JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Emerging Problems in Infectious Diseases

Investigation of clonal relationship in *Klebsiella pneumoniae* strains grown in invasive specimens obtained from intensive care units

Hüseyin Güdücüoğlu[1], İsmail Davarcı[1], Elif Seren Tanrıverdi[2], Furkan Arabacı[1], Eda Kırılmaz[3], Feza İrem Aldı[1], Barış Otlu[2]

[1] Trakya University, Faculty of Medicine, Department of Medical Microbiology, Edirne, Turkiye
[2] Inonu University, Faculty of Medicine, Department of Medical Microbiology, Malatya, Turkiye
[3] Cankiri State Hospital, Medical Microbiology Laboratory

Abstract

Introduction: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a bacterium in "critical" category on the World Health Organization's list of "priority pathogens". The aim of our study is to identify the carbapenem resistance genes of *K. pneumoniae* isolates obtained from blood and cerebrospinal fluid samples sent from the intensive care units of our hospital and to investigate the clonal relationship among them.

Methodology: *K. pneumoniae* strains isolated from blood and CSF samples routinely collected from the intensive care units of our hospital over a two-year period were included in the study. Carbapenemase screening of CRKP strains was performed using Carba NP and CIM tests. Additionally, carbapenemase resistance genes (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, and *bla*$_{OXA-48}$) and clone analysis were performed using AP-PCR on these strains.

Results: A total of 186 strains with reduced susceptibility to at least one carbapenem were detected. In carbapenemase screening, the sensitivity of the CIM test was 98.3% (169/172), specificity was 7.1% (1/14); the sensitivity of Carba NP was 90.7% (156/172), specificity was 78.6% (11/14). 83.9% of the strains were *bla*$_{OXA-48}$, 4.8% *bla*$_{NDM}$, and 3.8% *bla*$_{KPC}$ positive. *bla*$_{IMP}$ and *bla*$_{VIM}$ resistance genes were not detected. One hundred and eighty-six *K. pneumoniae* isolates were identified with 62 different genotypes, and isolates showing clustering were grouped into 30 different clusters. The clustering rate of these isolates was 82.8%.

Conclusions: Resistant bacteria can cause small outbreaks in ICUs. Therefore, to identify high-risk clones and prevent further spread, there is a need to increase capacity to support outbreak investigations and surveillance with real-time whole genome sequencing.

Key words: bloodstream infection; carbapenem-resistant; clonal relationship; *Klebsiella pneumoniae*; multidrug-resistant.

J Infect Dev Ctries 2025; 19(11):1577-1583. doi:10.3855/jidc.21195

(Received 13 December 2024 – Accepted 20 May 2025)

Introduction

Klebsiella pneumoniae was described as a bacterium causing pneumonia by Carl Friedländer in 1882 and has been recognized as an important pathogen since then [1]. This bacterium, which belongs to the Enterobacterales family, is usually an encapsulated, immobile, and Gram-negative bacillus [2]. Today, it remains one of the most common Gram-negative pathogens in nosocomial septic patients worldwide [1]. Therefore, bloodstream infections (BSI) represent 40% of community-acquired and hospital-acquired sepsis and septic shock cases and approximately 20% of intensive care unit (ICU)-acquired cases [3]. Hospital-acquired BSI accounts for the highest burden of healthcare-associated infections [4]. These infections are highly prevalent in ICU patients and are associated with a mortality rate of 36-42% [5]. Since the bacteria are not airborne in such settings, direct or person-to-person contact is usually required for the spread of *Klebsiella* infection [1,2]. The arbitrarily primed polymerase chain reaction (AP-PCR) method used to explain this contact is one of the methods used for clone analysis, and its main advantage is that it provides highly specific DNA profiles without knowing the DNA sequence of the gene region. In addition, this method is a rapidly reproducible and highly sensitive method used to examine genetic polymorphism [6].

Bacteria acquire resistance through two main mechanisms: mutations in chromosomal genes and resistance genes acquired through mobile genetic elements that can spread horizontally between bacteria [7]. *K. pneumoniae* can carry resistance genes against various drugs and, therefore, develop resistance to antibiotics such as ß-lactams, tetracyclines, and quinolones. Carbapenems have long been recognized as an effective treatment against this bacterium. However, the widespread and uncontrolled use of antibiotics has caused the bacteria to produce enzymes such as

extended-spectrum ß-lactamase (ESBL) and carbapenemase, leading to increased resistance to carbapenems. Current treatment options for carbapenem-resistant *K. pneumoniae* (CRKP) are very limited, and this poses a severe public health threat [1].

Antimicrobial resistance (AMR) is projected to cause 10 million deaths worldwide each year by 2050, with severe economic consequences [8]. Although these estimates have been criticized by some authors [9,10], the first comprehensive global assessment of the burden of AMR conducted in 2019 confirmed this worrying trend [11]. Today, while the concept of "One Health" is becoming increasingly important, the World Health Organization (WHO) established a list of "priority pathogens" in 2017, ranked from highest priority to lowest priority according to antibiotic resistance levels. CRKP was included in the highest critical category in this list [12,13]. AMR can cause delays in treatment, increased mortality rates, resource consumption, and costs. This leads to significant increases in the use of broad-spectrum antimicrobials [5].

Our study aims to identify the carbapenem resistance genes of *K. pneumoniae* isolates from blood and cerebrospinal fluid (CSF) samples sent from our hospital's intensive care units, investigate the clonal relationship between them, and show whether these bacteria can cause recurrent infections in ICUs.

Methodology

Ethics committee approval

The study received ethical approval from the Non-Interventional Scientific Research Ethics Committee of the Faculty of Medicine of our University with the protocol code GOBAEK 2023/30.

Study Setting

This study was conducted at a university hospital with a total capacity of 942 beds. The hospital includes a 7-bed Reanimation ICU, a 22-bed Internal ICU (Units 1 and 2), a 12-bed Surgical ICU, a 4-bed secondary level Postoperative ICU, a 26-bed Cardiology ICU (Units 1 and 2), a 20-bed Neonatal ICU, a 6-bed Pediatric ICU, and a 20-bed Chest Diseases ICU (Units 1 and 2). The institution provides advanced-level intensive care services with a total of 117 ICU beds. This is a single-center study conducted at this university hospital.

Inclusion criteria

Between 01.10.2020 and 01.10.2022, *K. pneumoniae* isolates grown in blood and CSF samples obtained routinely from our hospital's intensive care units were included in the study. A single isolate was obtained from each patient.

Storage and revitalization of strains

K. pneumoniae growths in the samples included in the study were passaged onto skim milk medium for storage. Passaged colonies were frozen at -80 °C. In the analytical phase, frozen colonies were thawed at room temperature. When thawing was complete, 1 mL of the medium was taken and inoculated onto 5% sheep blood agar. Blood agar Petri dishes were then incubated at 37 °C for 24 hours.

Resistance profile determination by the disk diffusion method

Inoculum with 0.5 McFarland turbidity was prepared from the colonies in Petri dishes. This inoculum was inoculated on Mueller-Hinton Agar (MHA) with a sterile cotton swab. Antibiotic disks with a diameter of 6 mm were placed on the inoculated MHA Petri dishes using a disk dispenser (BioAnalyse, Turkey). MHA Petri dishes were incubated at 35 °C for 18 hours. At the end of the incubation period, the diameters of the zones of inhibition were measured, and EUCAST Breakpoint Tables Version 14.0 was used to interpret these diameters [14].

Carba NP

After 24 hours of incubation on Mueller-Hinton agar (BBL/Becton Dickinson, France), a 10 µL sample was suspended in a 100 µL volume of 20 mmol/L Tris-HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific Pierce, USA). The mixture was vortexed for 1 minute and incubated at room temperature for 30 minutes. 30 µL of supernatant from each isolate was placed in two wells on a 96-well microplate. The first well served as a negative control and contained 100 µL of phenol red (pH 7.8) solution to which 0.1 mmol/L ZnSO4 (Fluka, Germany) was added. The second well contained 100 µL of phenol red solution containing 6 mg/mL imipenem/cilastatin and 0.1 mmol/L ZnSO4. The phenol red solution was prepared by mixing 2 mL of 0.5% (wt/vol) phenol red solution with 16.6 mL of distilled water and adjusting the pH to 7.8 with 1N NaOH. Instead of imipenem monohydrate according to the original protocol, 6 mg/mL imipenem/cilastatin (Tienam, MSD) was used in the modified protocol. Tubes were incubated at 37 °C for 2 hours and monitored every 15 minutes; a color change from red to yellow was considered a positive result. Discordant results were repeated three times

[15].

CIM (Carbapenem Inactivation Method) test

The principle of this method is to detect the enzymatic hydrolysis of a carbapenem by incubating it with a bacterial suspension. In the CIM assay, meropenem disks are used as substrate. A suspension is prepared by taking a 10 µL sample of *K. pneumoniae* colonies grown on Muller-Hinton Agar (MHA) and suspending it in 400 µL of water. A susceptibility test disk containing 10 µg meropenem (BioAnalyse, Turkey) was then immersed in this suspension and incubated at 35 °C for two hours. After incubation, the disk is placed on an MHA inoculated three-way with *Escherichia coli* ATCC 25922. If bacterial enzymatic inactivation is present, no zone of inhibition is formed. In the absence of carbapenemase activity, a zone of inhibition is formed because the meropenem on the disk is not hydrolyzed [16].

Detection of carbapenemase genes:

DNA isolation from bacterial isolates was performed using the QIAsymphony automated DNA extraction system (Qiagen, Hilden, Germany) and QIAamp DNA Midi Kit (Qiagen, Hilden, Germany). Detection of carbapenemase genes was performed by multiplex polymerase chain reaction (PCR) targeting bla_{KPC}, bla_{NDM}, bla_{VIM}, $bla_{IMP,}$ and bla_{OXA-48} as described by Poirel *et al.* [17]

DNA amplification was performed using the GeneAmp PCR System 9700 (Applied Biosystems, Waltham, MA, USA). The resulting amplicons were electrophoresed on a 1.5% agar gel at 90V for 2 hours, stained with ethidium bromide, and visualized under UV light using a Kodak Gel Logic 200 system (Kodak Company, Rochester, USA).

Analysis of clonal relationships of Klebsiella pneumoniae isolates:

Clonal relationships of *K. pneumoniae* isolates were assessed using AP-PCR as previously described by Menekşe *et al.* [18]. Band patterns were analyzed with GelCompar II software (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium). The Dice correlation coefficient was used to calculate similarity for band analysis, and clustering was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Isolates with similarity coefficients greater than 90% were considered to belong to the same or related clones, while those with similarity below 90% were classified as different or unrelated clones.

Results

Between October 2020 and September 2022, 20756 samples, including 16531 blood cultures and 1131 CSF, were sent to our laboratory for routine culture testing. Significant growth was observed in 2931 samples, 2803 in blood culture, and 128 in CSF. *K. pneumoniae* (n=268; 9.1%) was found to be the second most common agent among the grown samples.

The number of *K. pneumoniae* included in the study was 268. The number of *Klebsiella* strains with decreased susceptibility to at least one carbapenem was 186 (69.4%). Of these samples, 182 were blood, and 4 were CSF. The antimicrobial resistance rates of these strains are shown in Table 1. Over 94% resistance to cephalosporin group antimicrobials and 91% resistance to quinolone group antimicrobials were observed. Amikacin resistance was 50%, while the most sensitive antimicrobial was ceftazidime-avibactam (CZA) (14.5%).

The CIM test positivity rate of these strains was 97.8% (182/186), and the Carba NP positivity rate was 85.5% (159/186). In carbapenemase screening of these strains in which five different resistance genes were investigated, the sensitivity of the CIM test was 98.3% (169/172), and specificity was 7.1% (1/14); the sensitivity of Carba NP was 90.7% (156/172), and specificity was 78.6% (11/14) (Table 2). 83.9% of the strains were bla_{OXA-48}, 4.8% bla_{NDM}, and 3.8% bla_{KPC} positive. bla_{IMP} and bla_{VIM} resistance genes were not detected. 100% (7/7) of bla_{KPC} carriers, 55.6% (5/9) of

Table 1. Distribution of Antimicrobial Resistance Rates Among Strains Harboring Resistance Genes by ICU Type (%).

	n	MEM	IMP	ETP	CXM	CAZ	CRO	CTX	FEP	AMC	TZP	CZA	CIP	LEV	CN	AK	SXT
Surgical ICU	67	83.6	79.1	100.0	95.5	91.0	94.0	97.0	95.5	98.5	98.5	17.9	91.0	89.6	65.7	44.8	77.6
Internal ICU	55	96.4	70.9	100.0	98.2	94.5	92.7	94.5	98.2	98.2	100.0	14.5	98.2	94.5	85.5	52.7	81.8
Chest Diseases ICU	30	93.3	90.0	100.0	100.0	93.3	96.7	100.0	100.0	100.0	100.0	0.0	100.0	100.0	93.3	50.0	86.7
Reanimation ICU	23	78.3	78.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	21.7	87.0	78.3	73.9	52.2	78.3
Post-op ICU	5	60.0	60.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	20.0	100.0	100.0	80.0	40.0	80.0
Pediatric ICU	4	100.0	75.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	25.0	100.0	100.0	100.0	100.0	100.0
Neonatal ICU	1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0
Cardiology ICU	1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0
Total	186	88.2	78.0	100.0	97.8	94.1	95.2	97.3	97.8	98.9	99.5	14.5	94.6	91.4	78.0	50.0	80.6

ICU: Intensive Care Unit; MEM: Meropenem; IMP: Imipenem; ETP: Ertapenem; CXM: Cefuroxime; CAZ: Ceftazidime; CRO: Ceftriaxone; CTX: Cefotaxime; FEP: Cefepime; AMC: Amoxicillin/clavulanic acid; TZP: Piperacillin/tazobactam; CZA: Ceftazidime/avibactam; CIP: Ciprofloxacin; LEV: Levofloxacin; CN: Gentamicin; AK: Amikacin; SXT: Trimethoprim/sulphamethoxazole.

Table 2. Phenotypic Test Results (CIM and Carba NP) According to the Distribution of Carbapenemase Resistance Genes.

Resistance gene	CIM		Carba NP		Total
	-	+	-	+	
bla_{OXA-48}	3	153	13	143	156
bla_{NDM}	0	9	3	6	9
bla_{KPC}	0	7	0	7	7
Negative	1	13	11	3	14
Total	4	182	27	159	186

bla_{NDM} carriers, and 85.9% (134/156) of bla_{OXA-48} carriers were found to be CZA sensitive.

AP-PCR was performed on 186 *K. pneumoniae* isolates with reduced susceptibility to at least one carbapenem. No single dominant epidemic clone was detected in the isolates, and clusters were found in them. One hundred and eighty-six *K. pneumoniae* isolates were identified with 62 different genotypes, and isolates showing clustering were grouped into 30 different clusters (tolerance 1.0, optimization 1.0, cutoff 90%). One hundred fifty-four of the isolates were included in any cluster, and the clustering rate was 82.8% (Supplementary Figure 1).

When the ICUs, in which genotype 1, which constituted the largest cluster in the study, was isolated within months, were analyzed, it was observed that it caused small outbreaks at four different times (Figure 1). Thus, it was determined that *Klebsiella* strains with the same genotype were isolated both in the same ICU and in different ICUs on different dates. Although the number of genotypes in other clusters was less, they were found to have similar characteristics.

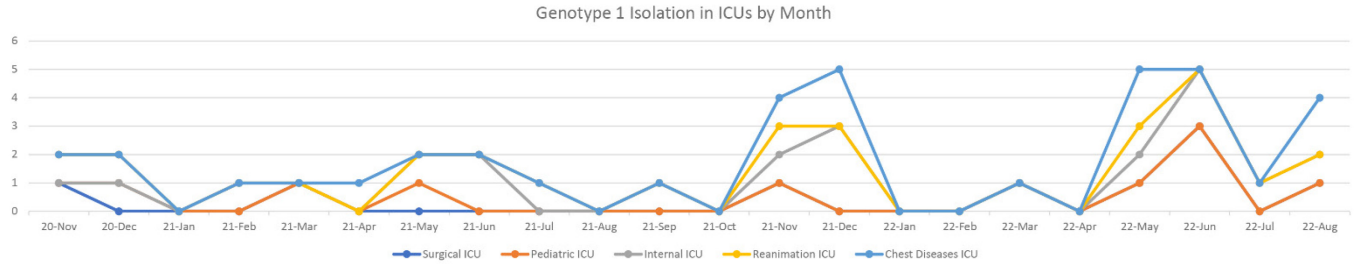
Discussion

Multidrug-resistant (MDR) bacterial infections pose an increasing burden on healthcare services and have severe economic impacts [19]. O'Neill's 2014 and 2015 studies project that without policy changes and improvements in research and drug development, 150 million premature deaths could occur worldwide in the next 50 years. These projections are based on only three of the seven resistant bacteria that the WHO considers the greatest threat – *K. pneumoniae*, *E. coli,* and *Staphylococcus aureus*. When the other four bacteria are taken into account, this threat and impact could be even more severe [20,21]. In 2019, another study estimated 541,000 deaths associated with bacterial AMR and 133,000 deaths attributable to bacterial AMR across Europe. Bloodstream infections caused the largest lethal burden of AMR in the region. Among the 319,000 deaths involving bloodstream infections, 195,000 were associated with AMR, and 47,200 deaths were attributable to AMR. *K. pneumoniae* was the third most responsible pathogen for approximately 457,000 resistance-related deaths in 53 countries in this region (68,994 deaths) [22]. Compared to 2019, the EU incidence of CRKP-associated bloodstream infections in 2022 increased by nearly 50%. This suggests that the EU is not making sufficient progress to meet its target of a 5% reduction in incidence by 2030 [23]. Carbapenem resistance is almost always combined with resistance to several other important antimicrobial groups, leaving extremely limited options in the treatment of serious infections caused by this bacterial species. In our study, carbapenem-resistant isolates were highly resistant to other antimicrobial drug groups. This suggests the need for continuous close monitoring and increased efforts to respond effectively to this threat to public health.

Various methods are used for the detection of carbapenemases. The CIM test and the Carba NP test are two of them. Although the CIM test was found to be more sensitive than Carba NP in the detection of carbapenemases in our study, the high specificity results of the Carba NP test give Carba NP an advantage over the CIM test. Therefore, we recommend that laboratories that cannot perform molecular tests should use Carba NP for carbapenemase detection instead of the CIM test.

Carbapenemase enzymes include class A serine

Figure 1. Timeline of Genotype 1 Isolation in ICUs by Month.



enzymes (bla_{KPC}), class B metallo beta-lactamases (bla_{VIM}, bla_{IMP}, bla_{NDM}), and class D enzymes ($bla_{OXA\text{-}48\text{-}like}$). Class D beta-lactamases identified in *K. pneumoniae* include bla_{OXA}-type carbapenemases ($bla_{OXA\text{-}48}$, $bla_{OXA\text{-}51}$, $bla_{OXA\text{-}181}$, $bla_{OXA\text{-}237}$) and ESBL-type oxacillinases ($bla_{OXA\text{-}11}$, $bla_{OXA\text{-}15}$). However, $bla_{OXA\text{-}48\text{-}like}$ carbapenemases pose a particular challenge in laboratory detection because they hydrolyze carbapenems poorly [23]. The $bla_{OXA\text{-}48}$ enzyme in *K. pneumoniae* was first identified in our country in 2001 [19,24]. The fact that the most frequently detected resistance gene in our study was $bla_{OXA\text{-}48}$ is consistent with other studies conducted in our country [25].

Carbapenems (imipenem or meropenem) are recommended as antimicrobial agents in patients with severe BSI and third-generation cephalosporin-resistant infections. For serious infections caused by carbapenem-resistant Enterobacterales (CRE), meropenem-vaborbactam or ceftazidime-avibactam are recommended if they show in vitro activity [26]. However, it should be taken into consideration that ceftazidime-avibactam is effective in vitro against Ambler class A (bla_{KPC}) and some class D ($bla_{OXA\text{-}48}$) carbapenemases but ineffective against MBL-producing strains [26]. In our study, CZA resistance was 14.5%, and 83.9% of the strains were $bla_{OXA\text{-}48}$ positive. In another study conducted in Turkey, CZA resistance was found to be 10.7%, and $_{OXA\text{-}48}$ positivity was 96.4% [23]. The high sensitivity rate of bla_{KPC} and $bla_{OXA\text{-}48}$-carrying strains to CZA is an expected result. Although, surprisingly, approximately half of the bla_{NDM}-carrying strains were sensitive to CZA, similar results were obtained in previous studies [23]. The fact that this rate is higher than expected may be due to the low number of bla_{NDM} strains in our hospital, but it also shows that strains carrying bla_{NDM} may be susceptible to CZA, albeit rarely. On the other hand, the fact that CZA was the first drug selected for CRKP infections in our hospital shows that it was the right decision.

There are eight different ICUs in our hospital. When ICUs were evaluated within themselves, no dominant clone was found in any ICU, but 30 different clusters were observed. The fact that an isolated genotype can be isolated from another patient in the same ICU or in another ICU months later is an indication that these strains spread within the hospital. However, ward patients were not included in our study. Considering that patients are transferred from the ICU to the ward or from the ward to the ICU, this spread may occur with patient transfer or hospital staff. In one study, the most frequently contaminated areas included gauze pads around the endotracheal tube, nasal catheter, oxygen mask, suction machine, bed sheet around the pillow, floor on both sides of the bed, bed railings, mobile nurse trolley railing, and the outer surface of the bedside drainage bag. These areas function as environmental reservoirs of CRKP in intensive care settings and are frequently contacted by healthcare workers during routine patient care [27]. These findings emphasize the importance of standardized cleaning regimes for surfaces and objects in the immediate vicinity of patients and the need to be aware of the role of these surfaces and objects in the spread of CRE. In the hospital setting, meticulous cleaning of surfaces and objects is of paramount importance to prevent the transmission of such infections to other patients and healthcare staff. In addition, molecular rapid diagnostic tests play a key role as they allow earlier adequate antimicrobial treatment and rapid identification of the origin of strains [5]. Therefore, molecular rapid diagnostic tests should be applied together with active surveillance so that genotypes showing clustering can be recognized earlier and measures can be taken.

Limitations

Our study has some limitations. Our study is retrospective, and active surveillance was not performed. Therefore, clone follow-up could not be performed when patients changed beds. In addition, environmental sampling was not performed to identify the source. However, the clonal relationship of *K. pneumoniae* strains causing infection in the ICU was revealed, shedding light on future comprehensive studies.

Conclusions

In conclusion, the WHO and numerous other researchers agree that the spread of antimicrobial resistance is an urgent problem that requires a global, coordinated plan of action [28,29] because, if left unchecked, antimicrobial resistance could make many bacterial pathogens much more deadly in the future than they are today [11]. CRKP isolates can be transferred between patients, intensive care unit staff, and the environment. Environmental contamination and CRKP-positive patients are the major sources of transmission to the hands, gloves, or gowns of intensive care unit staff. Compliance with contact precautions and more aggressive environmental cleaning and disinfection can reduce the transmission of CRKP isolates. A multidisciplinary approach is required in the treatment of critically ill patients with hospital-acquired BSI [5]. To identify high-risk clones and prevent further spread, there is a need to increase capacity to support

outbreak investigations and surveillance with real-time whole genome sequencing. Options for action to this end include timely and accurate diagnosis, implementation of high infection prevention and control standards, and effective antimicrobial stewardship.

Acknowledgements

This Project was supported by Project No: 2023-141 within the scope of Trakya University Scientific Projects. We would also like to thank the Trakya University Medical Microbiology staff.

Authors contributions

Idea/Hypothesis: HG Design: HG, İD, Data Collection/Data Processing: HG, İD, EST, FA, EK, FİA, BO Data Analysis: HG, İD, EST, FA, EK, FİA, BO Manuscript Preparation: HG, İD, EST, FA, EK, FİA, BO

Corresponding author

İsmail Davarci,
Trakya University Rectorate 22030
Balkan Campus / EDIRNE
Tel: +90 (284) 223 42 10-11-12-13
E-mail: ismaildavarci@trakya.edu.tr

Conflict of interest

No conflict of interest is declared.

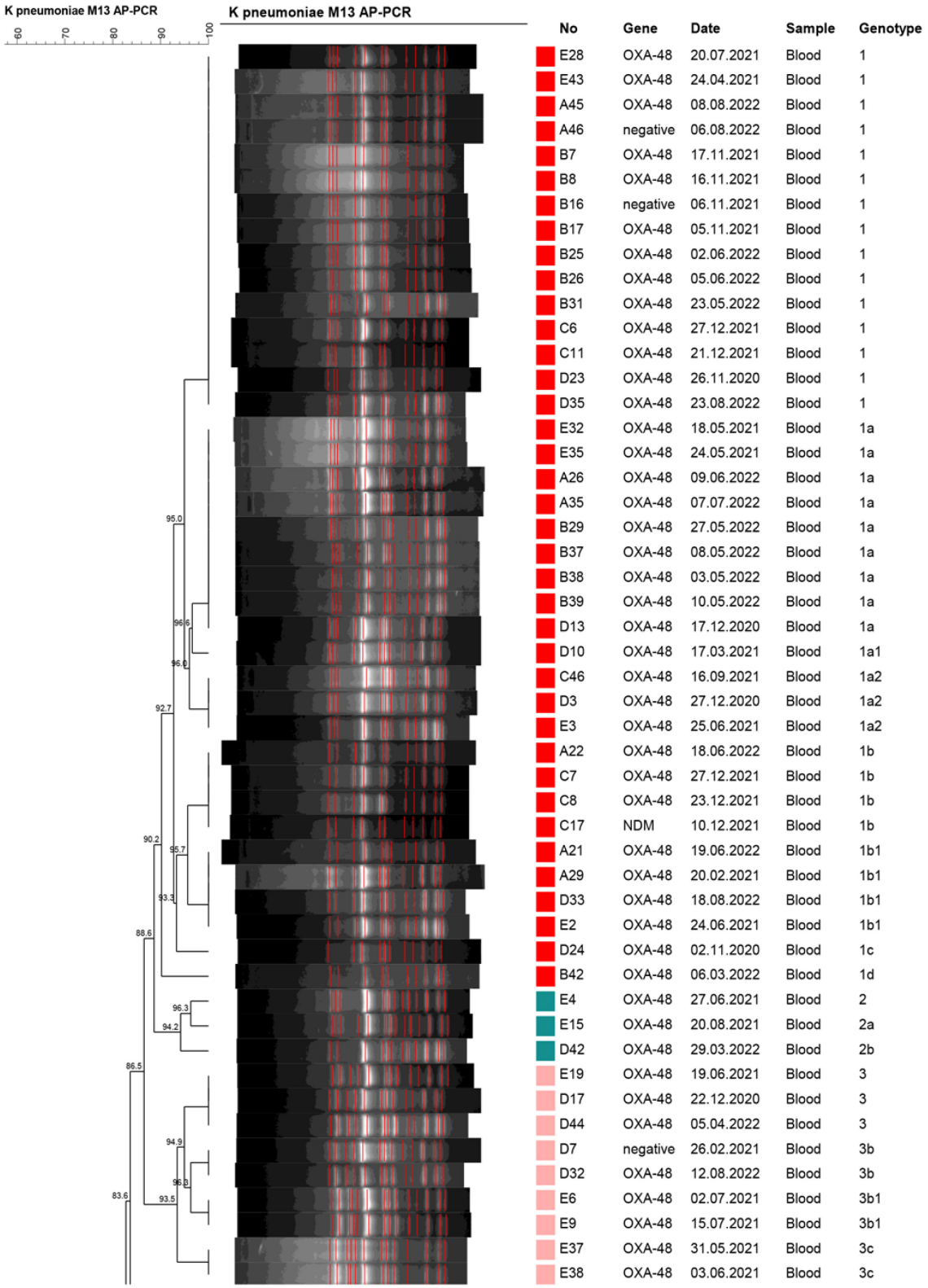
References

1. Kang J, Li G, Ma M, Lan M, Kang Y, Yang N, Jia W, Zhao Z (2022) Evidence of sharing of carbapenem-resistant *Klebsiella pneumoniae* strains between intensive care unit patients and the environment. Infect Drug Resist 15: 7831-7839. doi: 10.2147/idr.S388085.
2. Mancuso G, Midiri A, Gerace E, Biondo C (2021) Bacterial antibiotic resistance: the most critical pathogens. Pathogens 10: 1310. doi: 10.3390/pathogens10101310
3. Timsit JF, Ruppé E, Barbier F, Tabah A, Bassetti M (2020) Bloodstream infections in critically ill patients: an expert statement. Intensive Care Med 46: 266-284. doi: 10.1007/s00134-020-05950-6.
4. Cassini A, Plachouras D, Eckmanns T, Abu Sin M, Blank HP, Ducomble T, Haller S, Harder T, Klingeberg A, Sixtensson M, Velasco E, Weiß B, Kramarz P, Monnet DL, Kretzschmar ME, Suetens C (2016) Burden of six healthcare-associated infections on European population health: estimating incidence-based disability-adjusted life years through a population prevalence-based modelling study. PLoS Med 13: e1002150. doi: 10.1371/journal.pmed.1002150.
5. Tabah A, Buetti N, Staiquly Q, Ruckly S, Akova M, Aslan AT, Leone M, Conway Morris A, Bassetti M, Arvaniti K, Lipman J, Ferrer R, Qiu H, Paiva JA, Povoa P, De Bus L, De Waele J, Zand F, Gurjar M, Alsisi A, Abidi K, Bracht H, Hayashi Y, Jeon K, Elhadi M, Barbier F and Timsit JF on behalf of the EUROBACT-2 Study Group, ESICM, ESCMID ESGCIP and the OUTCOMEREA Network (2023) Epidemiology and outcomes of hospital-acquired bloodstream infections in intensive care unit patients: the EUROBACT-2 international cohort study. Intensive Care Med 49: 178-190. doi: 10.1007/s00134-022-06944-2.
6. Chen C, Slots J (1994) Clonal analysis of porphyromonas gingivalis by the arbitrarily primed polymerase chain reaction. Oral Microbiol Immunol 9: 99-103. doi: 10.1111/j.1399-302x.1994.tb00042.x.
7. European Centre for Disease Prevention and Control (2023) Antimicrobial resistance surveillance in Europe 2023 – 2021. Available: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2023-2021-data. Accessed: 15 November 2024.
8. Wellcome Trust and the UK Department of Health (2016) Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance. Available: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf. Accessed: 15 November 2024.
9. de Kraker ME, Stewardson AJ, Harbarth S (2016) Will 10 million people die a year due to antimicrobial resistance by 2050? PLoS Med 13: e1002184. doi: 10.1371/journal.pmed.1002184.
10. NOAH (National Office of Animal Health) (2016) Response to final O Neill AMR review report July 2016. Available: https://www.noah.co.uk/wp-content/uploads/2016/07/FINAL-NOAH-response-to-final-O-Neill-review-25-07-16-cle.pdf. Accessed: 15 November 2024.
11. Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399: 629-655. doi: 10.1016/S0140-6736(21)02724-0.
12. Breijyeh Z, Jubeh B, Karaman R (2020) Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 25. doi: 10.3390/molecules25061340.
13. Güdücüoğlu H (2024) A one health approach to zoonotic diseases in extraordinary and ordinary circumstances. Balkan Med J 41: 236–238. doi: 10.4274/balkanmedj.galenos.2024.2024-140624.
14. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2024) Breakpoint tables for interpretation of MICs and zone diameters, Version 14.0. Available: https://www.eucast.org/ast_of_bacteria/previous_versions_of_documents. Accessed: 15 November 2024
15. Nordmann P, Poirel L, Dortet L (2012) Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerging infectious diseases 18:1503. doi: 10.3201/eid1809.120355.
16. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM (2015) The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PloS one 10: e0123690. doi: 0.1371/journal.pone.0123690.
17. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70: 119-23. doi: 10.1016/j.diagmicrobio.2010.12.002.
18. Menekşe Ş, Tanrıverdi ES, Oğuş H, Altınay E, Kaya Ç, Çağlayan E, Aydoğan AA, Otlu B, Kırali MK (2023) Stenotrophomonas maltophilia outbreak with a commercial blood gas injector as the culprit and interventions for source and prevention:A possible passage between patient and ECMO water heater device. Am J Infect Control 51: 533-538. doi: 10.1016/j.ajic.2022.07.012.

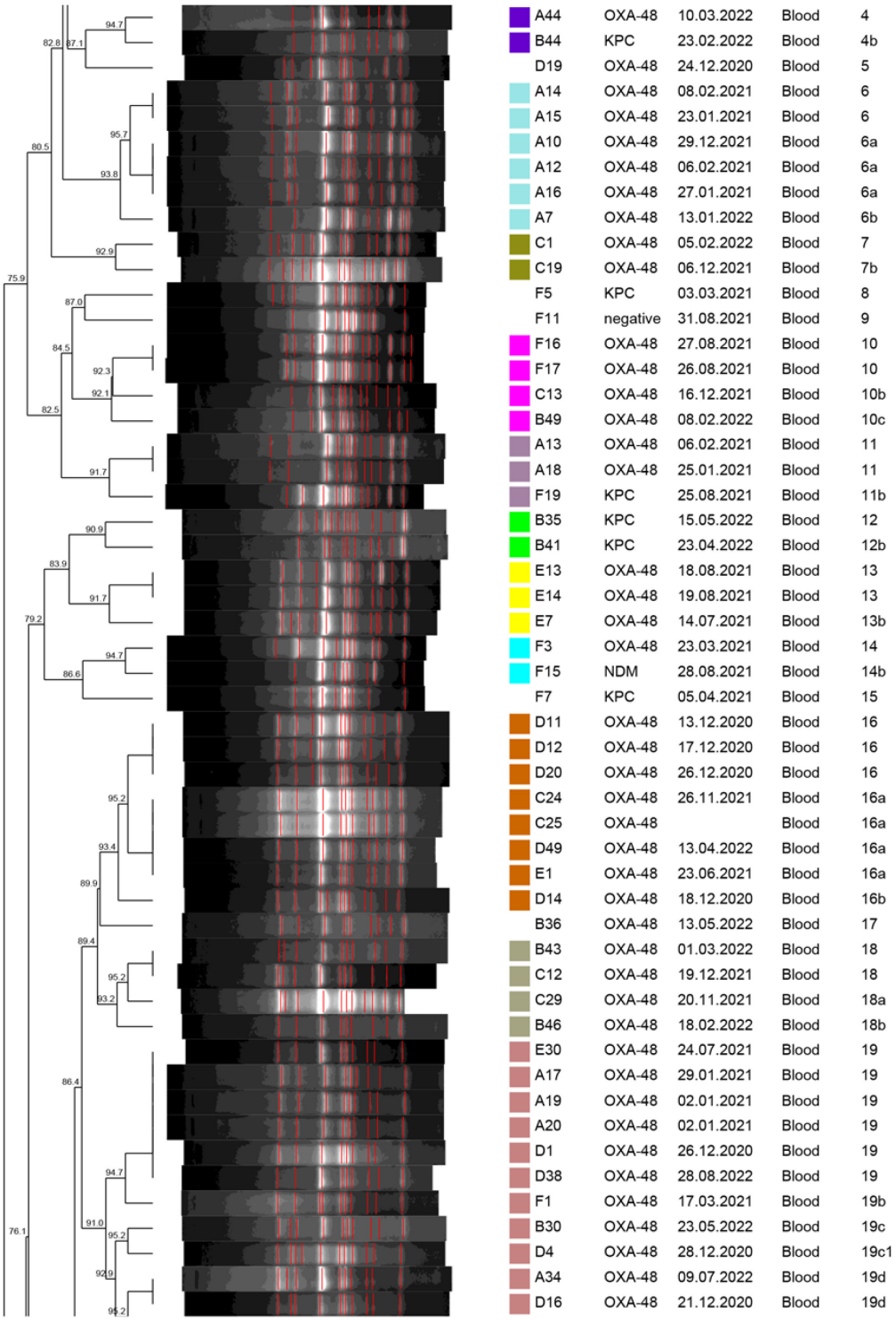
19. Gauba A, Rahman KM (2023) Evaluation of antibiotic resistance mechanisms in gram-negative bacteria. Antibiotics 12: 1590. doi: 10.3390/antibiotics12111590.
20. Wellcome Trust and the UK Department of Health (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on antimicrobial resistance. Available: https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf. Accessed: 15 November 2024.
21. Wellcome Trust and the UK Department of Health (2015) Securing new drugs for future generations: the pipeline of antibiotics. Review on antimicrobial resistance. Available: https://amr review.org/sites/default/files/SECURING%20NEW%20DRUGS%20FOR%20FUTURE%20GENERATIONS%20FINAL%20WEB_0.pdf. Accessed: 15 November 2024.
22. European Antimicrobial Resistance Collaborators (2022) The burden of bacterial antimicrobial resistance in the WHO European region in 2019: a cross-country systematic analysis. Lancet Public Health 7: e897-e913. doi: 10.1016/S2468-2667(22)00225-0.
23. European Centre for Disease Prevention and Control (2022) Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2022. Available: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2022. Accessed: 15 November 2024.
24. Poirel L, Héritier C, Tolün V, Nordmann P (2004) Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrobial agents and chemotherapy 48: 15-22. doi: 10.1128/aac.48.1.15-22.2004.
25. Arıcı N, Tanrıverdi ES, Çalık Ş, Kansak N, Adaleti R, Otlu B, Aksaray S (2023) Carbapenem-resistant *Klebsiella pneumoniae* outbreak with monoclonal spread: Evaluation of resistance genes and ceftazidime-avibactam susceptibility. Indian J Med Microbiol 46: 100431. doi: 10.1016/j.ijmmb.2023.100431.
26. Paul M, Carrara E, Retamar P, Tängdén T, Bitterman R, Bonomo RA, de Waele J, Daikos GL, Akova M, Harbarth S, Pulcini C, Garnacho-Montero J, Seme K, Tumbarello M, Lindemann PC, Gandra S, Yu Y, Bassetti M, Mouton JW, Tacconelli E, Rodríguez-Baño J (2022) European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant Gram-negative bacilli (endorsed by European society of intensive care medicine). Clin Microbiol Infect 28: 521-547. doi: 10.1016/j.cmi.2021.11.025.
27. Yan Z, Zhou Y, Du M, Bai Y, Liu B, Gong M, Song H, Tong Y, Liu Y (2019) Prospective investigation of carbapenem-resistant *Klebsiella pneumonia* transmission among the staff, environment and patients in five major intensive care units, Beijing. J Hosp Infect 101: 150-157. doi: 10.1016/j.jhin.2018.11.019.
28. World Health Organisation (2023) Antimicrobial resistance. Available: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. Accessed: 15 November 2024.
29. US Department of Health and Human Services (2019) Antibiotic resistance threats in the United States. Available: https://www.cdc.gov/antimicrobial-resistance/media/pdfs/2019-ar-threats-report-508.pdf. Accessed: 15 November 2024.

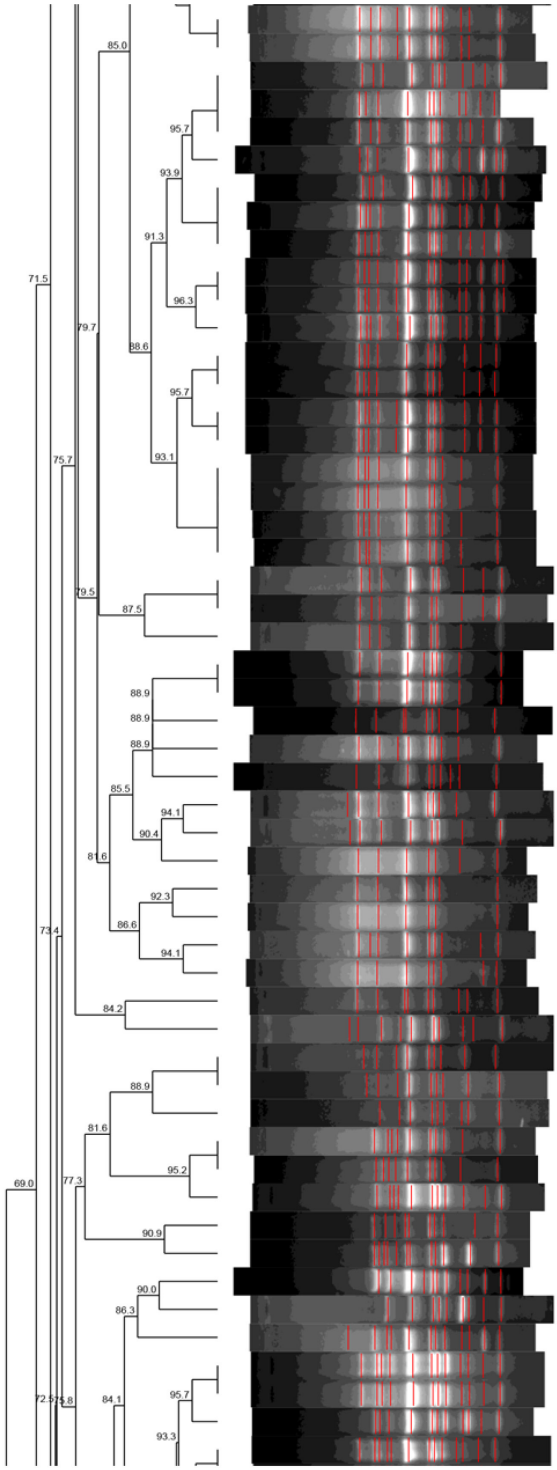
Annex – Supplementary Items

Supplementary Figure 1. AP-PCR results of 186 strains.

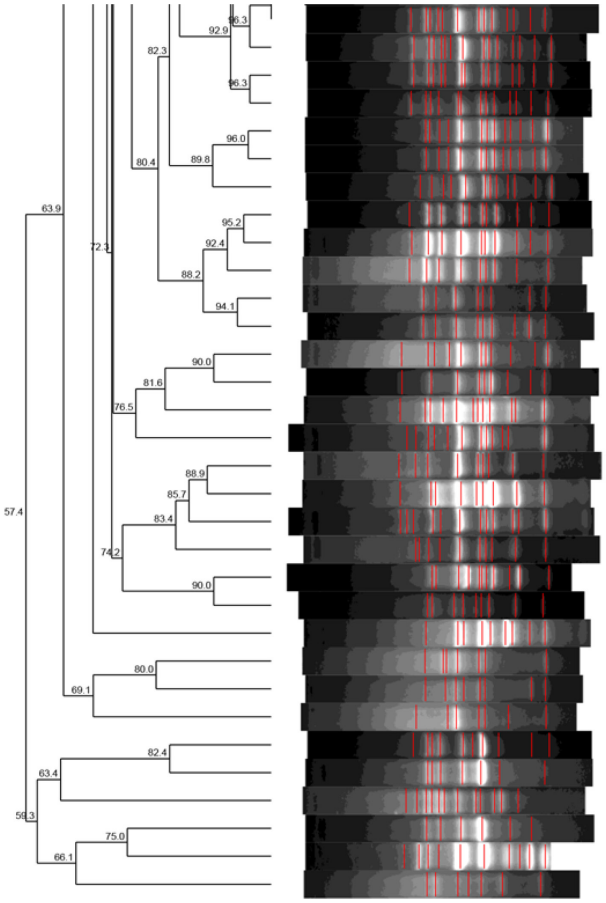


No	Gene	Date	Sample	Genotype
E28	OXA-48	20.07.2021	Blood	1
E43	OXA-48	24.04.2021	Blood	1
A45	OXA-48	08.08.2022	Blood	1
A46	negative	06.08.2022	Blood	1
B7	OXA-48	17.11.2021	Blood	1
B8	OXA-48	16.11.2021	Blood	1
B16	negative	06.11.2021	Blood	1
B17	OXA-48	05.11.2021	Blood	1
B25	OXA-48	02.06.2022	Blood	1
B26	OXA-48	05.06.2022	Blood	1
B31	OXA-48	23.05.2022	Blood	1
C6	OXA-48	27.12.2021	Blood	1
C11	OXA-48	21.12.2021	Blood	1
D23	OXA-48	26.11.2020	Blood	1
D35	OXA-48	23.08.2022	Blood	1
E32	OXA-48	18.05.2021	Blood	1a
E35	OXA-48	24.05.2021	Blood	1a
A26	OXA-48	09.06.2022	Blood	1a
A35	OXA-48	07.07.2022	Blood	1a
B29	OXA-48	27.05.2022	Blood	1a
B37	OXA-48	08.05.2022	Blood	1a
B38	OXA-48	03.05.2022	Blood	1a
B39	OXA-48	10.05.2022	Blood	1a
D13	OXA-48	17.12.2020	Blood	1a
D10	OXA-48	17.03.2021	Blood	1a1
C46	OXA-48	16.09.2021	Blood	1a2
D3	OXA-48	27.12.2020	Blood	1a2
E3	OXA-48	25.06.2021	Blood	1a2
A22	OXA-48	18.06.2022	Blood	1b
C7	OXA-48	27.12.2021	Blood	1b
C8	OXA-48	23.12.2021	Blood	1b
C17	NDM	10.12.2021	Blood	1b
A21	OXA-48	19.06.2022	Blood	1b1
A29	OXA-48	20.02.2021	Blood	1b1
D33	OXA-48	18.08.2022	Blood	1b1
E2	OXA-48	24.06.2021	Blood	1b1
D24	OXA-48	02.11.2020	Blood	1c
B42	OXA-48	06.03.2022	Blood	1d
E4	OXA-48	27.06.2021	Blood	2
E15	OXA-48	20.08.2021	Blood	2a
D42	OXA-48	29.03.2022	Blood	2b
E19	OXA-48	19.06.2021	Blood	3
D17	OXA-48	22.12.2020	Blood	3
D44	OXA-48	05.04.2022	Blood	3
D7	negative	26.02.2021	Blood	3b
D32	OXA-48	12.08.2022	Blood	3b
E6	OXA-48	02.07.2021	Blood	3b1
E9	OXA-48	15.07.2021	Blood	3b1
E37	OXA-48	31.05.2021	Blood	3c
E38	OXA-48	03.06.2021	Blood	3c


94.7
82.8
87.1
95.7
80.5
93.8
92.9
75.9
87.0
84.5
92.3
92.1
82.5
91.7
90.9
83.9
91.7
79.2
94.7
86.6
95.2
93.4
89.9
89.4
95.2
93.2
86.4
94.7
91.0
76.1
95.2
92.9
95.2
A44 OXA-48 10.03.2022 Blood 4
B44 KPC 23.02.2022 Blood 4b
D19 OXA-48 24.12.2020 Blood 5
A14 OXA-48 08.02.2021 Blood 6
A15 OXA-48 23.01.2021 Blood 6
A10 OXA-48 29.12.2021 Blood 6a
A12 OXA-48 06.02.2021 Blood 6a
A16 OXA-48 27.01.2021 Blood 6a
A7 OXA-48 13.01.2022 Blood 6b
C1 OXA-48 05.02.2022 Blood 7
C19 OXA-48 06.12.2021 Blood 7b
F5 KPC 03.03.2021 Blood 8
F11 negative 31.08.2021 Blood 9
F16 OXA-48 27.08.2021 Blood 10
F17 OXA-48 26.08.2021 Blood 10
C13 OXA-48 16.12.2021 Blood 10b
B49 OXA-48 08.02.2022 Blood 10c
A13 OXA-48 06.02.2021 Blood 11
A18 OXA-48 25.01.2021 Blood 11
F19 KPC 25.08.2021 Blood 11b
B35 KPC 15.05.2022 Blood 12
B41 KPC 23.04.2022 Blood 12b
E13 OXA-48 18.08.2021 Blood 13
E14 OXA-48 19.08.2021 Blood 13
E7 OXA-48 14.07.2021 Blood 13b
F3 OXA-48 23.03.2021 Blood 14
F15 NDM 28.08.2021 Blood 14b
F7 KPC 05.04.2021 Blood 15
D11 OXA-48 13.12.2020 Blood 16
D12 OXA-48 17.12.2020 Blood 16
D20 OXA-48 26.12.2020 Blood 16
C24 OXA-48 26.11.2021 Blood 16a
C25 OXA-48 Blood 16a
D49 OXA-48 13.04.2022 Blood 16a
E1 OXA-48 23.06.2021 Blood 16a
D14 OXA-48 18.12.2020 Blood 16b
B36 OXA-48 13.05.2022 Blood 17
B43 OXA-48 01.03.2022 Blood 18
C12 OXA-48 19.12.2021 Blood 18
C29 OXA-48 20.11.2021 Blood 18a
B46 OXA-48 18.02.2022 Blood 18b
E30 OXA-48 24.07.2021 Blood 19
A17 OXA-48 29.01.2021 Blood 19
A19 OXA-48 02.01.2021 Blood 19
A20 OXA-48 02.01.2021 Blood 19
D1 OXA-48 26.12.2020 Blood 19
D38 OXA-48 28.08.2022 Blood 19
F1 OXA-48 17.03.2021 Blood 19b
B30 OXA-48 23.05.2022 Blood 19c
D4 OXA-48 28.12.2020 Blood 19c1
A34 OXA-48 09.07.2022 Blood 19d
D16 OXA-48 21.12.2020 Blood 19d



E33	OXA-48	20.05.2021	Blood	19d1
E40	OXA-48	07.06.2021	Blood	19d1
B33	OXA-48	22.05.2022	Blood	20
C28	OXA-48	21.11.2021	Blood	20
D40	OXA-48	06.09.2022	Blood	20
A5	negative	24.01.2022	Blood	20a
E20	OXA-48	30.07.2021	Blood	20b
B47	OXA-48	16.02.2022	Blood	20b
D36	OXA-48	23.08.2022	Blood	20b
C4	OXA-48	31.01.2022	Blood	20c
C5	OXA-48	31.01.2022	Blood	20c
B48	OXA-48	12.02.2022	Blood	20c1
C14	NDM	17.12.2021	Blood	21
C15	NDM	17.12.2021	Blood	21
C2	OXA-48	03.02.2022	Blood	21a
C3	OXA-48	02.02.2022	Blood	21a
B12	OXA-48	10.11.2021	Blood	21b
B13	OXA-48	09.11.2021	Blood	21b
B19	OXA-48	01.11.2021	Blood	21b
B23	OXA-48	22.10.2021	Blood	21b
A42	OXA-48	18.03.2022	Blood	22
B27	negative	28.05.2022	Blood	22
A43	OXA-48	16.03.2022	Blood	23
F8	OXA-48	07.04.2021	Blood	24
F9	OXA-48	12.04.2021	Blood	25
D29	OXA-48	11.12.2020	Blood	26
E44	OXA-48	04.05.2021	CSF	27
A25	OXA-48	10.06.2022	Blood	28
A30	negative	18.02.2021	Blood	29
A38	OXA-48	24.06.2022	Blood	29b
B2	OXA-48	04.01.2022	Blood	29c
E47	OXA-48	07.05.2021	Blood	30
B4	OXA-48	27.12.2021	Blood	30b
E42	OXA-48	26.04.2021	CSF	31
B1	OXA-48	03.01.2022	Blood	31b
F2	OXA-48	23.03.2021	Blood	32
A41	negative	22.03.2022	Blood	33
A47	OXA-48	04.08.2022	Blood	34
B28	NDM	27.05.2022	Blood	34
B40	NDM	25.04.2022	Blood	35
E39	OXA-48 +.	05.06.2021	Blood	36
B24	OXA-48	21.10.2021	Blood	36
C41	OXA-48	01.10.2021	Blood	36a
D30	OXA-48	13.12.2020	Blood	37
D31	OXA-48	10.08.2022	Blood	37b
F12	OXA-48	31.08.2021	Blood	38
A40	negative	23.06.2022	Blood	38b
E41	KPC	26.04.2021	Blood	39
C31	OXA-48	17.10.2021	Blood	40
C35	OXA-48	12.10.2021	Blood	40
D43	OXA-48 +.	02.04.2022	Blood	40a
D6	OXA-48	21.02.2021	Blood	40b



D15	OXA-48	18.12.2020	Blood	40b
E11	OXA-48	15.08.2021	Blood	40b1
E22	OXA-48	03.08.2021	Blood	40c
E27	OXA-48	18.07.2021	Blood	40c1
D45	OXA-48	05.04.2022	Blood	41
D48	OXA-48	13.04.2022	Blood	41a
E18	KPC	11.06.2021	Blood	42
E26	OXA-48	12.08.2021	Blood	43
C30	OXA-48	18.11.2021	Blood	43a
E36	OXA-48	25.05.2021	Blood	43b
E50	OXA-48	14.05.2021	Blood	44
D5	OXA-48	29.12.2020	Blood	44b
E31	KPC	18.05.2021	Blood	45
D18	OXA-48	22.12.2020	Blood	45b
C20	OXA-48	04.12.2021	Blood	46
A3	OXA-48	26.01.2022	Blood	47
A32	OXA-48	14.07.2022	Blood	48
C18	NDM	07.12.2021	Blood	49
A2	negative	31.12.2020	Blood	50
A50	OXA-48	21.07.2022	Blood	51
F13	NDM	29.08.2021	Blood	52
C16	NDM	17.12.2021	Blood	52b
C21	OXA-48	04.12.2021	Blood	53
B9	negative	13.11.2021	Blood	54
B14	negative	08.14.2021	Blood	55
B6	OXA-48	17.11.2021	Blood	56
E23	OXA-48	05.08.2021	Blood	57
C45	OXA-48	18.09.2021	Blood	58
E48	OXA-48	12.05.2021	Blood	59
C50	negative	23.08.2021	Blood	60
C27	OXA-48	25.11.2021	Blood	61
B10	negative	11.11.2021	Blood	62