\text{OXA-244}}$ on the chromosome and included a 147 kbp resistance plasmid containing *sul1*, *sul2*, *strA*, *strB*, *tet(A)*, *aadA5*, $bla_{\text{CTX-M-27}}$, *mph(A)*, and *dfrA17* and a 211 kbp plasmid without any resistance genes. The $bla_{\text{OXA-244}}$ gene generally appeared on very short contigs in the short read data assemblies, so it is not possible to determine if was located on the chromosome for all isolates in the main cluster, but it is probable, given the close genetic relationships within the cluster.

The isolates in the other clusters carried both the $bla_{\text{CTX-M-14b}}$ and $bla_{\text{OXA-244}}$ genes, both on the chromosome as indicated by three different long read sequenced genomes matching these clusters, plus a variable set of plasmid-borne resistance genes and virulence genes. One of the smaller clusters contained several historical isolates from the 2013-2015 EuSCAPE study [8], and had an overrepresentation of isolates from the United Kingdom (33 out of 53 isolates from the United Kingdom belonged to this cluster). The outlier isolates (not shown in Figure 2) carried $bla_{\text{OXA-244}}$ plus a variable set of additional resistance genes, and corresponded to cases with a relatively high proportion of travel to Egypt and Turkey within the past 12 months. Three long read sequenced genomes were among these outliers, all carrying $bla_{\text{OXA-244}}$ 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/i2earohjD

Table 1. Epidemiological and genetic characteristics of the main cluster, other isolates and outliers of OXA-244 producing *E. coli* ST38

	Main cluster (n=114)	Other isolates (n=137)	Outliers (n=64)
Affected countries	Australia (n=1) Denmark (n=12) Finland (n=4) France (n=24) Germany (n=29) Ireland (n=10) Luxembourg (n=3) Netherlands (n=4) Norway (n=4) Sweden (n=19) United Kingdom (n=3) United States (n=1)	Australia (n=1) Canada (n=2) Colombia (n=1) Denmark (n=9) Finland (n=1) France (n=11) Germany (n=25) Ireland (n=5) Lithuania (n=1) Luxembourg (n=1) Netherlands (n=9) Norway (n=1) Singapore (n=3) Sweden (n=14) Thailand (n=2) Turkey (n=1) United Kingdom (n=42) United States (n=1) Asia, unknown country (n=6) Missing/unknown (n=1)	Denmark (n=15) Ireland (n=17) Netherlands (n=21) Norway (n=2) United Kingdom (n=8) United States (n=1)
Year of isolation	2016 (n=3) 2017 (n=6) 2018 (n=38) 2019 (n=66) Missing/unknown (n=1)	2013 (n=3) 2014 (n=10) 2015 (n=14) 2016 (n=21) 2017 (n=14) 2018 (n=30) 2019 (n=25) 2020 (n=1) Missing/unknown (n=19)	2013 (n=2) 2014 (n=2) 2015 (n=6) 2016 (n=7) 2017 (n=6) 2018 (n=15) 2019 (n=25) Missing/unknown (n=1)
Type of sample	Blood (n=6) Urine (n=53) Screening (n=43) Other (n=8) Missing/unknown (n=4)	Blood (n=7) Urine (n=50) Screening (n=30) Other (n=16) Missing/unknown (n=34)	Blood (n=1) Urine (n=17) Screening (n=42) Other (n=2) Missing/unknown (n=2)
Median age, 25th-75th percentile	54.5 years (29 – 70 years)	59 years (42 – 71 years)	59 years (37 – 74 years)
Sex	Female (n=42) Male (n=13) Missing/unknown (n=59)	Female (n=52) Male (n=30) Missing/unknown (n=55)	Female (n=42) Male (n=19) Missing/unknown (n=3)
Prior travel abroad (12 months)	Turkey (n=6) Egypt (n=4) Iraq (n=2) Other countries (n=6) None (=11) Missing/unknown (n=85)	Egypt (n=6) Other countries (n=9) None (n= 7) Missing/unknown (n=115)	Egypt (n=9) Turkey (n=5) Turkey/Egypt (n=2) Other countries (n=3) None (n=4) Missing/unknown (n=41)
Prior hospitalisation abroad (12 months)	Turkey (n=2) Egypt (n=2) Turkey/Italy (n=1) Other countries (n=3) None (n=1) Missing/unknown (n=105)	Germany (n=1) Turkey (n=1) Egypt (n=1) Other countries (n=1) None (n=4) Missing/unknown (n=129)	Egypt (n=9) Turkey (n=5) Other countries (n=3) None (n=3) Missing/unknown (n=44)

Data from the public domain were included.

Disease background

Disease characteristics

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 [9]. OXA-244 is characterised by a single Arg214Gln mutation compared to OXA-48 [10] 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 [11]. In 2013, the first OXA-244-producing *E. coli* isolate was reported in Germany [12]. OXA-244 has also previously been reported in isolates from France [10], the UK [13], Russia [14], Turkey [15], the Netherlands (in a Dutch traveller to Indonesia and her spouse) [16], and very recently in Colombia [17]. It was also found in *E. coli* isolated from wastewater in Algeria [18] and from an estuary in Lebanon [19]. The $bla_{OXA-244}$ gene has been found on both plasmids and integrated into the chromosome [20]. The latter was found in *E. coli* ST38, the same sequence type that has been responsible for the increase in Europe. Due to the low-level hydrolytic activity of the OXA-244 carbapenemase against carbapenems, OXA-244-producing *E. coli* may be difficult to detect when using routine methods in clinical laboratories [20] as these isolates do not grow on media used to detect Enterobacterales producing other carbapenemases such as *K. pneumoniae* carbapenemase (KPC) or New Delhi metallo-beta-lactamase (NDM). However, OXA-244-producing *E. coli* frequently also carry genes encoding for extended-spectrum beta-lactamases (ESBLs) and grow on screening media to detect ESBL-producing Enterobacterales. Thus, these isolates may be categorised as ESBL-producing *E. coli* only, unless specific methods are used to screen for OXA-48-like carbapenemases, which include OXA-244. Furthermore, isolates may be reported as susceptible to meropenem and/or imipenem based on clinical breakpoints, but are less frequently susceptible to ertapenem.

E. coli is the most common pathogen responsible for urinary tract infections in the community. According to 2018 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 Bulgaria, Cyprus and Greece reporting 1% to <5% of *E. coli* isolates as carbapenem-resistant [21]. In all other EU/EEA countries, this proportion was below 1%. It should be noted that carbapenem resistance proportions reported by EARS-Net are based on meropenem and/or imipenem, and thus do not take into account ertapenem resistance. 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.) Nevertheless, in a multinational study including Finland, Germany, Latvia, Poland, Sweden and Russia, resistance to meropenem was not reported in 775 *E. coli* urinary isolates collected between October 2015 and January 2017 [22], whereas in the EuSCAPE survey most participating countries submitted at least one carbapenem-resistant *E. coli* isolate. Urine was the most common site of isolation [8]. In the intensive care unit, *E. coli* accounted for 32.1% of all urinary tract infections, 13.5% of pneumonia episodes and 9.2% of bloodstream infections. Overall, carbapenem resistance was only found in 0.8% of all isolates [23].

E. coli ST38 occurs globally and often carries genes encoding for extended-spectrum-beta-lactamases (ESBLs) [24]. *E. coli* ST38 has been responsible for urinary tract infections [24,25] and bloodstream infections in human patients [26], has been shown to be present in wastewater, and has been isolated from different types of food (vegetables and chicken meat) [27,28]. ST38 was the main sequence type among all submitted *E. coli* isolates and constituted the largest cluster in this analysis. *E. coli* ST38 with a chromosomally encoded bla_{OXA-48} gene has been reported from Europe [29,30], North Africa and the Middle East [9,31]. *E. coli* ST38 was the leading sequence type in OXA-48-producing isolates cultured from Syrian patients admitted to Israeli hospitals [32]. *E. coli* ST38 isolates with chromosomal bla_{OXA-48} (and closely related variants including $bla_{OXA-244}$) submitted to Public Health England in 2014 were closely related, despite originating from different healthcare institutions across the country.

Monitoring of AMR in zoonotic and commensal bacteria

Since 2015, antimicrobial resistance in zoonotic and commensal bacteria has been monitored across the European Union (Commission Decision 2013/652/EU) [33], with this monitoring being performed by the competent authorities and laboratories from the veterinary and food sector. The AMR monitoring includes mandatory ESBL/AmpC/carbapenemase monitoring for all EU Member States. *Salmonella* spp. and indicator commensal *E. coli* isolates collected from food-producing animal matrices and their meat are tested for susceptibility to meropenem. In addition, voluntary carbapenemase-specific monitoring is also performed, using selective pre-enrichment and subsequent selective plating on carbapenem-containing media that also allow 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-producers are whole genome-sequenced by the EURL-AR (DTU, Copenhagen) or the Member States. No OXA-244-producing isolate has been detected from the data reported until 2018. However, OXA-162 was detected in poultry and poultry meat as a result of the monitoring [34]. Data from the monitoring are reported to EFSA which prepares the EUSR-AR reports in collaboration with ECDC [34].

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 current 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 [21], with most countries reporting less than 1% of all invasive isolates as carbapenem-resistant. However, OXA-244-producing *E. coli* might not be reported as carbapenem-resistant in surveillance systems such as EARS-Net because they are frequently susceptible to meropenem and/or imipenem, based on clinical breakpoints. Furthermore, these isolates might be classified only as ESBL-producing instead of carbapenemase-producing *E. coli*, unless they are specifically tested for OXA-48-like carbapenemases. All countries that could contribute data had isolates belonging to the main cluster, indicating a pan-European problem.

Treatment options

OXA-244-producing *E. coli* isolates may be susceptible to meropenem and imipenem, according to EUCAST clinical breakpoints. Data are missing on the clinical consequences of carbapenems being used for treatment of OXA-244-producing *E. coli* infections in this specific situation. Many isolates may be susceptible to other classes of antibiotics and treatment decisions should be made on an individual basis, in consultation with an infectious diseases specialist or 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. This increases the likelihood of patient-to-patient transmission in hospitals. In the reported clusters, nosocomial transmission of OXA-244-producing *E. coli* might have occurred on a few occasions, for example in Germany where an overlap in time and place was identified for a few cases. However, the dispersed distribution in ten countries and different regions within countries without linked hospital referral networks argues against nosocomial transmission as the main explanation for the observed increase, even though there might be gaps in surveillance. In addition, while *E. coli* can cause healthcare-associated infections and hospital outbreaks, it is often associated with community-acquired infection.

Travel

Epidemiological data on travel or previous hospitalisation were scarce, thus precluding conclusions on the importance of travel as a risk factor. Nevertheless, whenever such information was available, Turkey and Egypt were relatively commonly reported previous travel destinations or countries of hospitalisation. OXA-48-like carbapenemases are endemic in both countries [9]. In the phylogenetic analysis, 64 isolates were either found to be singletons, or belonged to very small clusters with little genetic similarity between them. These included 19 cases with a travel history, 16 of which had travelled to Egypt and/or Turkey within the past 12 months. Both countries are popular tourist destinations for European citizens, and therefore the travel history obtained from the OXA-244-producing *E. coli* cases may simply reflect the most frequent travel destinations among the populations of those countries included. Travel-related importation may therefore have occurred sporadically, but is unlikely to explain the majority of closely related isolates in the main cluster.

Environment

E. coli is an indicator for faecal contamination in the environment originating from both humans and animals. Data on several related isolates that originated from environmental sources were retrieved from open-access databases, (e.g. an isolate from a wastewater inflow in the United States.) OXA-244-producing *E. coli* has been isolated from wastewater in Algeria [18]. OXA-48-producing *E. coli* ST38 has also been found in wastewater in Basel, Switzerland [35], and in Lebanon [19]. Related *E. coli* ST38 isolates from both water and humans have previously been described [36]. Furthermore, *E. coli* ST38 has been documented in pets, livestock and wild animals [37-41]. Flies on a cattle farm in Japan have been shown to carry ESBL-producing *E. coli* ST38 [42]. However, the close genetic relatedness, combined with dispersed geographical distribution in at least ten European countries is unlikely to be compatible with an environmental source of transmission, unless there is widespread environmental contamination in multiple countries.

Food and animal products

Transmission via food (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 Europe. Imported food or animal products from regions where OXA-48-like carbapenemases are endemic, or even food from domestic sources, therefore cannot be either excluded or confirmed as a possible contributing factor.

Previous detection of *E. coli* ST38 (not carrying *bla*$_{OXA-244}$)

E. coli ST38 isolates that did not carry the *bla*$_{OXA-244}$) gene have previously been found in meat, fowl and humans in Germany [28], and in retail chicken in Norway, with related isolates also found in humans [39]. In a Swiss study investigating imported vegetables for the presence of ESBL-producing Enterobacterales, *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 the samples were not investigated for the presence of genes encoding for carbapenemases [27]. Clonal *E. coli* ST38 isolates have also been found in Swiss retail meat [43]. In Algeria and Lebanon, OXA-48-producing *E. coli* ST38 has been found in fowl [44,45]. In a prospective study in the United States, retail meat was shown to be a possible source for community-acquired urinary tract infections caused by *E. coli*, with ST38 being a common sequence type in both humans and retail meat [46]. Further investigation of crops, animals and foods as a possible source of OXA-244-producing *E.coli* should be considered, although at present there are no reports on the detection of OXA-244-producing *E.coli* 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 chromosomally encoded, 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 likely to be high, given the rapid and simultaneous increase in multiple countries despite the difficulties with laboratory detection. 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 might also partially be attributable to increased awareness, changes in laboratory testing methodology and the availability of molecular methods including WGS.

If awareness, sampling frequency and capacity to detect OXA-244 is improved in clinical microbiology laboratories, and appropriate infection prevention and control measures are implemented when detected, the risk of transmission within healthcare settings will be low. However, without adapted 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. There may be a risk that carbapenems will be lost as an effective option for the treatment of serious infections in the EU/EEA. 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 from a food source. There is therefore an urgent need for further investigations 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 control measures for carbapenem-resistant Enterobacterales (CRE) in general, please refer to the ECDC Rapid Risk Assessment on Carbapenem-resistant Enterobacteriaceae – second update, 26 September 2019 [47].

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. A systematic collection of OXA-244 carbapenemase-producing *E. coli* isolates at the national reference laboratories would improve the understanding of the extent of their spread. Strategies and protocols to screen for OXA-244-producing *E. coli* need to be developed. Few data are available to provide guidance on optimal strategies for detecting OXA-244. In Switzerland, guidance for screening in both outbreak and non-outbreak situations has been issued by the Swiss national reference laboratory which recommends using screening media with a low concentration of ertapenem [3]. Commercial rapid diagnostic tests developed for the detection of OXA-48 also seem to detect OXA-244 [20,48]. Both commercial and in-house PCR protocols have been documented to identify *bla*$_{OXA-244}$ [48,49]. Alternatively, WGS may be used for direct detection of *bla*$_{OXA-244}$. It would also be important to determine the risk factors for carriage of OXA-244-producing *E. coli* that would allow for the targeted screening of specific risk groups. Use of EUCAST's screening breakpoints for carbapenemase-producing Enterobacterales [4] is recommended, since most isolates for which data was available would have been detected using these breakpoints, whereas clinical breakpoints are not suitable for detection of OXA-244 producing *E. coli.* However, as data was missing for most isolates, this needs to be confirmed with additional antimicrobial susceptibility testing results.

Prospective data collection

Despite the collection of WGS and epidemiological data from ten European countries, strong evidence on common exposures and routes of transmission that could explain the observed increase in OXA-244-producing *E. coli* is missing. The lack of precise metadata for isolates 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 in order 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 history and previous hospitalisation, covering a sufficiently long period to account for persistent intestinal colonisation, food shopping and eating habits. A questionnaire covering these risk factors has been developed by epidemiologists at the Robert Koch Institute to investigate the increase in cases in Germany. This questionnaire can be requested from ECDC or RKI.

ECDC encourages Member States to collect further information and perform analytical studies to identify the source and mode of transmission of the cluster of OXA-244-producing *E. coli* ST38.

One-health approach

As the source of exposure may hypothetically be related to the environment, crops, animals and food for at least a subset of cases, ECDC contacted the European Food Safety Authority (EFSA), which in turn contacted the EURL-AR who contacted the NRLs-AR with a request to share any data that they may have at the national level. However, so far ECDC has not received any reports of isolates fulfilling the case definition (OXA-244-producing *E. coli* ST38). Further studies should be considered to determine the likelihood of the environment, crops, animals and foods having been contaminated with OXA-244-producing *E. coli* which may have contributed to this outbreak.

Preventing spread in healthcare settings

Despite the absence of evidence for healthcare-associated spread, prevention measures for OXA-244-producing *E. coli* in healthcare settings should be implemented similar to those 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' [47].

Consulted experts

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The European Food Safety Authority was also consulted for the final version of 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 851/2004 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.

This report was written with the coordination and assistance of an Internal Response Team at the European Centre for Disease Prevention and Control. All data published in this risk assessment are correct to the best of our knowledge at the time of publication. Maps and figures published do not represent a statement on the part of ECDC or its partners on the legal or border status of the countries and territories shown.

References

1. Robert Koch Institut. Datenbank Resistenzübersicht 2018 [cited 14.10.2019]. Available from: https://ars.rki.de/Content/Database/ResistanceOverview.aspx
2. Hans JB, Kramer R, Kremer K, Pfennigwerth N, Haller S, Reuss A, Pfeifer Y et al. Ongoing outbreak of OXA-244 carbapenemase-producing Enterobacterales in Germany is driven by clonal expansion of *Escherichia coli* ST38. 12th International Meeting on Microbial Epidemiological Markers; Dubrovnik, Croatia, 2019.
3. Nordmann PK, Poirel L. Warning: Carbapenemase OXA-244 in *E. coli* in Switzerland 2019. Available from: https://www.nara-antibiotic-resistance.ch/wp-content/uploads/Carbapenemase-OXA-244-in-E.-coli-in-Switzerland.pdf
4. European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, version 2.0 July 2017: EUCAST; 2017. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf
5. Zhou Z, Alikhan NF, Mohamed K, Fan Y, Agama Study G, Achtman M. The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity. Genome Res. 2020 Jan;30(1):138-52.
6. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012 Nov;67(11):2640-4.
7. Argimon S, Abudahab K, Goater RJE, Fedosejev A, Bhai J, Glasner C, et al. Microreact: visualizing and sharing data for genomic epidemiology and phylogeography. Microbial Genomics. 2016 Nov;2(11):e000093.
8. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasevic AT, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis. 2017 Feb;17(2):153-63.
9. Pitout JDD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The Global Ascendency of OXA-48-Type Carbapenemases. Clin Microbiol Rev. 2019 Dec 18;33(1).
10. Potron A, Poirel L, Dortet L, Nordmann P. Characterisation of OXA-244, a chromosomally-encoded OXA-48-like beta-lactamase from *Escherichia coli*. Int J Antimicrob Agents. 2016 Jan;47(1):102-3.
11. Oteo J, Hernandez JM, Espasa M, Fleites A, Saez D, Bautista V, et al. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. J Antimicrob Chemother. 2013 Feb;68(2):317-21.
12. Valenza G, Nickel S, Pfeifer Y, Eller C, Krupa E, Lehner-Reindl V, et al. Extended-spectrum-beta-lactamase-producing *Escherichia coli* as intestinal colonizers in the German community. Antimicrob Agents Chemother. 2014;58(2):1228-30.
13. Findlay J, Hopkins KL, Loy R, Doumith M, Meunier D, Hill R, et al. OXA-48-like carbapenemases in the UK: an analysis of isolates and cases from 2007 to 2014. J Antimicrob Chemother. 2017 May 1;72(5):1340-9.
14. Fursova NK, Astashkin EI, Knyazeva AI, Kartsev NN, Leonova ES, Ershova ON, et al. The spread of bla OXA-48 and bla OXA-244 carbapenemase genes among *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter* spp. isolated in Moscow, Russia. Ann Clin Microbiol Antimicrob. 2015 Nov 2;14:46.
15. Kahraman EP, Toptan H, Otlu B, Koroglu M, Altindis M. [Investigation of blaOXA-48-like genes in carbapenemase producing *Klebsiella spp*. isolates]. Mikrobiyol Bul. 2019 Apr;53(2):134-43.
16. van Hattem JM, Arcilla MS, Bootsma MC, van Genderen PJ, Goorhuis A, Grobusch MP, et al. Prolonged carriage and potential onward transmission of carbapenemase-producing Enterobacteriaceae in Dutch travelers. Future Microbiol. 2016 Jul;11:857-64.
17. Abril D, Bustos Moya IG, Marquez-Ortiz RA, Josa Montero DF, Corredor Rozo ZL, Torres Molina I, et al. First Report and Comparative Genomics Analysis of a blaOXA-244-Harboring *Escherichia coli* Isolate Recovered in the American Continent. Antibiotics (Basel). 2019 Nov 13;8(4).
18. Tafoukt R, Touati A, Leangapichart T, Bakour S, Rolain JM. Characterization of OXA-48-like-producing Enterobacteriaceae isolated from river water in Algeria. Water Res. 2017 Sep 1;120:185-9.
19. Diab M, Hamze M, Bonnet R, Saras E, Madec JY, Haenni M. Extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae in water sources in Lebanon. Vet Microbiol. 2018 Apr;217:97-103.
20. Hoyos-Mallecot Y, Naas T, Bonnin RA, Patino R, Glaser P, Fortineau N, et al. OXA-244-Producing *Escherichia coli* Isolates, a Challenge for Clinical Microbiology Laboratories. Antimicrob Agents Chemother. 2017 Sep;61(9).
21. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2018. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2019. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2018.pdf
22. Ny S, Edquist P, Dumpis U, Grondahl-Yli-Hannuksela K, Hermes J, Kling AM, et al. Antimicrobial resistance of *Escherichia coli* isolates from outpatient urinary tract infections in women in six European countries including Russia. Journal of Global Antimicrobial Resistance. 2019 Jun;17:25-34.

23. European Centre for Disease Prevention and Control. Healthcare-associated infections acquired in intensive care units - Annual Epidemiological Report for 2017. 2019.
24. Brolund A, Edquist PJ, Makitalo B, Olsson-Liljequist B, Soderblom T, Wisell KT, et al. Epidemiology of extended-spectrum beta-lactamase-producing *Escherichia coli* in Sweden 2007-2011. Clin Microbiol Infect. 2014 Jun;20(6):O344-52.
25. Alghoribi MF, Gibreel TM, Farnham G, Al Johani SM, Balkhy HH, Upton M. Antibiotic-resistant ST38, ST131 and ST405 strains are the leading uropathogenic *Escherichia coli* clones in Riyadh, Saudi Arabia. J Antimicrob Chemother. 2015 Oct;70(10):2757-62.
26. van der Bij AK, Peirano G, Goessens WH, van der Vorm ER, van Westreenen M, Pitout JD. Clinical and molecular characteristics of extended-spectrum-beta-lactamase-producing *Escherichia coli* causing bacteremia in the Rotterdam Area, Netherlands. Antimicrob Agents Chemother. 2011 Jul;55(7):3576-8.
27. Zurfluh K, Nuesch-Inderbinen M, Morach M, Zihler Berner A, Hachler H, Stephan R. Extended-spectrum-beta-lactamase-producing Enterobacteriaceae isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. Appl Environ Microbiol. 2015 May 1;81(9):3115-20.
28. Pietsch M, Irrgang A, Roschanski N, Brenner Michael G, Hamprecht A, Rieber H, et al. Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. BMC Genomics. 2018 Aug 9;19(1):601.
29. Skalova A, Chudejova K, Rotova V, Medvecky M, Studentova V, Chudackova E, et al. Molecular Characterization of OXA-48-Like-Producing Enterobacteriaceae in the Czech Republic and Evidence for Horizontal Transfer of pOXA-48-Like Plasmids. Antimicrob Agents Chemother. 2017 Feb;61(2).
30. Turton JF, Doumith M, Hopkins KL, Perry C, Meunier D, Woodford N. Clonal expansion of *Escherichia coli* ST38 carrying a chromosomally integrated OXA-48 carbapenemase gene. J Med Microbiol. 2016 Jun;65(6):538-46.
31. Beyrouthy R, Robin F, Delmas J, Gibold L, Dalmasso G, Dabboussi F, et al. IS1R-mediated plasticity of IncL/M plasmids leads to the insertion of bla OXA-48 into the *Escherichia coli* Chromosome. Antimicrob Agents Chemother. 2014 Jul;58(7):3785-90.
32. Lerner A, Solter E, Rachi E, Adler A, Rechnitzer H, Miron D, et al. Detection and characterization of carbapenemase-producing Enterobacteriaceae in wounded Syrian patients admitted to hospitals in northern Israel. Eur J Clin Microbiol Infect Dis. 2016 Jan;35(1):149-54.
33. European Commission. Commission implementing decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. Official Journal of the European Union [Internet]. 2013. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32013D0652
34. EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. EFSA Journal. 2019;17(2)(5598).
35. Zurfluh K, Bagutti C, Brodmann P, Alt M, Schulze J, Fanning S, et al. Wastewater is a reservoir for clinically relevant carbapenemase- and 16s rRNA methylase-producing Enterobacteriaceae. Int J Antimicrob Agents. 2017 Sep;50(3):436-40.
36. Hu YY, Cai JC, Zhou HW, Chi D, Zhang XF, Chen WL, et al. Molecular typing of CTX-M-producing *Escherichia coli* isolates from environmental water, swine feces, specimens from healthy humans, and human patients. Appl Environ Microbiol. 2013 Oct;79(19):5988-96.
37. Kawamura K, Sugawara T, Matsuo N, Hayashi K, Norizuki C, Tamai K, et al. Spread of CTX-Type Extended-Spectrum beta-Lactamase-Producing *Escherichia coli* Isolates of Epidemic Clone B2-O25-ST131 Among Dogs and Cats in Japan. Microbial Drug Resistance (Larchmont, NY). 2017 Dec;23(8):1059-66.
38. Gekenidis MT, Qi W, Hummerjohann J, Zbinden R, Walsh F, Drissner D. Antibiotic-resistant indicator bacteria in irrigation water: High prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. PLoS One. 2018;13(11):e0207857.
39. Berg ES, Wester AL, Ahrenfeldt J, Mo SS, Slettemeas JS, Steinbakk M, et al. Norwegian patients and retail chicken meat share cephalosporin-resistant *Escherichia coli* and IncK/blaCMY-2 resistance plasmids. Clin Microbiol Infect. 2017 Jun;23(6):407 e9- e15.
40. Guenther S, Semmler T, Stubbe A, Stubbe M, Wieler LH, Schaufler K. Chromosomally encoded ESBL genes in *Escherichia coli* of ST38 from Mongolian wild birds. J Antimicrob Chemother. 2017 May 1;72(5):1310-3.
41. Bouaziz A, Loucif L, Ayachi A, Guehaz K, Bendjama E, Rolain JM. Migratory White Stork (Ciconia ciconia): A Potential Vector of the OXA-48-Producing *Escherichia coli* ST38 Clone in Algeria. Microbial Drug Resistance (Larchmont, NY). 2018 May;24(4):461-8.
42. Usui M, Iwasa T, Fukuda A, Sato T, Okubo T, Tamura Y. The role of flies in spreading the extended-spectrum beta-lactamase gene from cattle. Microbial Drug Resistance (Larchmont, NY). 2013 Oct;19(5):415-20.
43. Vogt D, Overesch G, Endimiani A, Collaud A, Thomann A, Perreten V. Occurrence and genetic characteristics of third-generation cephalosporin-resistant *Escherichia coli* in Swiss retail meat. Microbial Drug Resistance (Larchmont, NY). 2014 Oct;20(5):485-94.
44. Al Bayssari C, Olaitan AO, Dabboussi F, Hamze M, Rolain JM. Emergence of OXA-48-producing *Escherichia coli* clone ST38 in fowl. Antimicrob Agents Chemother. 2015 Jan;59(1):745-6.
45. Belmahdi M, Bakour S, Al Bayssari C, Touati A, Rolain JM. Molecular characterisation of extended-spectrum beta-lactamase- and plasmid AmpC-producing *Escherichia coli* strains isolated from broilers in Bejaia, Algeria. Journal of Global Antimicrobial Resistance. 2016 Sep;6:108-12.

46. Yamaji R, Friedman CR, Rubin J, Suh J, Thys E, McDermott P, et al. A Population-Based Surveillance Study of Shared Genotypes of *Escherichia coli* Isolates from Retail Meat and Suspected Cases of Urinary Tract Infections. mSphere. 2018 Aug 15;3(4).
47. European Centre for Disease Prevention and Control. Carbapenem-resistant Enterobacteriaceae - second update. Stockholm ECDC; 2019. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/carbapenem-resistant-enterobacteriaceae-risk-assessment-rev-2.pdf
48. Dortet L, Jousset A, Sainte-Rose V, Cuzon G, Naas T. Prospective evaluation of the OXA-48 K-SeT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. J Antimicrob Chemother. 2016 Jul;71(7):1834-40.
49. Hoyos-Mallecot YN, Bonnin R, Fortineau N, Dortet L. OXA-244-producers, the nightmare of clinical microbiology labs: insights into the genome. 27th European Congress of Clinical Microbiology and Infectious Diseases; Vienna 2017.