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## A current threat to public health: hospital acquired infections caused by multidrug resistant microorganisms, such as *Klebsiella pneumoniae*

### Abstract

This paper presents a bacteria case study involving the isolation of a *Klebsiella pneumoniae* strain producing both KPC and OXA-48 carbapenemases, cultured from clinical material obtained from a patient hospitalised with suspected bacterial infection. Microbiological diagnostics included culture on selective media, strain identification using MALDI-TOF technology (Autobio<sup>®</sup>), and antimicrobial susceptibility testing by broth microdilution with the Thermo Scientific<sup>TM</sup> Sensititre<sup>TM</sup> automated system. The isolate exhibited resistance to a broad spectrum of antibiotics, including carbapenems, cephalosporins, aminoglycosides, and colistin, while remaining susceptible only to cefiderocol. This case highlights the urgent need for implementing rational antibiotic therapy and coordinated efforts by antimicrobial stewardship teams. The paper also discusses national and international programs aimed at combating antibiotic resistance and evaluates their effectiveness in preserving the efficacy of infection treatment.

**Keywords:** Enterobacteriaceae, epidemiology, OXA-48 carbapenemases

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### Introduction

The increasing resistance of Enterobacteriaceae to antibiotics, particularly carbapenems, has become a major global concern. Of particular importance is *Klebsiella pneumoniae* (Schroeter) Trevisan producing carbapenemases such as KPC (*Klebsiella pneumoniae* carbapenemase) and OXA-48, which significantly limit treatment options for healthcare-associated infections (Queenan, Bush, 2007; Munoz-Price et al., 2013).

These pathogens have been listed among the highest priority organisms by the World Health Organization (WHO, 2017).

KPC enzymes belong to class A  $\beta$ -lactamases and can hydrolyse nearly all  $\beta$ -lactams, including carbapenems, making them especially problematic (Bush, Bradford, 2020). Meanwhile, OXA-48 carbapenemases, belonging to class D, have a lower hydrolytic activity but often coexist with other resistance mechanisms, further complicating treatment (Poirel et al., 2012; Evans, Amyes, 2014). The co-occurrence of KPC and OXA-48 in a single isolate leads to strains classified as extensively drug-resistant (XDR) or even pandrug-resistant (PDR), resistant to all available  $\beta$ -lactams and often to other antibiotic classes (Muñoz-Price et al., 2013; ECDC, 2018). Treating infections caused by such strains poses a significant challenge. Despite the emergence of new therapeutic options, such as ceftazidime/avibactam or cefiderocol, clinical efficacy remains limited in some cases (Hayden et al., 2020; Yu et al., 2024). Therefore, rapid identification of resistance mechanisms using molecular and phenotypic methods, along with close collaboration between microbiologists and clinicians, is essential (Caliskan-Aydogan, Alocilja, 2023; EUCAST, 2025).

Infections caused by multidrug-resistant *K. pneumoniae* are associated with high mortality rates, prolonged hospital stays, and substantial treatment costs (Laxminarayan et al., 2013; Prestinaci et al., 2015). In this context, not only the development of new antimicrobials but also strengthening global infection control strategies, educational efforts, and antimicrobial stewardship programs in line with the One Health approach are crucial (Pulcini et al., 2019; WHO, 2025).

### **ESKAPE pathogens – a threat to modern medicine**

The group of pathogens known as ESKAPE comprises bacteria characterised by particularly high virulence and the ability to develop multidrug resistance (MDR), extensive drug resistance (XDR), and in some cases even pan-drug resistance (PDR). These microorganisms are among the main etiological agents of hospital-acquired infections and pose a significant challenge in the treatment of severe infections, particularly in patients admitted to Intensive Care Units, individuals with compromised immunity, and those undergoing invasive medical procedures (Santajit, Indrawattana, 2016; Tacconelli et al., 2018).

ESKAPE pathogens not only hinder effective treatment due to the limited availability of active antibiotics but also contribute to prolonged hospitalisation, the need to use last-resort therapies (e.g. colistin), and an increased risk of epidemic outbreaks in healthcare settings. The economic burden associated with treating infections caused by these pathogens is substantial. According to the World Health Organization (WHO), research into novel methods of combating these microorganisms is a global priority (WHO, 2023). The name ESKAPE is an acronym created from the Latin names of

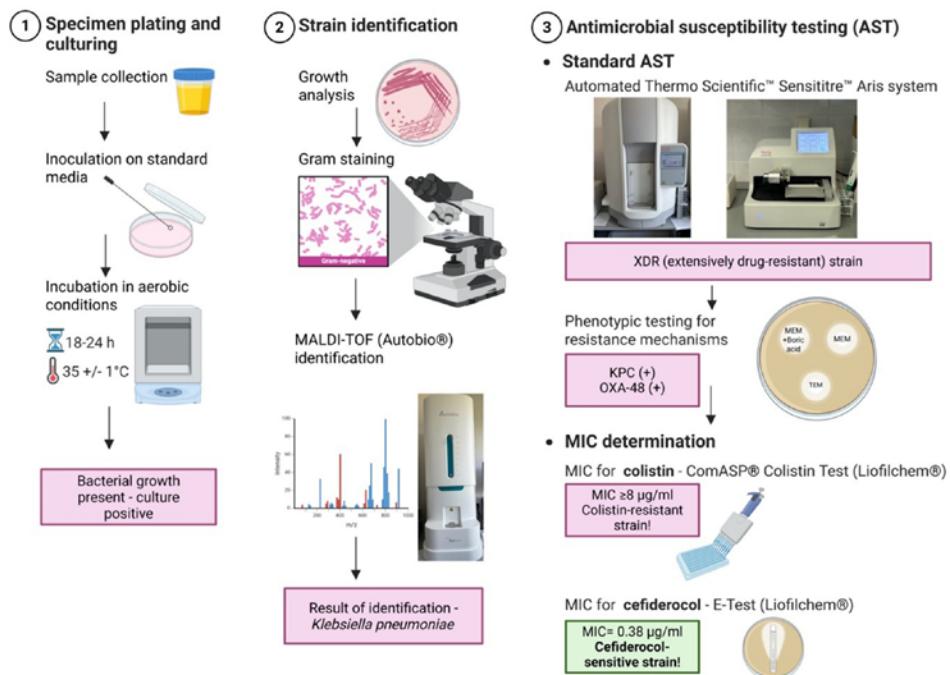
six key opportunistic pathogens: E – *Enterococcus faecium* (Orla-Jensen) Schleifer & Kilpper-Bälz, S – *Staphylococcus aureus* F.J. Rosenbach, K – *Klebsiella pneumoniae*, A – *Acinetobacter baumannii* Brisou & Prévot, P – *Pseudomonas aeruginosa* (Schröter) Migula, E – *Enterobacter* spp. Each of these pathogens makes use of unique resistance mechanisms that significantly complicate effective antibiotic therapy: *Enterococcus faecium* – strains resistant to vancomycin (VRE), greatly complicate treatment (Tacconelli et al., 2018); *Staphylococcus aureus* – methicillin resistance (MRSA) makes it one of the most dangerous nosocomial pathogens (Otto, 2023); *Klebsiella pneumoniae* – capable of producing extended-spectrum  $\beta$ -lactamases (ESBL) and carbapenemases (KPC), rendering a broad spectrum of  $\beta$ -lactams ineffective (Nordmann et al., 2021); *Acinetobacter baumannii* – shows particularly high resistance to carbapenems and many other antibiotic classes (Kubin et al., 2025); *Pseudomonas aeruginosa* – often multidrug-resistant due to mechanisms such as efflux pumps and enzymatic drug inactivation (Potron et al., 2013); *Enterobacter* spp. – resistance mainly associated with efflux pump deregulation and the presence of  $\beta$ -lactam hydrolysing enzymes (Hu et al., 2023). The ability of ESKAPE bacteria to evade antibiotic action through diverse resistance mechanisms makes them one of the greatest challenges of modern infectious disease medicine. Their increasing prevalence in hospital environments necessitates urgent action in infection control, resistance monitoring, and the development of new therapeutic strategies (Tacconelli et al., 2018; WHO, 2023).

The aim of this study is to characterise a *Klebsiella pneumoniae* strain producing KPC and OXA-48 carbapenemases, with a focus on microbiological analysis and antimicrobial susceptibility. In addition, it also discusses national and international programs combating antibiotic resistance and assesses their effectiveness in treating infections.

## Material and methods

This study was conducted as part of routine microbiological diagnostics in a hospital located in the Małopolska region, Poland. The analysed specimen was a urine sample collected from a hospitalised patient with suspected urinary tract infection. The scheme in figure (1) covers the successive stages of microbiological diagnosis.

The sample was cultured on standard microbiological media according to the EUCAST (2025) recommendations, including Columbia Agar with 5% sheep blood (Thermo Scientific). Incubation was carried out under aerobic conditions at  $35 \pm 1^\circ\text{C}$  for 18–24 hours. Colonies with typical morphology were selected for further analysis. Species identification was performed using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS, Autobio<sup>®</sup> MS 2000). Protein spectra generated by the instrument were compared against the manufacturer's reference database, which allowed for the definitive identification of the isolate.



**Fig. 1.** The stages of the diagnostic procedure that led to the detection of a multidrug-resistant isolate (Created with Biorender 2025)

Antimicrobial susceptibility testing was performed using the automated Thermo Scientific™ Sensititre™ ARIS System. Minimum inhibitory concentrations (MIC) were determined for a broad range of antibiotics in compliance with EUCAST/CLSI standards. Phenotypic tests for carbapenemase detection were also performed.

Given the clinical relevance, reference MIC testing for colistin was performed using the broth microdilution (BMD) method according to EUCAST and CLSI (2024) guidelines. A commercial ComASP® Colistin kit (Liofilchem®), covering the concentration range of 0.25–16  $\mu\text{g}/\text{mL}$ , was applied. Additionally, MIC testing for cefiderocol was performed using the gradient diffusion method (E-test, Liofilchem®) on Mueller-Hinton agar. The bacterial suspension was prepared to a 0.5 McFarland standard and incubated at 35°C for 18–20 hours.

## Results

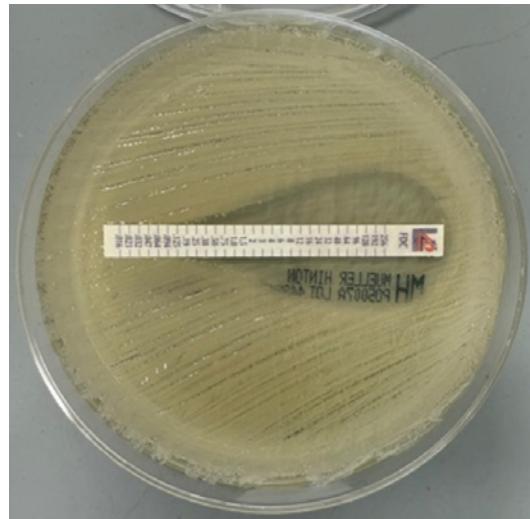
On standard culture media, colonies consistent with Enterobacteriaceae morphology were obtained. MALDI-TOF MS analysis (Autobio® MS 2000) identified the isolate as *Klebsiella pneumoniae*. Antimicrobial Susceptibility Testing using the Sensititre™ ARIS system demonstrated resistance to most clinically relevant antibiotic classes,

Antibiotic	MIC (µg/mL)	OR	GR	R
Amikacin	>32	○ R	● R	R
Amoxicillin/ Clav. Acid ...	>32	○ R	● R	R
Ampicillin	>16	○ R	● R	R
Cefalexin	>32	○ NI	● NI	NI
Cefepime	>8	○ R	● R	R
Cefixime	>2	○ NI	● NI	NI
Cefotaxime	>4	○ R	● R	R
Cefoxitin	>16	○ NI	● NI	NI
Ceftazidime	>8	○ R	● R	R
Cefuroxime (sodium)	>16	○ R	● R	R
Ciprofloxacin	>1	○ R	● R	R
Ertapenem	>2	○ R	● R	R
Gentamicin	>8	○ R	● R	R
Levofloxacin	>2	○ R	● R	R
Meropenem	>16	○ R	● R	R
Nalidixic Acid	>16	○ NI	● NI	NI
Nitrofurantoin	>64	○ NI	● NI	NI
Piperacillin/Tazobactam	>32	○ R	● R	R
Positive Growth Control	-	○ OK	● OK	OK
Ticarcillin	>32	○ NI	● R	R
Tigecycline	1	○ NI	● NI	NI
Tobramycin	>8	○ R	● R	R
Trimethoprim/Sulfame...	>8	○ R	● R	R

**Fig. 2.** Antimicrobial susceptibility testing (AST) of *Klebsiella pneumoniae* (Schroeter) Trevisan using the Sensititre™ ARIS system, showing extensive drug resistance (XDR); the columns represent: antibiotic name, MIC value (minimum inhibitory concentration), interpretation of the result (NI – no interpretation, OK – growth control valid, R – resistant strain), and additional columns confirming the result or consistency with other system criteria (Photo. P. Likus)

According to EUCAST (2025) criteria, the isolate was classified as extensively drug-resistant (XDR). Phenotypic carbapenemase testing confirmed the presence of class A (KPC) and class D (OXA-48) enzymes.

The reference broth microdilution test (ComASP® Colistin) yielded MIC  $\geq 8$  µg/mL, classifying the isolate as resistant to colistin according to EUCAST criteria. E-test analysis showed a cefiderocol MIC of 0.38 µg/mL. Based on EUCAST (2025) breakpoints, the isolate was interpreted as susceptible to cefiderocol (Fig. 3).

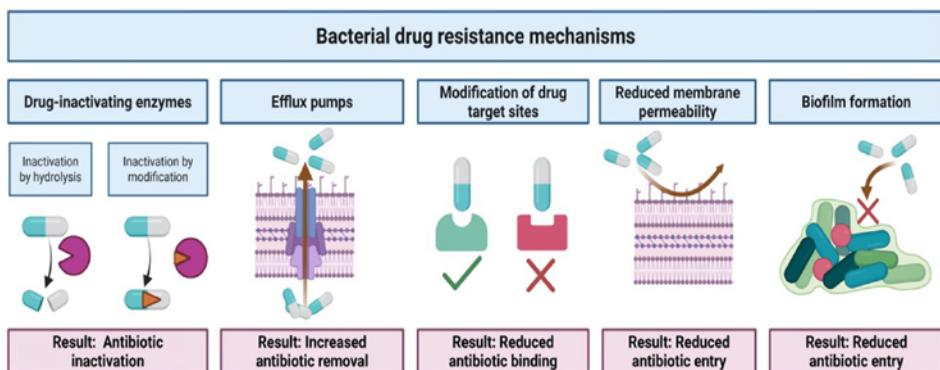


**Fig. 3.** Demonstration of sensitivity of *Klebsiella pneumoniae* (Schroeter) Trevisan to cefiderocol (Photo. P. Likus)

## Discussion

### Mechanisms of antimicrobial resistance in bacteria

Among many bacterial defence strategies, four mechanisms are considered key to the development of antibiotic resistance: the production of drug-inactivating enzymes, active efflux of drugs from the cell via efflux pumps, modification of drug target sites, and reduced membrane permeability (Walsh, 2000; Crofts et al., 2017; Belay et al., 2024) – Fig. 4. In recent years, increased attention has been paid to biofilm formation – protective structures that hinder drug penetration and promote the persistence and horizontal transfer of resistance genes (Belay et al., 2024; Costerton et al., 1999).



**Fig. 4.** The main mechanisms of antibiotic resistance found in bacteria (Created with Biorender 2025)

The production of antibiotic-inactivating enzymes is one of the most common resistance mechanisms. Bacteria synthesise diverse enzymes that chemically degrade or modify drug molecules, rendering them inactive (Gabibov et al., 2020; Belay et al., 2024). This group includes  $\beta$ -lactamases, which hydrolyse the  $\beta$ -lactam ring found in penicillins, cephalosporins (including 3<sup>rd</sup> and 4<sup>th</sup> generations), and monobactams such as aztreonam (Fair, Tor, 2014; Blair et al., 2015). Similar mechanisms apply to other drug classes – e.g., chloramphenicol acetyltransferases, aminoglycoside-modifying enzymes, and phosphotransferases that add chemical groups to the antibiotic structure, preventing its activity (Blair et al., 2015; Mehta et al., 2015).

Reduced membrane permeability, especially in Gram-negative bacteria, is another defence strategy. Limiting porin expression reduces antibiotic penetration, lowering their efficacy (Crofts et al., 2017). Efflux pumps belonging to the RND, MFS, ABC, or MATE families actively expel antibiotics from the cell, preventing them from reaching therapeutic concentrations. High expression of these systems is responsible for multidrug resistance. Structural changes at drug target sites – such as mutations in DNA gyrase or topoisomerase IV (relevant for fluoroquinolones) or rRNA methylation that blocks macrolide binding – lead to reduced antibiotic affinity and treatment efficacy (Blair et al., 2015).

Biofilm formation is a complex mechanism that enhances bacterial resistance to antibiotics and the immune system while promoting the persistence of so-called persister cells – metabolically inactive cells that can survive treatment (Belay et al., 2024, Costerton et al., 1999). Biofilms also facilitate horizontal gene transfer (Gillings, 2017).

In conclusion, bacterial resistance to antibiotics arises from diverse and often overlapping molecular mechanisms. Thorough understanding and monitoring of these processes are essential for developing new drugs and treatment strategies amid the growing antibiotic resistance crisis (Tacconelli et al., 2018; Belay et al., 2024).

### **Resistance mechanisms of KPC (Class A) and OXA-48 (Class D)**

Class A and D carbapenemases produced by Gram-negative bacteria are of critical clinical importance due to their ability to hydrolyse carbapenems and confer multidrug resistance (MDR), significantly limiting therapeutic options. According to the Ambler classification, *Klebsiella pneumoniae* carbapenemase (KPC) belongs to class A, while OXA-48 is categorised under class D (Bush, Bradford, 2020).

#### *KPC – Class A Carbapenemases*

KPC enzymes are class A serine  $\beta$ -lactamases capable of hydrolysing nearly all  $\beta$ -lactam antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems (Queenan, Bush, 2007; Nordmann et al., 2011). The resistance mechanism involves serine-catalysed hydrolysis of the  $\beta$ -lactam ring, leading to antibiotic inactivation (Queenan, Bush, 2007).

The  $\text{bla}_{\text{KPC}}$  gene, most commonly encoding the KPC-2 and KPC-3 variants, is typically located on plasmids with high interspecies transfer potential, which facilitates the rapid spread of resistance in hospital environments (Munoz-Price et al., 2013). KPC-producing strains often exhibit resistance, not only to  $\beta$ -lactams, but also to other antibiotic classes such as fluoroquinolones, aminoglycosides, and even colistin, further complicating treatment (van Duin, Doi, 2017).

### OXA-48 – Class D Carbapenemases

OXA-48 carbapenemases belong to class D  $\beta$ -lactamases and display a distinctive substrate profile: they have limited hydrolytic activity against cephalosporins but are active against penicillins and carbapenems (Poirel et al., 2012; Evans, Amyes, 2014). Their mechanism of resistance also involves serine hydrolase activity; however, their catalytic efficiency ( $k_{\text{cat}}$ ) against carbapenems is lower than that of class A or B enzymes, making phenotypic detection more difficult (Evans, Amyes, 2014).

The  $\text{bla}_{\text{OXA-48}}$  gene is usually located on mobile plasmids frequently associated with transposons (e.g., Tn1999), promoting its dissemination, particularly among Enterobacteriaceae (Potron et al., 2013). Many OXA-48-producing strains remain susceptible to third-generation cephalosporins, which may lead to false-negative results in standard phenotypic assays if not complemented by genotypic methods (Tzouvelekis et al., 2012).

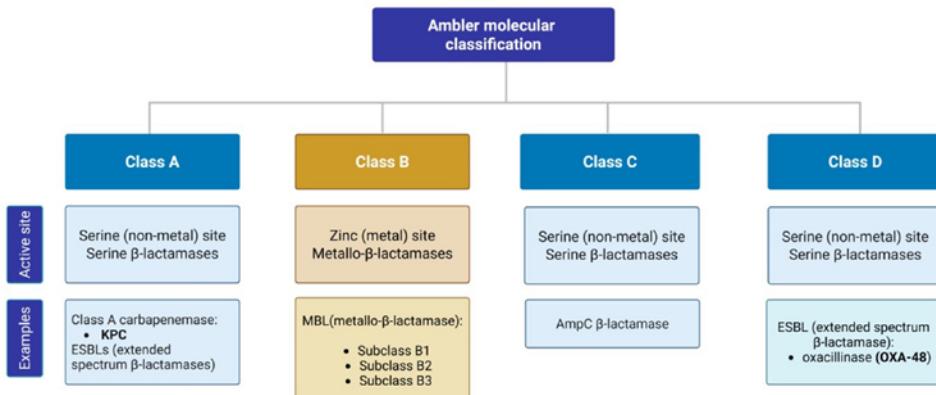


Fig. 5. Classification of  $\beta$ -lactamase enzymes according to Ambler (Created with Biorender 2025)

According to Ambler's classification,  $\beta$ -lactamases are divided into four classes (A, B, C and D) based on their amino acid sequence and mechanism of action. Classes A, C and D use serine as a catalyst, whereas class B (metallo- $\beta$ -lactamases) requires zinc ions. The figure (5) illustrates examples of enzymes that belong to specific classes (Bush, Bradford, 2020).

## Epidemiology of multidrug-resistant Enterobacterales infections in Europe

Infections caused by carbapenem-resistant Enterobacterales, including *Klebsiella pneumoniae*, remain a critical issue in European Union and European Economic Area countries. The latest assessment by the European Centre for Disease Prevention and Control (ECDC, 2025) indicates a worsening epidemiological situation and an increase in carbapenem-resistant Enterobacterales infections in several European countries.

In Poland, *K. pneumoniae* strains producing KPC and OXA-48 carbapenemases pose a particular threat and are spreading across multiple hospitals. The diversity of resistance mechanisms and the capacity to acquire additional genetic determinants increase the risk of difficult-to-treat infections (Kot et al., 2025). Whole-genome sequencing (WGS) analysis of 934 carbapenemase-producing Enterobacterales isolates (VIM/IMP) revealed a high prevalence of *Enterobacter hormaechei* Jordan and several high-risk clones (ST90, ST89, ST121), responsible for multifocal and multiregional infection outbreaks. WGS enabled precise identification of resistance genes and their association with various plasmids and integrons (Izdebski et al., 2023).

The presented study described a *K. pneumoniae* strain harboring both KPC and OXA-48 carbapenemases, which was susceptible only to cefiderocol. Similar observations have been reported by other authors, highlighting the crucial role of cefiderocol as a last-line therapy against multidrug-resistant Gram-negative infections. Oueslati et al. (2022) assessed the *in vitro* activity of cefiderocol against carbapenem-resistant *K. pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* Jordan, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Minimal inhibitory concentrations (MIC) were determined using broth microdilution in iron-adjusted Mueller-Hinton broth and interpreted according to EUCAST and CLSI criteria. Results confirmed the superior efficacy of cefiderocol among the tested antibiotics, supporting its role as a last-resort agent.

In Poland, cefiderocol also demonstrated high activity against MDR and XDR strains. Zalas-Więcek et al. (2022) applied disk diffusion and MIC test strip methods, interpreting results based on EUCAST guidelines, and concluded that cefiderocol represented a new therapeutic option for *E. coli*. Similarly, Tarski et al. (2024) documented cefiderocol's effectiveness in challenging clinical cases where other antibiotics failed. At the European level, a study from Lithuania in 2023 identified 106 *K. pneumoniae* OXA-48 isolates belonging to high-risk clones ST45, ST392, and ST395. WGS confirmed that these strains were responsible for multifocal outbreaks in multiple hospitals and regions (Greičius et al., 2024). These findings underscore the necessity for systematic epidemiological surveillance and rapid identification of high-risk clones.

The resistance mechanisms observed in the studied *K. pneumoniae* strain (KPC and OXA-48) are consistent with experimental findings, demonstrating that these

enzymes confer resistance to most  $\beta$ -lactam antibiotics while cefiderocol remains effective, thereby confirming its role as a last-line therapy for multidrug-resistant Gram-negative infections (Oueslati et al., 2022; Zalas-Więcek et al., 2022; Tarski et al., 2024).

### **Diagnostics, treatment, and prevention of carbapenemase-producing organisms**

In recent years, diagnostics for carbapenemase-producing organisms (CPOs) have advanced significantly with the introduction of rapid molecular techniques such as PCR and LAMP, as well as immunochromatographic assays. These tools improve the detection of key enzymes like KPC and OXA-48, even when phenotypic expression is low (Nordmann et al., 2012; Dortet et al., 2014). Despite these improvements, identifying OXA-48 producers remains challenging, particularly in strains with minimal enzyme activity. To enhance diagnostic sensitivity and specificity, a combination of phenotypic and genotypic methods is recommended (Nordmann et al., 2012).

As carbapenem resistance increases and the effectiveness of traditional treatments declines, new  $\beta$ -lactamase inhibitors have expanded therapeutic options. Ceftazidime/avibactam, active against class A and C enzymes and partially effective against class D (including many KPC- and OXA-48-producers), has become a key agent (Yu et al., 2024). Other novel combinations – such as meropenem/vaborbactam and imipenem/relebactam – target class A carbapenemases and some class D enzymes (Hayden et al., 2020). Cefiderocol, a siderophore cephalosporin that exploits iron-uptake systems, offers broad activity against multiple carbapenemase types, including KPC and OXA-48 (Ito et al., 2017). Despite these advances, last-resort antibiotics like colistin and fosfomycin are still used in severe cases, although rising resistance and toxicity concerns limit their utility (Falagas, Kasiakou, 2024). Furthermore, emerging mutations in *bla*<sub>KPC</sub> and *bla*<sub>OXA-48</sub> genes threaten the efficacy of newer inhibitors, including ceftazidime/avibactam, underscoring the need for continuous surveillance and research (ECDC, 2018).

Class A (KPC) and class D (OXA-48) carbapenemases remain the dominant resistance mechanisms among Gram-negative bacteria and are frequently associated with multidrug resistance. While modern diagnostics and agents like cefiderocol offer critical therapeutic tools, the rapid evolution of resistance highlights the importance of sustained monitoring and innovation (Koenig, Kuti, 2024).

Excessive antibiotic use in both human and veterinary medicine continues to drive antimicrobial resistance. Gram-negative bacteria, in particular, increasingly produce ESBLs and carbapenemases, leading to resistance against cephalosporins and other  $\beta$ -lactams (Prestinaci et al., 2015; Laxminarayan et al., 2013). This contributes to prolonged hospital stays and higher mortality rates in infections caused by multidrug-resistant organisms (Levy, Marshall, 2004). Consequently, accurate microbiological diagnostics, including MIC testing in accordance with EUCAST guidelines, are essential to guide appropriate therapy (WHO, 2020).

In response to the global threat of antimicrobial resistance (AMR), international efforts such as the WHO's Global Action Plan on AMR and the work of the Global Leaders Group promote a One Health approach and multisectoral coordination (WHO, 2025; Laxminarayan et al., 2020). In Poland, national efforts are led by the National Reference Centre for Antimicrobial Susceptibility (KORLD) and supported by the National Programme for Antibiotic Protection (NPOA). These initiatives include the development of training programs and support for antimicrobial stewardship teams in healthcare institutions.

Antimicrobial Stewardship Programs (ASP) have demonstrated clear clinical and epidemiological benefits, including reduced inappropriate antibiotic use, shorter hospital stays, and fewer infections caused by multidrug-resistant organisms such as MRSA, KPC, and ESBL producers (Baur et al., 2017). Their success depends on structured collaboration among healthcare professionals, access to rapid diagnostics, and continuous monitoring of local resistance trends (Huttnner et al., 2014). WHO-led initiatives such as the Antimicrobial Stewardship Coaching Programme, launched in 2025, are support hospitals across Central and Eastern Europe in implementing these practices. In Poland, AMS teams are being developed in line with NPOA recommendations and national infection control strategies, with emphasis on staff education, therapeutic audits, and adherence to EUCAST and national guidelines (Pulcini et al., 2019).

## Conclusions

The case of the described *Klebsiella pneumoniae* strain producing both KPC and OXA-48 carbapenemases highlights the scale of the threat posed by the increasing resistance of Gram-negative bacteria to available antimicrobial agents. High-quality microbiological diagnostics – including accurate MIC determination and identification of resistance mechanisms – are essential for the implementation of targeted therapy. In the context of limited therapeutic options, antimicrobial stewardship programs and coordinated national and global efforts are of paramount importance in reducing the selection of resistant strains. An integrated approach – based on the One Health principle – remains the only effective strategy in the fight against the growing problem of antimicrobial resistance.

## Conflict of interest

The authors declare no conflict of interest related to this article.

## References

Bahr, G., González, L.J., Vila, A.J. (2021). Metalloβlactamases in the Age of Multidrug Resistance: From Structure and Mechanism to Evolution, Dissemination, and Inhibitor Design. *Chemical Reviews Journal*, 121(13), 7957–8094. <https://doi.org/10.1021/acs.chemrev.1c00138>

Baur, D., Gladstone, B.P., Burkert, F., Carrara, E., Foschi, F., Döbele, S., Tacconelli, E. (2017). Effect of antibiotic stewardship on the incidence of infection and colonisation with antibiotic-resistant bacteria and *Clostridium difficile* infection: a systematic review and meta-analysis. *Lancet Infectious Diseases Journal*, 17(9), 990–1001. [https://doi.org/10.1016/S1473-3099\(17\)30325-0](https://doi.org/10.1016/S1473-3099(17)30325-0)

Belay, W.Y., Getachew, M., Tegegne, B.A., Teffera, Z.H., Dagne, A., Zeleke, T.K., Abebe, R.B., Gedif, A.A., Fenta, A., Yirdaw, G., Tilahun, A., Aschale, Y. (2024). Mechanism of antibacterial resistance, strategies and next generation antimicrobials to contain antimicrobial resistance: a review. *Pharmacology*, 15, 144478. <https://doi.org/10.3389/fphar.2024.1444781>

Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O., Piddock, L.J.V. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1), 42–51. <https://doi.org/10.1038/nrmicro3380>

Bush, K., Bradford, P.A. (2020).  $\beta$ -Lactams and  $\beta$ -lactamase Inhibitors: An Overview. *Cold Spring Harbor Perspectives in Medicine*, 6(8), a025247. <https://doi.org/10.1101/cshperspect.a025247>

Caliskan-Aydogan, O., Alocilja, E.C. (2023). A review of carbapenem resistance in Enterobacteriales and its detection techniques. *Microorganisms*, 11(6), 1491. <https://doi.org/10.3390/microorganisms11061491>

CLSI, (2024). Performance standards for antimicrobial susceptibility testing: 34<sup>th</sup> edition (CLSI Supplement M100). Clinical and Laboratory Standards Institute, Wayne, PA.

Costerton, J.W., Stewart, P.S., Greenberg, E.P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science Direct Journal*, 284(5418), 1318–1322. <https://doi.org/10.1126/science.284.5418.1318>

Crofts, T.S., Gasparrini, A.J., Dantas, G. (2017). Next-generation approaches to understand and combat the antibiotic resistome. *Nature Reviews Microbiology*, 15(7), 422–434. <https://doi.org/10.1038/nrmicro.2017.28>

Dortet, L., Bréchard, L., Poirel, L., Nordmann, P. (2014). Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures. *Clinical Microbiology and Infection*, 20(4), 340–344. <https://doi.org/10.1111/1469-0999.12318>

Duin, D., Doi, Y. (2017). The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence*, 8(4), 460–469. <https://doi.org/10.1080/21505594.2016.1222343>

EUCAST, (2025). Breakpoint tables for interpretation of MICs and zone diameters, Version 15.0. European Committee on Antimicrobial Susceptibility Testing. <https://www.eucast.org>

European Centre for Disease Prevention and Control. ECDC, (2025). *Antimicrobial resistance surveillance in Europe 2023–2025 data*. <https://www.ecdc.europa.eu/en>

European Centre for Disease Prevention and Control (ECDC), (2018). Emergence of resistance to ceftazidime-avibactam in carbapenem-resistant Enterobacteriaceae. <https://www.ecdc.europa.eu/sites/default/files/documents/RRA-Emergence-of-resistance-to%20CAZ-AVI-in-CRE-Enterobacteriaceae.pdf>

Evans, B.A., Amyes, S.G. (2014). OXA  $\beta$ -lactamases. *Clinical Microbiology Reviews Journal*, 27(2), 241–263. <https://doi.org/10.1128/CMR.00117-13>

Fair, R.J., Tor, Y. (2014). Antibiotics and bacterial resistance in the 21<sup>st</sup> century. *Perspectives in Medicinal Chemistry*, 28(6), 25–64. <https://doi.org/10.4137/PMC.S14459>

Falagas, M.E., Kasiakou, S.K. (2005) Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clinical Infectious Diseases*, 78(4), 545–553. <https://doi.org/10.1086/429323>

Fernández, L., Hancock, R.E.W. (2012). Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clinical Microbiology Reviews Journal*, 25(4), 661–681. <https://doi.org/10.1128/CMR.00043-12>

Gabibov, A.G., Dontsova, O.A., Egorov, A.M. (2020). Overcoming antibiotic resistance in microorganisms: molecular mechanisms. *Biochemistry (Moscow)*, 85(11), 1289–1291. <https://doi.org/10.1134/S0006297920110012>

Gillings, M.R. (2017). Class 1 integrons as invasive species. *Current Opinion Microbiology*, 38, 10–15. <https://doi.org/10.1016/j.mib.2017.03.002>

Greičius, P., Linkevičius, M., Stankevičius, M., Razmuk, J., Sinotova, J., Alm, E., Svartström, O., Bortolai, V., Kudirkienė, E., Roer, L., Hendriksen, R.S., Tamoliūnaitė, G., Palm, D., Monnet, D.L., Kohlberg, A., Griškevičius, A. (2024). Emergence of OXA-48-producing *Klebsiella pneumoniae* in Lithuania, 2023: a multi-cluster, multi-hospital outbreak. *Euro Surveillance*, 29(16), 2400188. <https://doi.org/10.2807/1560-7917.ES.2024.29.16.2400188>

Hayden, D., White, B.P., Bennett, K. (2020). Review of ceftazidime-avibactam, meropenem-vaborbactam, and imipenem/cilastatin-relebactam to target *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriales. *Journal of Pharmacy Technology*, 17, 36(5), 202–210. <https://doi.org/10.1177/8755122520934726>

Hu, S., Xie, W., Cheng, Q., Zhang, X., Dong, X., Jing, H., Wang, J. (2023). Molecular epidemiology of carbapenem-resistant *Enterobacter cloacae* complex in a tertiary hospital in Shandong, China. *MBC Microbiology*. 177. <https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-023-02913-x>

Huttner, B., Harbarth, S., Nathwani, D. (2014). Success stories of implementation of antimicrobial stewardship: a narrative review. *Clinical Microbiology Infection*, 20(10), 954–962. <https://doi.org/10.1111/1469-0691.12803>

Ito, A., Sato, T., Ota, M., Takemura, M., Nishikawa, T., Toba, S., Kohira, N., Miyagawa, S., Ishibashi, N., Matsumoto, S., Nakamura, R., Tsuji, M., Yamano, Y. (2017). *In vitro* antibacterial properties of cefiderocol, a novel siderophore cephalosporin, against gram-negative bacteria. *Antimicrobial Agents Chemotherapy*, 62(1), e01254-17. <https://doi.org/10.1128/AAC.01454-17>

Izdebski, R., Baraniak, A., Herda, M., Fiett, J., Bonten, M.J.M., Carmeli, Y., Goossens, H., Hryniwicz, W., Brun-Buisson, C., Dumpis, U., Gniadkowski, M., Glasner, C., Gniadkowski, M. (2023). Genome-Based Epidemiologic Analysis of VIM/IMP Carbapenemase-Producing *Enterobacter* spp. *Emerging Infectious Diseases*, 29(8), 1618–1626. <https://doi.org/10.3201/eid2908.230199>

Koenig, C., Kuti, J.L. (2024). Evolving resistance landscape in gram-negative pathogens: an update on  $\beta$ -lactam and  $\beta$ -lactam-inhibitor treatment combinations for carbapenem-resistant organisms. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 44, 8. <https://doi.org/10.1002/phar.2950>

Kot, B., Witeska, M., Szweda, P., Piechota, M., Kondera, E., Horoszewicz, E., Balak, I., Bin Hafeez, A., Synowiec, A. (2025). Antibiotic resistance, virulence genes, and molecular diversity of clinical *Klebsiella pneumoniae* isolates from patients of district hospital in central Poland. *Pathogens*, 14(7), 648. <https://doi.org/10.3390/pathogens14070648>

Kubin, C.J., Garzia, C., Uhlemann, A-C. (2025). *Acinetobacter baumannii* treatment strategies: a review of therapeutic challenges and considerations. *Antimicrobial Agents and Chemotherapy*, 69, 8. <https://doi.org/10.1128/aac.01063-24>

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K.M., Wertheim, H.F.L., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., Greko, C., So, A., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, Q., Qamar, F.N., Mir, F., Kariuki, S., Bhutta, Z.A., Coates, A., Bergstrom, R., Wright, G.D., Brown, E.D., Cars, O. (2013). Antibiotic resistance – the need for global solutions. *The Lancet Infectious Diseases*, 13(12), 1057–1098. [https://doi.org/10.1016/S1473-3099\(13\)70318-9](https://doi.org/10.1016/S1473-3099(13)70318-9)

Levy, S.B., Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine*, 10(12), S122–129. <https://doi.org/10.1038/nm1145>

Li, X-Z., Plésiat, P., Nikaido, H. (2015). The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clinical Microbiology Reviews*, 28(2), 337–418. <https://doi.org/10.1128/CMR.00117-14>

Liofilchem, (2024). ComASP® Colistin Product Insert, REF 75001. Liofilchem S.r.l., Italy.

Mehta, S.C., Rice, K., Palzkill, T. (2015). Natural variants of the KPC-2 carbapenemase have evolved increased catalytic efficiency for ceftazidime hydrolysis at the cost of enzyme stability. *PLoS Pathogens*, 12(5), e1005930. <https://doi.org/10.1371/journal.ppat.1004949>

Munoz-Price, L.S., Poirel, L., Bonomo, R.A., Schwaber, M.J., Daikos, G.L., Cormican, M., Giuseppe, C., Garau, J., Gniadkowski, M., Hayden, M.K., Kumarasamy, K., Livermore, D.M., Maya, J.J., Nordmann, P., Patel, J.B., Paterson, D.L., Pitout, J., Villegas, M.V., Wang, H., Woodford, N., Quinn, J.P. (2013). Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *The Lancet Infectious Diseases Journal*, 13(9), 785–796. [https://doi.org/10.1016/S1473-3099\(13\)70190-7](https://doi.org/10.1016/S1473-3099(13)70190-7)

*Narodowy Program Ochrony Antybiotyków – przegląd działań w Polsce* (2025). <https://antybiotyki.edu.pl/category/2025/> [In Polish]

Nordmann, P., Naas, T., Poirel, L. (2011). Global spread of carbapenemase-producing Enterobacteriaceae. *Emerging Infectious Diseases*, 17(10), 1791–1798. <https://doi.org/10.3201/eid1710.110655>

Nordmann, P., Poirel, L., Dortet, L. (2012). Rapid detection of carbapenemase – producing Enterobacteriaceae. *Emerging Infectious Diseases*, 18(9), 1503–1507. <https://doi.org/10.3201/eid1809.120355>

Otto, M., Dickey, S., Wolz, C. (2023). Editorial: Quorum-sensing in gram-positive pathogens – mechanisms, role in infection, and potential as a therapeutic target. *Frontiers in Cellular and Infection Microbiology*, 19(13), 1236705. <https://doi.org/10.3389/fcimb.2023.1236705>

Queslati, S., Bogaerts, P., Dortet, L., Bernabeu, S., Ben Lakhal, H., Longshaw, C., Glupczynski, Y., Naas, T. (2022). *In vitro* activity of ceferidrocol and comparators against carbapenem-resistant Gram-negative pathogens from France and Belgium. *Antibiotics*, 11(10), 1352. <https://doi.org/10.3390/antibiotics11101352>

Poirel, L., Potron, A., Nordmann, P. (2012). OXA-48-like carbapenemases: the phantom menace. *Journal of Antimicrobial Chemotherapy*, 67(7), 1597–1606. <https://doi.org/10.1093/jac/dks121>

Potron, A., Poirel, L., Nordmann, P. (2015). Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *International Journal Antimicrobial Agents*, 42(5), 415–423. <https://doi.org/10.1016/j.ijantimicag.2015.03.001>

Prestinaci, F., Pezzotti, P., Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Journal of Global Health*, 109(7), 309–318. <https://doi.org/10.1179/204773215Y.0000000030>

Pulcini, C., Binda, F., Lamkang, A.S., Trett, A., Charani, E., Goff, D.A., Harbarth, S., Hinrichsen, S.L., Levy-Hara, G., Mendelson, M., Nathwani, D., Gunturu, R., Singh, S., Srinivasan, A., Thamlikitkul, V., Thursky, K., Vlieghe, E., Wertheim, H., Zeng, M., Gandra, S., Laxminarayan, R. (2019). Developing core elements and checklist items for global hospital antimicrobial stewardship programmes: a consensus approach. *Clinical Microbiology and Infection Journal*, 21(5), e276–e287. <https://doi.org/10.1016/j.cmi.2018.03.033>

Queenan, A.M., Bush, K. (2007). Carbapenemases: the versatile β-lactamases. *Clinical Microbiology Reviews*, 20(3), 440–458. <https://doi.org/10.1128/CMR.00001-07>

Santajit, S., Indrawattana, N. (2016). Mechanisms of antibiotic resistance in ESKAPE pathogens. *Biomed Research International*, 2016, 2475067. <https://doi.org/10.1155/2016/2475067>

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavalieri, M., Cox, E.M., Houchens, C.R., Grayson, M.L., Hansen, P., Singh, N., Theuretzbacher, U., Magrini, N. (2018). WHO Pathogens Priority List Working Group. Discovery, research and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria. *The Lancet Infectious Diseases Journal*, 18(3), 318–327. [https://doi.org/10.1016/S1473-3099\(17\)30753-3](https://doi.org/10.1016/S1473-3099(17)30753-3)

Tarski, I., Śmiechowicz, J., Duszyńska, W. (2024). Cefiderocol in the Successful Treatment of Complicated Hospital-Acquired *Klebsiella pneumoniae* NDM, OXA-48 Intraabdominal Infection. *Infection and Drug Resistance*, 17, 5163–5170. <https://doi.org/10.2147/IDR.S485450>

Tzouvelekis, L.S., Markogiannakis, A., Psichogiou, M., Tassios, P.T., Daikos, G.L. (2012). Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clinical Microbiology Reviews*, 25(4), 682–707. <https://doi.org/10.1128/CMR.05035-11>

Walsh, C. (2000). Molecular mechanisms that confer antibacterial drug resistance. *Nature*, 406(6797), 775–781. <https://doi.org/10.1038/35021219>

WHO, (2017). *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*. Geneva: World Health Organization. <https://remed.org/wp-content/uploads/2017/03/global-priority-list-of-antibiotic-resistant-bacteria-2017.pdf>

WHO, (2020). *Antimicrobial stewardship programmes in health-care facilities in low- and middle-income countries: a WHO practical toolkit*. Geneva: World Health Organization. <https://www.who.int>

WHO, (2023). *Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report*. Geneva: World Health Organization. <https://www.who.int/publications/i/item/9789240062702>

WHO, (2025). *Fifth meeting of the Strategic and Technical Advisory Group on Antimicrobial Resistance (STAG-AMR)*. Geneva: World Health Organization. June 2025. <https://www.who.int>

Yu, C-H., Tsai, M-S., Liao, C-H., Yang, C-J. (2024). Ceftazidime-avibactam for the treatment of carbapenem-resistant *Klebsiella pneumoniae* infection: a retrospective, single center study. *Infection and Drug Resistance*, 5(17), 5363–5374. <https://doi.org/10.2147/IDR.S475679>

Zalas-Więcek, P., Płachta, M., Gospodarek-Komkowska, E. (2022). Cefiderocol against multidrug and extensively drug-resistant *Escherichia coli*: an *in vitro* study in Poland. *Pathogens*, 11(12), 1508. <https://doi.org/10.3390/pathogens11121508>

## Aktualne zagrożenie dla zdrowia publicznego: zakażenia szpitalne wywołane przez drobnoustroje oporne na wiele leków, takie jak *Klebsiella pneumoniae*

### Streszczenie

W niniejszej pracy opisano przykład izolacji szczepu *Klebsiella pneumoniae* wytwarzającego karbapenemazy typu KPC i OXA-48, opornego na większość stosowanych antybiotyków. Diagnostyka obejmowała posiewy na podłożach selektywnych, identyfikację metodą MALDI-TOF oraz oznaczenie lekowraźliwości przy użyciu automatycznego systemu mikrorozcieńczeń. Szczep wykazywał oporność na karbapenemy, aminoglikozydy i kolistynę, wykazując wrażliwość jedynie na cefiderokol – antybiotyk ostatniej szansy. Opisany przypadek ilustruje kliniczne wyzwanie związane z zakażeniami wywoływanymi przez bakterie wielolekooporne.

Równocześnie zwraca uwagę narastające znaczenie szczepów łączących różne mechanizmy oporności, m.in. produkcję  $\beta$ -laktamaz typu ESBL, karbapenemaz klasy A, B i D oraz mechanizmy zmniejszonej przepuszczalności błon i aktywnego wypompowywania leku (efflux). Zakażenia tymi patogenami nie tylko ograniczają możliwości terapeutyczne, lecz także generują poważne konsekwencje epidemiologiczne i ekonomiczne. Podkreśla to potrzebę zintegrowanych działań diagnostycznych, terapeutycznych i profilaktycznych w ramach programów racjonalnej antybiotykoterapii oraz aktywnego nadzoru mikrobiologicznego.

**Słowa kluczowe:** Enterobacteriaceae, epidemiologia, karbapenemazy OXA-48

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