Emergence of OXA-48-producing Klebsiella pneumoniae in Lithuania, 2023
a multi-cluster, multi-hospital outbreak

Greičius, Paulius; Linkevičius, Marius; Razmuk, Jelena; Sinotova, Jekaterina; Alm, Erik; Svartström, Olov; Bortolaia, Valeria; Kudirkienė, Eglė; Roer, Louise; Hendriksen, Rene S

Total number of authors:
15

Published in:
Eurosurveillance

Link to article, DOI:
10.2807/1560-7917.ES.2024.29.16.2400188

Publication date:
2024

Document Version
Publisher’s PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

Paulius Greičius¹, Marius Linkevicius²,* Jelena Razmuk¹, Jekaterina Sinotova¹, Erik Alm², Olov Svarthström², Valeria Bortolaia², Eglė Kudirkienė¹, Louise Roer², Rene S Hendriksen⁴, Gabija Tamoliūnaitė¹, Daniel Palm², Dominique L Monnet², Valeria Bortolaia³, Eglė Kudirkienė³, Louise Roer³, Rene S Hendriksen⁴, Gabija Tamoliūnaitė¹, Daniel Palm², Dominique L Monnet², Anke Kohlenberg², Algirdas Griškevičius¹

1. National Public Health Surveillance Laboratory (NVSPL), Vilnius, Lithuania
2. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
3. Statens Serum Institut (SSI), Copenhagen, Denmark
4. Technical University of Denmark, National Food Institute (DTU Food), Kongens Lyngby, Denmark

* These authors contributed equally to this work and share first authorship.

Correspondence: Marius Linkevicius (marius.linkevicius@ecdc.europa.eu)

Citation style for this article:

In 2023, an increase of OXA-48-producing *Klebsiella pneumoniae* was noticed by the Lithuanian National Public Health Surveillance Laboratory. Whole genome sequencing (WGS) of 106 OXA-48-producing *K. pneumoniae* isolates revealed three distinct clusters of carbapenemase-producing *K. pneumoniae* high-risk clones, including sequence type (ST) 45 (n = 35 isolates), ST392 (n = 32) and ST395 (n = 28), involving six, six and nine hospitals in different regions, respectively. These results enabled targeted investigation and control, and underscore the value of national WGS-based surveillance for antimicrobial resistance.

Implementation of whole genome sequencing (WGS) for surveillance and control of multidrug-resistant bacteria was initiated at the Lithuanian National Public Health Surveillance Laboratory (NVSPL) in 2023. Among carbapenem-resistant *Klebsiella pneumoniae* isolates routinely referred from clinical laboratories, NVSPL noticed a sudden increase of isolates carrying *bla* OXA-48-like in 2023. To validate laboratory procedures while generating real-time genomic data for public health purposes, 106 isolates of *K. pneumoniae* carrying *bla* OXA-48-like were selected for a pilot study. The aim of this study was to determine the genetic relatedness of isolates for tracking transmission pathways in involved hospitals for improved infection prevention and control (IPC) measures and to describe the molecular characteristics of the involved clones. Here, we report preliminary epidemiological, microbiological and genomic findings from the ongoing OXA-48-producing *K. pneumoniae* outbreak.

Data collection and analysis
Submission of carbapenem-resistant Enterobacterales (CRE) for reference testing to NVSPL is mandatory in Lithuania since 2014 [1]. Confirmatory antimicrobial susceptibility testing and carbapenemase gene detection by PCR of *bla* OXA-48-like, *bla* KPC, *bla* NDM and *bla* VIM genes is routinely performed at NVSPL. For this investigation, minimal inhibitory concentrations (MICs) for meropenem were determined using Bruker Micronaut-S plates and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [2].

Of 308 *K. pneumoniae* isolates confirmed by NVSPL to carry *bla* OXA-48-like genes in 2022–23, 106 (34.4%) were selected for sequencing. These isolates originated from 17 public hospitals between 28 April 2022 and 29 November 2023, covering the period of emergence. At least one isolate was selected per hospital. Supplementary Figure S1 provides time distribution of these isolates by sequencing status and major clusters. It also contains information on the isolate selection.

Paired-end sequencing was performed using Illumina platform. Reads were processed in Ridom SeqSphere v9.0.10 [3] using trimming parameters of ≥ 30 average quality in a window of 20 bases and SKESA assembler [4] and subjected to quality control. The Institute Pasteur scheme was used for typing [5]. Resistance genes were identified using NCBI AMRFinderPlus [6] within SeqSphere and Kleborate v2.4.1 [7] with standard parameters. A minimum spanning tree based on core genome multilocus sequence typing (cgMLST) scheme [3] was constructed in SeqSphere with samples...
that contained >95% cgMLST target loci excluded. Clusters were determined with a cut-off of ≤ 5 allelic differences (ADs).

**Detection of carbapenemase genes over time**

The carbapenemase gene distribution of submitted Enterobacterales isolates determined by NVSPL during 2017–23 is shown in the Table. Carbapenemase-producing Enterobacterales were rare in Lithuania until 2019, when a large outbreak of *K. pneumoniae* carrying *bla*<sub>KPC-2</sub> occurred (described in [8]). After the implementation of control measures, the number of identified carbapenemase-producing *K. pneumoniae* (CPKP) isolates declined but remained at a higher level than before the outbreak. In 2023, an increase in Enterobacterales carrying *bla*<sub>OXA-48-like</sub> (n = 319), mainly *K. pneumoniae* (n = 302, 94.7%), was identified (Table).

**Distribution of sequence types of *Klebsiella pneumoniae* carrying *bla*<sub>OXA-48</sub>**

The 106 sequenced *K. pneumoniae* isolates carrying *bla*<sub>OXA-48</sub> belonged to six *K. pneumoniae* sequence types (ST). The most frequent ST was ST45 (n = 35), followed by ST392 (n = 32) and ST395 (n = 31). A few isolates of other STs, i.e. ST147 (n = 4), ST307 (n = 3), ST29 (n = 1), also harboured *bla*<sub>OXA-48</sub> and four of these (ST307, n = 3; ST147, n = 1) co-carried *bla*<sub>NDM-1</sub>. Six clusters of suspected recent transmission were identified (Figure 1), including three large multi-hospital clusters with > 20 isolates (Cluster 1–3), described in further detail below.

**Multi-hospital clusters**

Cluster 1 included 35 isolates of *K. pneumoniae* ST45 involving six hospitals from two counties, indicating interregional spread in central-eastern Lithuania including the capital region. The first isolate was detected in June 2023 in hospital INST001 (Figure 2A) and 10 isolates were from blood samples. While no extended-spectrum beta-lactamase genes were detected, 25 isolates carried the plasmid-mediated AmpC gene *bla*<sub>DHA-1</sub>. Three isolates were resistant to meropenem (Figure 3A) of which one had a truncated outer membrane porin (OMP), OmpK36.

Cluster 2 included 32 isolates of *K. pneumoniae* ST392 involving six hospitals from three counties indicating interregional spread in central, northern and eastern Lithuania including the capital region. The first isolate was detected in hospital INSt004 in January 2023 (Figure 2B) and 16 isolates were from blood samples. Twenty-nine of these isolates also carried the *bla*<sub>CTX-M-15</sub> gene. Ten isolates were resistant to meropenem (Figure 3B), of which nine had truncated OMPs, either OmpK36 (n = 8) or OmpK35 (n = 1).

Cluster 3 included 28 isolates of *K. pneumoniae* ST395 involving nine hospitals from four counties indicating interregional spread in western Lithuania. The first isolate was detected in April 2022 in hospital INST017 (Figure 2C) and three isolates were from blood samples. All isolates carried truncated OmpK35 with duplication of OmpK36 Gly134-Asp135 (OmpK36GD) and were resistant to meropenem (Figure 3C). Additionally, 18 of these isolates carried the *bla*<sub>CTX-M-15</sub> gene and one isolate the *bla*<sub>CTX-M-3</sub> gene.

For Clusters 1, 2 and 3, the median ages of patients were 75 (range: 36–97), 64 (range: 29–94) and 74 (range: 30–88) years and the male-to-female ratios were 1.3, 0.9 and 1.5, respectively. Sample types varied by cluster, and the distribution is provided in Supplementary Figure S2. The 17 hospitals with confirmed CRE, including isolation and investigation of contact
Discussion

The worldwide spread of CPKP is driven by the transmission of high-risk non-susceptible clones among healthcare facilities [9,10]. A large outbreak of *K. pneumoniae* carrying bla*<sub>OXA-48-like</sub>* occurred in Lithuania in 2019 [8]. At that time, the increase of CPKP was largely based on clonal spread of *K. pneumoniae* ST392 and spread of an IncN plasmid harbouring bla*<sub>KPC-2</sub>* to other *K. pneumoniae* STs and Enterobacterales species [8]. The outbreak mainly occurred in one hospital with limited spread to other healthcare facilities via patient transfer [8]. In contrast, this genomic investigation highlights the changing epidemiology of CPKP in Lithuania, with diversification of involved high-risk clones and rapid interregional spread throughout the healthcare system in less than one year.

Cluster 1 – the largest cluster – was formed by *K. pneumoniae* ST45, which has been described as a major clone among carbapenem-non-susceptible *K. pneumoniae* isolates [7]; an outbreak of *K. pneumoniae* ST45 carrying bla<sub>GES-5/-1</sub> occurred in a hospital in Poland between 2017 and 2019 [11]. Cluster 2 involved *K. pneumoniae* ST392, which had caused the outbreak in Lithuania in 2019 [8] as well as outbreaks and cross-border spread in other European countries [12,13]. Finally, Cluster 3 consisted of *K. pneumoniae* isolates belonging to ST395, another well-known high-risk clone spreading in various countries [14] including in Eastern Europe [15]; ST395 has also been related to prior outbreaks and cross-border spread in other European countries [12,13]. This investigation has been enabled by the capacity building and harmonisation of WGS within the border spread in other European countries [12,13].

Isolates in different clusters showed varying levels of meropenem resistance with the highest resistance identified in isolates from Cluster 3 (ST395), which had alterations in major OMPs. Consistent with previous observation [17], combined OmpK35 truncation with OmpK35GD in OXA-48-producing isolates resulted in meropenem MICs exceeding the clinical breakpoint, as evident in isolates from Cluster 3 (ST395). This highlights the ability of CPKP to combine low-level carbapenem resistance mechanisms, i.e. altered OMPs (chromosomal feature) and OXA-48 production (mobile genetic element feature), resulting in clinically relevant phenotypes.

This investigation has been enabled by the capacity building and harmonisation of WGS within the European countries [12,13].
EURGen-RefLabCap project (https://www.eurgen-reflabcap.eu), funded by the European Commission. Lithuania is one of 16 European countries receiving specific training and bespoke advice to conduct genomic pilot studies and one of the first countries for which results have become available. This report may therefore provide an example for other countries currently setting up genomic AMR surveillance.

A limitation of this investigation is that it includes preliminary data from an ongoing outbreak investigation. Hence, at this stage, only epidemiological data collected by NVSPL under national surveillance regulations could be included. Additionally, it is too early to investigate the effectiveness of the implemented IPC measures. Furthermore, only a subset of *K. pneumoniae* isolates carrying *bla* _OXA-48_ has been sequenced and clusters may further increase in size and additional clusters may be detected once more genomic data become available.

**Conclusion**

This report demonstrates the value of national WGS-based surveillance for AMR. The newly established WGS capacity for AMR at NVSPL helped to disentangle an increase of *K. pneumoniae* carrying *bla* _OXA-48_ into at least three separate clonal outbreaks, facilitating targeted investigations and the strengthening of
national control efforts. This study also highlights the need to remain vigilant of repeated introduction and increasing spread of CPKP high-risk clones in healthcare systems and to ensure that hospitals are prepared to detect CPKP cases early and prevent onward transmission, as highlighted in the ECDC and WHO guidance documents.

**Ethical statement**

All data were anonymised and collected in accordance with the national legislation on data collection for communicable diseases in Lithuania. Ethical approval and informed consent were thus not required.

**Funding statement**

No specific funding.

**Data availability**

The whole genome sequencing data for this study were deposited in the European Nucleotide Archive under accession number PRJEB74083.

**Acknowledgements**

We would like to thank all the clinical microbiology laboratories for submitting isolates for national reference testing and the national working group on antimicrobial resistance investigation in Lithuania together with clinical microbiology laboratories for collaboration.

**Use of artificial intelligence tools**

None declared.

**Conflict of interest**

None declared.

**Authors’ contributions**

PG, ML, VB, EK, AK: design of the study; PG, LR: bioinformatic analysis; PG, ML, AK: drafting of manuscript; EK, GT: performance of whole genome sequencing; PG, ML, JR, JS, VB, EK, EA, OS, RSH, DP, DLM, AK, AG: interpretation of the results; all authors: review of manuscript.

**References**


**Figure 3**

Meropenem minimal inhibitory concentration values for *Klebsiella pneumoniae* isolates from the three major clusters, Lithuania, 2022–2023 (n = 95 isolates)

**MIC:** minimal inhibitory concentration; **ST:** sequence type.

Grey lines indicate European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [2] for meropenem in Enterobacteriales: susceptible ≤ 2 mg/L, resistant > 8 mg/L.


License, supplementary material and copyright

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY 4.0) Licence. You may share and adapt the material, but must give appropriate credit to the source, provide a link to the licence and indicate if changes were made.

Any supplementary material referenced in the article can be found in the online version.

This article is copyright of the authors or their affiliated institutions, 2024.