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Molecular and Phenotypic Diversity of Carbapenemase-Producing *Klebsiella pneumoniae* in Western Algeria

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Abstract

Background: *Klebsiella pneumoniae is a major opportunistic pathogen involved in hospital-acquired infections. The emergence of carbapenemase-producing strains severely compromises the effectiveness of last-resort antibiotics.*

Purpose: *To evaluate the phenotypic and genotypic profiles of carbapenem resistance among clinical isolates of K. pneumoniae recovered in Oran.*

Methods: *A total of 58 clinical carbapenemase-producing K. pneumoniae isolates were investigated. Antimicrobial susceptibility testing, determination of minimum inhibitory concentrations (MICs) for carbapenems and colistin, and molecular detection of carbapenemase-encoding genes by PCR were performed.*

Results: *A high level of resistance to most β-lactams and other antibiotic classes was observed. Carbapenem MIC values were markedly elevated, indicating a high level of resistance. The bla_{NDM-1} gene was predominant (82.75%), followed by OXA-48, IMP, and KPC at lower frequencies. Colistin resistance was detected in 8.62% of isolates.*

Conclusion: *The widespread dissemination of carbapenemase-producing K. pneumoniae in Oran represents a major public health threat and highlights the urgent need to strengthen surveillance systems and infection control strategies.*

Keywords: *Klebsiella pneumoniae; Carbapenemase; NDM-1; Antimicrobial resistance; Algeria.*

Introduction

Klebsiella pneumoniae is an opportunistic Enterobacterales species widely implicated in nosocomial infections, particularly pneumonia, septicemia, urinary tract infections, and infections associated with invasive medical devices[1]. Its remarkable ability to adapt to hospital environments, combined with its high potential for acquiring antimicrobial resistance genes, has led to its classification as a priority pathogen by the World Health Organization[2]. Over recent decades, the emergence and dissemination of multidrug-resistant strains have considerably complicated the therapeutic management of infections caused by this bacterium.

Initially, resistance of K. pneumoniae to β-lactam antibiotics was mainly associated with the production of extended-spectrum β-lactamases (ESBLs), particularly enzymes of the TEM, SHV, and CTX-M families[3,4]. This situation led to the increased use of carbapenems, which are considered last-resort antibiotics for the treatment of severe infections caused by ESBL-producing Enterobacterales [5]. However, the intensive use of these agents has promoted the emergence of new resistance mechanisms, notably the production of carbapenemases.

Carbapenemases are β-lactamases capable of hydrolyzing nearly all β-lactam antibiotics, including carbapenems. In K. pneumoniae, the most frequently reported enzymes belong to the NDM, OXA-48, KPC, and IMP families[6]. These enzymes are often encoded by genes located on plasmids, facilitating horizontal gene transfer between bacteria and contributing to their rapid dissemination in hospital settings[7]. The coexistence of multiple resistance mechanisms within the same strain frequently results in multidrug-resistant phenotypes, severely limiting available therapeutic options.

In Algeria, several studies have reported the emergence and spread of carbapenemase-producing K. pneumoniae in different hospital centers[8–11]. However, available data remain heterogeneous across regions, and microbiological surveillance is still insufficient to accurately assess the magnitude of this phenomenon. In this context, local analysis of resistance profiles and underlying molecular mechanisms is essential to guide therapeutic strategies and infection control policies.

Objectives

This study aimed to characterize the phenotypic and genotypic profiles of carbapenem resistance in 58 clinical *Klebsiella pneumoniae* isolates recovered in Oran. Specifically, it sought to determine antibiotic resistance levels, evaluate the minimum inhibitory concentrations (MICs) of carbapenems and colistin, and identify the carbapenemase genes involved.

Materials and Methods

Study population

This study included fifty-eight (58) clinical *Klebsiella pneumoniae* isolates obtained from hospitalized patients in Oran. The strains were collected from various biological specimens as part of routine microbiological diagnostic procedures. Each isolate corresponded to a distinct patient in order to avoid duplication.

Bacterial identification was performed using conventional microbiological methods based on morphological, biochemical, and cultural characteristics. Isolates identified as *K. pneumoniae* were preserved under appropriate conditions until phenotypic and molecular analyses were carried out. The included patients presented with diverse infections, predominantly hospital-acquired. No stratification was performed according to hospital ward, age, or sex in the descriptive analysis, as the primary objective of the study was the investigation of bacterial resistance mechanisms.

This study was conducted in accordance with ethical requirements, and all procedures complied with international recommendations for research involving human subjects.

Data stratification

Collected data were structured and stratified according to multiple analytical axes to ensure a clear and coherent interpretation of results.

First, isolates were classified based on their antibiotic susceptibility profiles determined by antimicrobial

susceptibility testing. Resistance rates were expressed as percentages for each antibiotic class tested, providing a global overview of multidrug resistance levels.

Second, MIC values for imipenem, ertapenem, and colistin were analyzed separately. Isolates were grouped according to observed MIC ranges in order to assess resistance levels and distribution patterns.

Finally, molecular PCR data allowed stratification of isolates according to the type of carbapenemase gene detected. Frequencies of different resistance genes were calculated and compared with phenotypic resistance profiles. This integrated approach enabled the establishment of an epidemiological resistance profile for *K. pneumoniae* strains circulating in the city of Oran.

Statistical analysis

Data analysis was performed using a descriptive statistical approach. Qualitative variables were expressed as counts and percentages, while quantitative variables, particularly MIC values, were presented as minimum values, maximum values, and frequency distributions.

No complex comparative analyses were conducted, as the primary objective of the study was descriptive characterization of resistance profiles and associated molecular mechanisms. Results were organized into tables and figures to facilitate interpretation.

The concordance between phenotypic and genotypic findings was qualitatively assessed by highlighting the correspondence between carbapenem resistance profiles and the presence of carbapenemase genes.

Results

Antibiotic resistance

Analysis of antimicrobial susceptibility testing revealed a high level of antibiotic resistance across all studied isolates. All strains exhibited resistance to the majority of tested β-lactam antibiotics. Resistance to imipenem reached 98.27%, with only a single isolate retaining susceptibility (Fig. 01).

Very high resistance rates were also observed for ciprofloxacin (98.6%), cotrimoxazole (96.5%), and gentamicin (86%). Amikacin showed a more moderate resistance rate, while fosfomycin and chloramphenicol retained the highest in vitro activity. Colistin resistance was detected in 8.62% of isolates. MIC values ranged from 4 to 64 µg/mL, with a predominance of high-level resistance values (Fig. 03).

These findings indicate a severe multidrug-resistant phenotype, significantly limiting available therapeutic options.

Carbapenem MICs

Determination of ertapenem and imipenem MICs revealed high values for the majority of isolates. The most frequently observed MICs were 8 µg/mL for ertapenem and 16 µg/mL for imipenem (Fig. 02).

Only one isolate exhibited intermediate susceptibility to ertapenem, while another remained susceptible to imipenem. These results confirm a high level of carbapenem resistance in the studied population.

Colistin resistance

Colistin resistance was detected in 8.62% of isolates. MIC values ranged from 4 to 64 µg/mL, with a predominance of high-level resistance values (Fig. 03).

Genotypic profiles of carbapenemases

Molecular analysis revealed a marked predominance of the bla_{NDM-1} gene, detected in 82.75% of isolates. The OXA-48, IMP, IMP-27, and KPC genes were detected at lower frequencies. Two isolates were negative for all tested carbapenemase genes (Fig. 04).

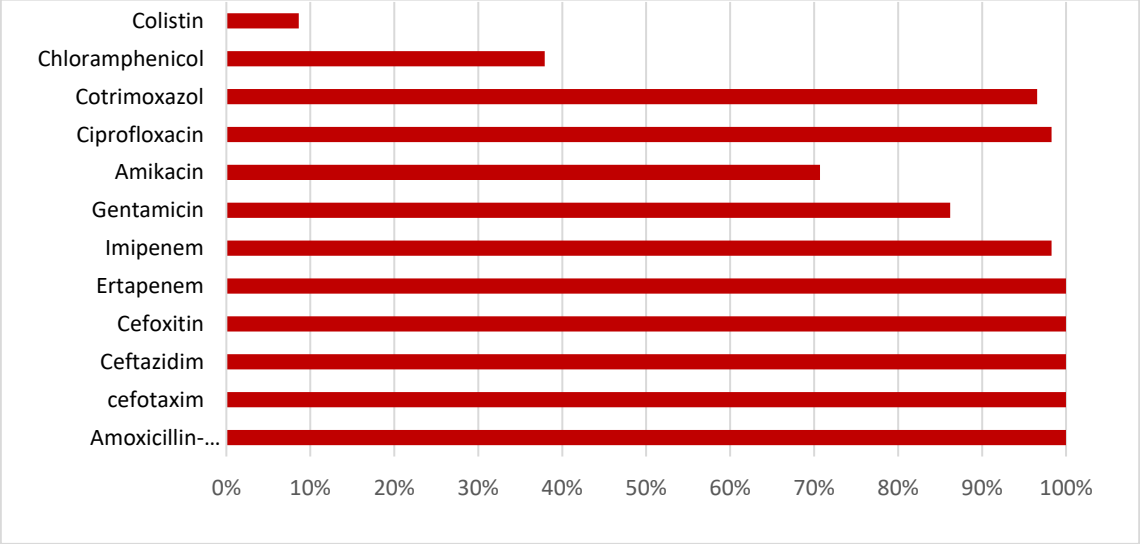

Fig. 01: Percentage of antibiotic resistance (R+1) in *K.pneumoniae* carbapenemase between 2019 and 2021 (n=58)

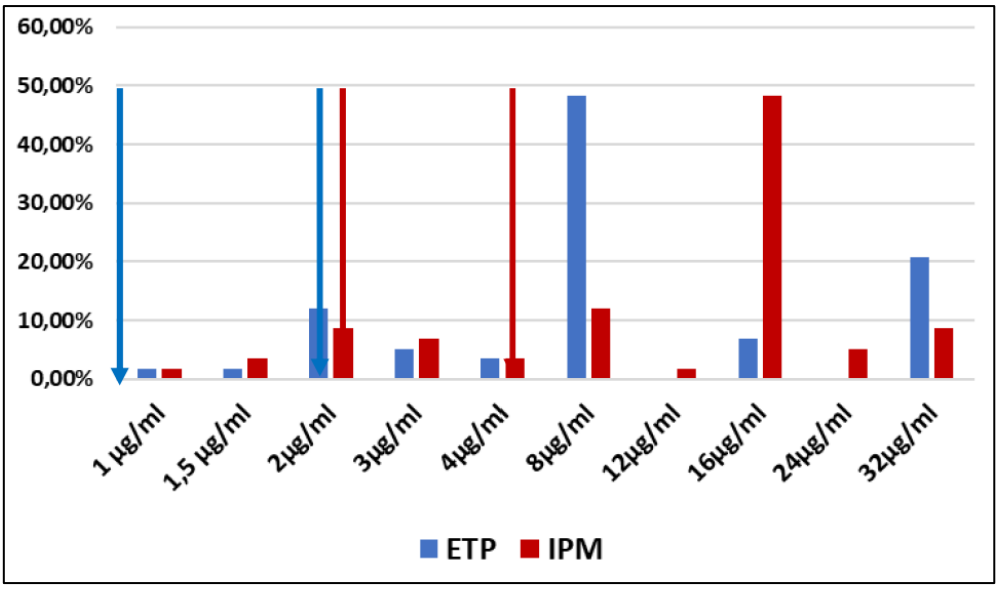

Fig. 02: Percentage of distribution of CMI ertapenem and imipenem of *K.pneumoniae* carbapenemase

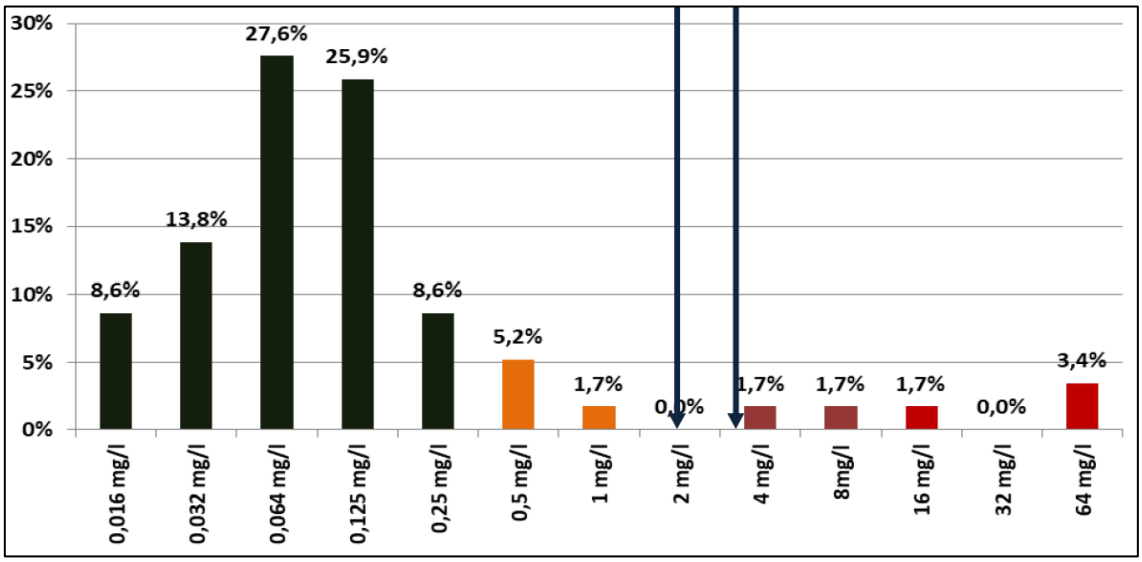

Fig. 03: Percentage of distribution of CMI Colistin of *K.pneumoniae* carbapenemase.

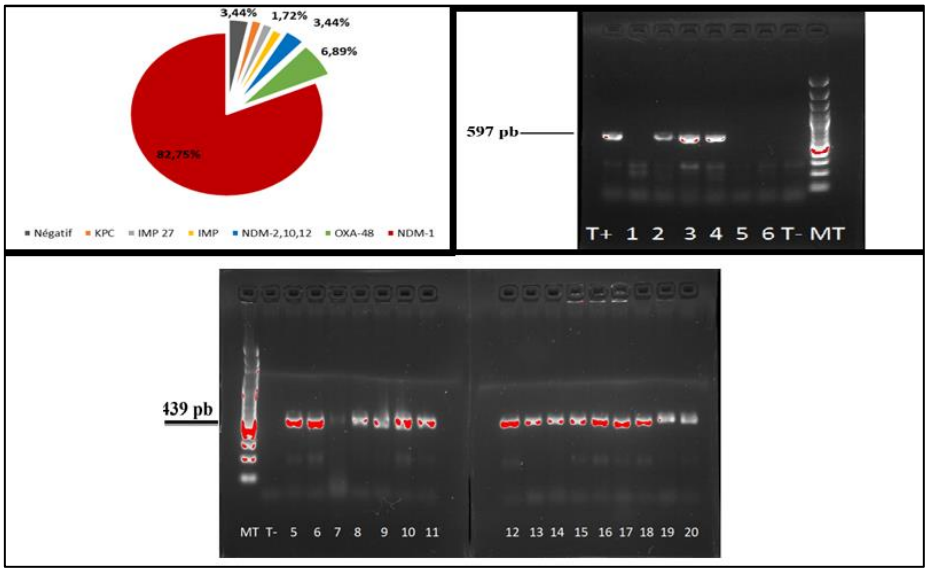

Fig. 04: Frequency and characterization of KpCBLSE carbapenemases.

Discussion

This study highlights a particularly alarming epidemiological situation characterized by a high prevalence of carbapenemase-producing *Klebsiella pneumoniae* within the investigated hospital setting. All 58 analyzed isolates exhibited severe multidrug-resistant profiles, with near-universal resistance to carbapenems, confirming the major role of this bacterial species in the current antimicrobial resistance crisis[12,13]. These findings are consistent with global observations that classify *K. pneumoniae* among the most serious threats to public health, notably due to its remarkable capacity to accumulate resistance determinants and to disseminate rapidly in hospital environments[14,15].

One of the major findings of this study is the overwhelming predominance of the bla_{NDM-1} gene, detected in 82.75% of isolates. This observation indicates endemic circulation of this resistance mechanism within the studied institution. NDM-1 carbapenemase confers high-level resistance to nearly all β-lactam antibiotics, with the exception of aztreonam, and is frequently associated with additional resistance genes, leading to extensively drug-resistant phenotypes[16,17]. The predominance of NDM-1 observed in this study is consistent with regional and international reports describing a progressive expansion of this genotype, particularly in Mediterranean countries and North Africa[18–20].

The dissemination of NDM-1 is especially concerning due to its frequent association with conjugative plasmids, which facilitate horizontal gene transfer both intra- and inter-species[21]. This characteristic may explain the widespread distribution observed in the studied bacterial population, independently of hospital wards or specimen types. The temporal increase in NDM-1–producing isolates during certain periods also suggests possible cross-transmission events in the hospital environment, underscoring the critical importance of infection prevention and control measures[22].

In addition to NDM-1, the detection of OXA-48-type carbapenemases, although less frequent (6.89%), remains epidemiologically significant. OXA-48 has historically been the most prevalent carbapenemase in Algeria and has been implicated in numerous hospital outbreaks[23–25]. The coexistence of multiple carbapenemase types within the same healthcare facility reflects substantial genetic diversity and further complicates both therapeutic management and epidemiological control. The sporadic presence of IMP, IMP-27, and KPC genes, although marginal, confirms the occasional introduction of rare but highly transmissible resistance mechanisms[26,27].

From a phenotypic perspective, antimicrobial susceptibility testing revealed extremely high resistance rates to most antibiotics evaluated. The near-total resistance to imipenem (98.27%) and the

elevated MIC values observed for carbapenems confirm a clinically significant level of resistance[28]. Even in isolates exhibiting residual susceptibility, high MIC values substantially reduce the likelihood of therapeutic success, particularly in severe infections[29]. These findings are consistent with published data on infections caused by carbapenemase-producing *K. pneumoniae*, in which carbapenems retain only limited activity and often require combination therapy strategies[30].

The observed multidrug resistance was not restricted to β-lactam antibiotics. High resistance rates to fluoroquinolones, aminoglycosides, and cotrimoxazole reflect the accumulation of multiple resistance mechanisms, frequently carried by the same mobile genetic elements[31,32]. This situation severely restricts available therapeutic options and promotes the use of last-resort antibiotics such as colistin.

The detection of colistin resistance in 8.62% of isolates represents a major warning signal. Although this proportion remains relatively low compared with some regions worldwide, the presence of high MIC values (up to 64 µg/mL) is particularly concerning[33]. These mechanisms are often associated with selective pressure resulting from the increasing clinical use of colistin in the treatment of multidrug-resistant Gram-negative infections[34].

The relatively preserved in vitro activity of fosfomycin and chloramphenicol observed in this study may represent potential therapeutic alternatives in specific clinical contexts. However, their use must be carefully controlled, taking into account the site of infection and pharmacokinetic and pharmacodynamic properties, in order to prevent the rapid emergence of secondary resistance[35,36].

The findings of this study also emphasize the importance of continuous microbiological surveillance and an integrated approach combining phenotypic and genotypic analyses. Early detection of carbapenemases not only guides appropriate antibiotic therapy but also enables the implementation of targeted infection prevention strategies to limit nosocomial transmission[37,38]. In resource-limited settings, strengthening diagnostic capacities and healthcare worker training constitute critical priorities[39].

Finally, this study presents certain limitations, notably its descriptive and single-center design. Nevertheless, it provides valuable local epidemiological data on carbapenemase-producing *K. pneumoniae* in Oran and contributes to a better understanding of the dissemination dynamics of these strains in Algeria. These findings strongly support the implementation of national antimicrobial resistance surveillance programs and antibiotic stewardship policies[40].

This study demonstrates a critical epidemiological situation characterized by the widespread dissemination of carbapenemase-producing *Klebsiella pneumoniae*, dominated by bla_{NDM-1}, and associated with severe multidrug resistance that severely restricts therapeutic options. However, the absence of molecular analysis of resistance genes to fluoroquinolones, aminoglycosides, and ESBLs, as well as the lack of molecular typing approaches (e.g., MLST, PFGE, WGS), limits the full understanding of resistance mechanisms and transmission dynamics. Despite these limitations, the findings provide valuable local epidemiological data supporting the urgent need for integrated surveillance, strengthened diagnostics, and coordinated antimicrobial stewardship and infection control strategies.

Conclusion

This study provides the first comprehensive characterization of carbapenemase-producing *Klebsiella pneumoniae* in the Oran region, revealing a high prevalence of multidrug-resistant strains dominated by bla_{NDM-1}. The findings highlight a critical public health threat, with severely limited therapeutic options and evidence of widespread dissemination in the hospital setting. These results underscore the urgent need for strengthened regional surveillance, advanced molecular diagnostics, and coordinated antimicrobial stewardship and infection control strategies.

Ethics approval and consent to participate

This study was approved by the *scientific committee of the medical faculty*.

Consent for publication

All authors have approved this article for publication.

Availability of data and materials

Data are available from the corresponding author upon request.

Competing interests

All authors declare that there is no conflict

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Financial interests

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References

1. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance. 2016.
2. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae. Proc Natl Acad Sci USA. 2015;112(27):E3574–E3581. https://doi.org/10.1073/pnas.1501049112
3. Russo TA, Marr CM. Hypervirulent Klebsiella pneumoniae. Clin Microbiol Rev. 2019;32(3):e00001-19. https://doi.org/10.1128/CMR.00001-19
4. Carrër A, Nordmann P. Emergence and spread of carbapenemases in Enterobacteriaceae. Pathol Biol. 2011;59(3):243–253. https://doi.org/10.1016/j.patbio.2010.12.003
5. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev. 2007;20(3):440–458. https://doi.org/10.1128/CMR.00001-07
6. Chen L, Mathema B, Pitout JD, DeLeo FR, Kreiswirth BN. Carbapenemase-producing Klebsiella pneumoniae: molecular and genetic insights. Trends Microbiol. 2014;22(12):686–696. https://doi.org/10.1016/j.tim.2014.07.003
7. Poirel L, Pitout JD, Nordmann P. Carbapenemases: molecular diversity and clinical consequences. Future Microbiol. 2007;2(5):501–512. https://doi.org/10.2217/17460913.2.5.501
8. Aggoune N, Klibi N, Bakour S, Mansour W, Rolain JM. First report of NDM-1-producing Klebsiella pneumoniae in Algeria. J Glob Antimicrob Resist. 2014;2(3):181–182.
9. Cuzon G, Naas T, Truong H, Villegas MV, Wisell KT, Carmeli Y, et al. First outbreak of OXA-48-positive Klebsiella pneumoniae in Algeria. Int J Antimicrob Agents. 2015;46(6):725–727. https://doi.org/10.1016/j.ijantimicag.2015.09.003
10. Loucif L, Benouda A, Bakour S, Rolain JM. Outbreak of OXA-48-producing Klebsiella pneumoniae in Algeria. Antimicrob Agents Chemother. 2016;60(12):7494–7497. https://doi.org/10.1128/AAC.01386-16
11. Abderrahim A, Touati A, Bakour S, Rolain JM. Antimicrobial resistance patterns and carbapenemase genes in Enterobacteriaceae isolated from clinical samples in Algeria. Microb Drug Resist. 2017;23(8):1013–1020. https://doi.org/10.1089/mdr.2017.0031
12. World Health Organization. Global priority list of antibiotic-resistant bacteria. WHO. 2017.
13. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis. 2017;215(Suppl 1):S28–S36. https://doi.org/10.1093/infdis/jiw282
14. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence. 2017;8(4):460–469.

https://doi.org/10.1080/21505594.2016.1222343

15. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791–1798. https://doi.org/10.3201/eid1710.110655
16. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int. 2014;2014:249856. https://doi.org/10.1155/2014/249856
17. Al-Zahrani IH, Alyousef AA, Alyousef NS, Al-Johani SM, Balkhy HH. Molecular epidemiology of NDM-producing Enterobacterales in North Africa. Infect Genet Evol. 2021;93:104964. https://doi.org/10.1016/j.meegid.2021.104964
18. Mathers AJ, Peirano G, Pitout JD. Carbapenemase-producing Enterobacteriaceae: epidemiology and control. Clin Microbiol Rev. 2015;28(4):871–902. https://doi.org/10.1128/CMR.00116-14
19. Poirel L, Heritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. J Antimicrob Chemother. 2012;67(7):1597–1606. https://doi.org/10.1093/jac/dks113
20. Pitout JD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The global ascendency of OXA-48-type carbapenemases. Clin Microbiol Rev. 2019;32(1):e00102-18. https://doi.org/10.1128/CMR.00102-18
21. Dortet L, Bréchard L, Cuzon G, et al. Emergence of KPC-producing Enterobacteriaceae in France. Antimicrob Agents Chemother. 2012;56(1):546–552. https://doi.org/10.1128/AAC.05116-11
22. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother. 2019;74(2):200–209. https://doi.org/10.1093/jac/dky438
23. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Treatment of infections caused by carbapenem-resistant Enterobacteriaceae. Clin Microbiol Rev. 2021;34(2):e00266-20. https://doi.org/10.1128/CMR.00266-20
24. van Duin D, Doi Y, Paterson DL. Multidrug-resistant Klebsiella pneumoniae: epidemiology, molecular mechanisms, and treatment options. Clin Microbiol Rev. 2013;26(4):676–701. https://doi.org/10.1128/CMR.00113-13
25. Wyres KL, Holt KE. Klebsiella pneumoniae population genomics and antimicrobial resistance. Nat Rev Microbiol. 2020;18(6):344–359. https://doi.org/10.1038/s41579-019-0315-1
26. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25(4):682–707. https://doi.org/10.1128/CMR.05035-11
27. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins. Clin Infect Dis. 2005;40(9):1333–1341. https://doi.org/10.1086/429323
28. [28] Jayol A, Nordmann P, Lehours P, et al. Mechanisms of colistin resistance in Klebsiella spp. Int J Antimicrob Agents. 2016;48(2):119–123. https://doi.org/10.1016/j.ijantimicag.2016.04.013
29. Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative infections. Lancet Infect Dis. 2006;6(9):589–601. https://doi.org/10.1016/S1473-3099(06)70580-1
30. Karaiskos I, Giamarellou H. Multidrug-resistant Gram-negative infections: current treatment options. Expert Rev Anti Infect Ther. 2014;12(10):1127–1141.

https://doi.org/10.1586/14787210.2014.957067
31. Centers for Disease Control and Prevention. Facility guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). CDC. 2015.
32. World Health Organization. Global action plan on antimicrobial resistance. WHO. 2015.
33. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of carbapenem-resistant Enterobacteriaceae. Lancet Infect Dis. 2013;13(9):785–796. https://doi.org/10.1016/S1473-3099(13)70190-7
34. Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014;5:643. https://doi.org/10.3389/fmicb.2014.00643
35. Falagas ME, Vouloumanou EK, Samonis G, Vardakas KZ. Fosfomycin. Clin Microbiol Rev. 2016;29(2):321–347. https://doi.org/10.1128/CMR.00068-15
36. Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. Molecular basis of chloramphenicol and florfenicol resistance in bacteria. FEMS Microbiol Rev. 2004;28(5):519–542. https://doi.org/10.1016/j.femsre.2004.02.002
37. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence. 2017;8(4):460–469. https://doi.org/10.1080/21505594.2016.1222343
38. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791–1798. https://doi.org/10.3201/eid1710.110655
39. Centers for Disease Control and Prevention. Facility guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). CDC. 2015.
40. World Health Organization. Global action plan on antimicrobial resistance. WHO. 2015.