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Original Article

Molecular characterization of the whole genome in clinical multidrug-resistant strains of *Klebsiella pneumoniae*

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Abstract

Introduction: Antimicrobial resistance (AMR) is a major public health concern. The spread of AMR-encoding genes between enterobacteria, especially in *Klebsiella pneumoniae* strains, lead to failure in the treatment of most individuals. The aim of this study was to characterize multi-drug resistant (MDR) clinical *K. pneumoniae* isolates that produce extended-spectrum β-lactamases (ESBLs) from Algeria.

Methodology: The isolates were identified using biochemical tests, and the identification was confirmed by mass spectrometry using VITEK® MS (BioMerieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was assessed by the disk diffusion method. Molecular characterization was performed by whole genome sequencing (WGS) using Illumina technology. Sequenced raw reads were processed using bioinformatics parameters: FastQC, ARIBA, and Shovill-Spades. Multilocus sequence typing (MLST) was used to estimate the evolutionary relationship between isolate strains.

Results: Molecular analysis resulted in the first detection of blaNDM-5 encoding *K. pneumoniae* in Algeria. Other resistance genes were *blaTEM*, *blaSHV*, *blaCTX*-M, *aac(6')-Ib-cr*, *qnrB1*, *qnrB4*, *qnrB19*, *qnrS1*, *gyrA* and *parC* variants.

Conclusions: Our data demonstrated a very high level of resistance in clinical *K. pneumoniae* strains which were resistant to most common antibiotic families. This was the first detection of *K. pneumoniae* with the *blaNDM-5* gene in Algeria. Surveillance of antibiotic use and measures for control should be implemented to reduce occurrence of AMR in clinical bacteria.

Key words: antimicrobial resistance, extended-spectrum β-lactamases, *Klebsiella. pneumoniae*, multi-drug resistant, New Delhi metallo-β-lactamases -5, sequenceType -307.

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Introduction

Prolonged exposure of Enterobacteriaceae to antibiotics and the selective pressure resulting from the excessive, clinically unnecessary, use of antibiotics contributes to the emergence of antibiotic resistance (AMR) that is a growing global health concern. The spread of infections resistant to the currently available antibiotics could contribute to a death every three seconds globally by 2050 [1]. Currently patients with infections caused by antibiotic-resistant bacteria are at high risk of long hospital stays, treatment failure, high mortality, and high health care costs [2].

The prevalence of multi drug resistant (MDR) Enterobacteriaceae, especially those which are resistant to β-lactams, has been increasing worldwide because these antibiotics are important therapeutic choices for treating infections in humans [3].

K. pneumoniae is a notorious bacterium, and by acquiring additional antimicrobial resistance, it could be the origin of increasing nosocomial infections. This species is known to be prone to MDR and hypervirulent strain emergence [18].

K. pneumoniae cause severe infections including liver abscess, pneumonia, and sepsis by the production of enzymes such as extended-spectrum β-lactamases (ESBLs) or plasmid-mediated AmpC β-lactamases (pAmpC) that are enzymes commonly isolated from Enterobacteriaceae [4]. Nowadays, enterobacteria producing ESBLs and β-lactamase enzymes are frequently isolated from nosocomial and community-acquired infections [5]. Therefore, acquired carbapenemase-encoding genes in enterobacteria constitute a real clinical concern for antimicrobial management. ESBL, pAmpC and the carbapenemases are largely produced by MDR enterobacteria such as *Klebsiella* spp isolates. The corresponding genes are located on plasmids and other mobile genetic elements [6]. This is alarming because these plasmids frequently

carry genes encoding resistance to other classes of drugs such as aminoglycosides, trimethoprim-sulfamethoxazole, fluoroquinolones, and can move horizontally among bacteria [7]. This is especially the case with *Klebsiella* spp which is one of the bacteria in need of development of new therapeutic compounds [8]. The objective of this study was to evaluate the level of antibiotic resistance and to perform the molecular characterization of clinical *K. pneumoniae* strains isolated from out-patients and in-patients admitted to different wards of the hospital in Oum El Bouaghi, Algeria.

Methodology

Samples collection

30 Bacterial isolates were provided by the laboratory of Microbiology of the hospital at Oum El Bouaghi, Algeria. They were isolated between December 2021-February 2022 from various clinical samples (urinary tract infections, hemoculture and pus) collected from out-patients and in-patients admitted to different wards of the hospital.

The isolates were grown on MacConkey agar (BioMerieux, Marcy l'Etoile, France) for 24 hrs at 37 ± 1 °C. The isolates were identified using biochemical tests, and the identification was confirmed by mass spectrometry using VITEK® MS (BioMerieux, Marcy l'Etoile, France). VITEK® MS PRIME is a Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) mass spectrometer. The instrument analyzes material from microbial cultures to identify the microorganism. The samples were exposed to multiple laser shots inside VITEK® MS PRIME [1], and this was designed to incorporate additional benefits and enhance the use of MALDI-TOF technology [1].

Antibiotic susceptibility testing

Antimicrobial drug susceptibility was determined using the disc diffusion method on Mueller-Hinton agar and interpreted according to the recommendations of Antibiogram Committee French Society for Microbiology (http://www.sfm-microbiologie.org). The antimicrobial agents tested were: β-lactamins (10µg of ampicillin, 30µg of amoxicillin-clavulanic acid, 10µg of imipenem and 30µg of cefotaxime), cyclines (30µg of tetracycline), sulfonamides (25µg of tri-methoprim-sulfamethoxazole), aminoglycosides (10µg of gentamicin), rifamycines (5µg of rifampicin), and quinolones (30µg of nalidixic acid and 5µg of ciprofloxacin).

The screening of ESBL production was performed by the double-disc synergy test (DDST) between clavulanic acid and third generation cephalosporins (cefotaxime, ceftazidime, aztreonam and cefepime) [9]. The test was considered positive when a "champagne cork" aspect was observed.

DNA sequencing

Genomic DNA was extracted using a QIAxtractor (Qiagen, Valencia, CA), and library preparation was performed by using a Nextera XT DNA sample preparation kit (Illumina, San Diego, CA) according to the Illumina protocol. The libraries were sequenced with the Illumina MiSeq platform (2x 300-bp paired-end reads) with a minimum of mapped-reads depth of 40-fold.

Contaminant searches and molecular identification were performed for each sample using the centrifuge. This method performs abundance analyses at all taxonomic levels (e.g. strain, species, genus) [10]. Quality control metrics were examined across the whole collection as a batch report to ensure a mean read base-pair quality score of $Q \geq 20$ and a read length of 70% of the original read length. Quality-filtered Illumina reads were assembled using Unicycler [11]. Contigs were annotated using Bakta.

Gene annotation

To define the presence of specific genes and their alleles, we used ARIBA [12], DIAMOND [13], and the following databases: Multilocus sequence typing MLST database, serotypeFinder O:H typing database, the fimH typing database, and curated databases of AMR genes/Single Nucleotide Polymorphism (SNPs) including Res-Finder, NDARO, and CARD.

Comparative genomic analysis

The filtered whole genome sequencing (WGS) reads were aligned against *K. pneumonae* core genome (https://www.cgmlst.org) to call SNPs using BactSNP [14]. A maximum-likelihood phylogeny was then generated by RAxML-ng v 0.9 [15] based on the resulting core genome alignment filtered for recombination using Gubbins v 2.2. [16]

Results

Multilocus sequence typing (MLST) and clonality analysis

According to data of MLST, all isolates belonged to the sequence type ST307. Clonality analysis performed by cgMLST on mean 2145635 bases (https://microreact.org/project/gekjDYwFLTkanVvTGBzwoi-confile) showed that the most similar isolates 03 and 13 (ST307) differ by 39 SNPs. The isolates did not

therefore emerge as a recent clonal spread but probably correspond to an endemic reservioir of ST307.

Discussion

The World Health Organization (WHO) has recommended that countries should develop AMR surveillance programs to collect and integrate antimicrobial use.

Our genomic data analysis, presented in Table 1, confirmed that all *K. pneumoniae* strains (n = 4) are ESBLs. A co-existence of three ESBL genes (*CTX-M*, *TEM*, and *SHV*) was found in two strains (S03 and S13), and this was already reported in Algeria [19], and in other countries [20]. Association of ESBL genes lead to selection of resistance genes in hospitals as well as in other sectors. In addition, the presence of multiple β-lactamase genes with several variants in *K. pneumoniae* isolates would also be the cause of MDR and other extremely drug resistant (XDR) strains emergence [21]. These may lead to high human mortality rates when it comes to clinical infections due to failures of antimicrobial therapies [22]. Both *blaSHV* and *blaCTX-M* genes are present in the two strains (S01 and S09) analyzed in *K. pneumoniae*. The coexistence of *blaSHV* and *blaCTX-M* genes was also observed in previous studies conducted in Algeria [23].

Through this investigation, the *CTX-M* genes have been identified in all *K. pneumoniae* (S01, S03, S09, and S13); *blaCTX-M-15* type variant (CTX-M-1 group) in particular is a well distributed strain throughout the world, including Algeria [24].

A *blaSHV-11* gene was found in S01 and S09 strains that procures a broad-spectrum resistance to antibiotics in the *K. pneumoniae* strain. Two strains (S03 and S13) harboring the *blaSHV28* and the *blaTEM-1* genes have been identified, and have already been a subject of study in Algeria [23]. No data was available on the functional aspect of variant gene *blaTEM-1*. Hyperproduction of TEM-1 β-lactamase mediated by the promoter *Pa/Pb* which was detected in two *K. pneumoniae* strains (S03 and S13) has never been addressed in Algeria, and according to other studies *blaTEM-1*(*PaPb*) is only responsible of high resistance to piperacillin-tazobactam (TZP) [25].

The *OXA* gene was found present in three strains of *K. pneumoniae*. The *OXA-1* gene was also detected in Algeria [26]. *OXA-1* variant gene was discovered in co-existence with ESBL genes. This result is consistent with previous studies that showed that these genes are commonly present in combination with *CTX-M-15*, *SHV-1*, *TEM-1* β-lactamases, PMQR (plasmid mediated quinolones resistance) determinants, and *aac(6')-Ib-cr* in a community of *K. pneumoniae* strain [27].

Previous studies have demonstrated that *NDM-5* offer greater resistance than *NDM-1* genes [34]. From what we observed, the detection of New Delhi Metallo-β-lactamases genes of type NDM-5 in *K. pneumoniae* (S09) strains is considered as a first in Algeria, However the blaNDM-5 gene has been reported only in other clinical isolates such as *Acinetobacter baumannii* [28], *Escherichia coli* [29], and *Enterobacter cloacae* [30].

In contrast, in other countries the *blaNDM-5* gene has been identified mainly in clinical Enterobacteriaceae. The carbapenems represent the latest threat to public health especially in the case of nosocomial transmission of the *blaNDM* gene which has occurred in many countries [31]. However, in Algeria, *NDM-5* gene was detected in *K. pneumoniae* from animal origins such as white storks [32]. This finding confirms that wildlife in Algeria could serve as reservoirs of MDR *K. pneumoniae*, in addition to its presence in fecal samples of companion animals [33].

Table 1. Antibiotic resistance-encoding genes found in clinical *K. pneumoniae* strains.

Strains	B-lactam resistance	B-lactam gene(s)	Aminoglycoside gene(s)	Quinolone gene(s)	Sulfonamide gene(s)	Trimethoprime gene(s)	Cycline gene(s)	Phenicole gene(s)	Fosfomycin gene(s)
Klebsiella pneumoniae (S1)	ESBL NB-L IRP	$bla_{CTX-M-15}$ bla_{SHV-11} bla_{OXA-1}	aac(3)-IIe aac(6')-Ib-cr aadA11	qnrB1 gyrA(S83I)	sul1 sul2	dfrA1 dfrB1	tet(G)	floR catB3	fosA
Klebsiella pneumoniae (S3)	P ESBL IRP NB-L	bla_{TEM-1} $bla_{CTX-M-15}$ bla_{OXA-1} bla_{SHV-28} $bla_{TEM(PaPb)}$	aph(3')-Ia aph(6)-Id aph(3'')-Ib	qnrB1 gyrA(S83I)	sul2	dfrA14	tetA TetR	-	fosA
Klebsiella pneumoniae (S9)	ESBL NB-L Cr	$bla_{CTX-M-15}$ bla_{SHV-11} bla_{NDM-5}	aph(3'')-Ib aph(3')-Ia aph(6)-Id	qnrS1	sul2	-	-	-	fosA
Klebsiella pneumoniae (S13)	ESBL IRP NB-L P	$bla_{CTX-M-15}$ bla_{OXA-1} bla_{SHV-28} bla_{TEM-1} $bla_{TEM(PaPb)}$	aac(3)-IIe aac(6')-Ib-cr aph(6)-Id aph(3'')-Ib	qnrB1 gyrA(S83I)	sul2	dfrA14	tet(A) TetR	-	fosA

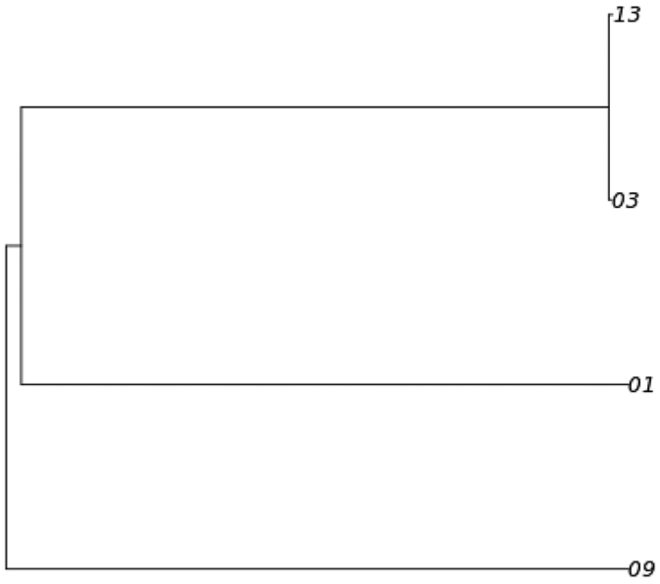
IRP: inhibitor-resistant penicillinase, NB-L: Natural-beta lactamase, P: penicillinase, C: cephalosporinase, Cr: carbapenemase, NC: Natural-cephalosporinase, ESBL: extended-spectrum β-lactamases.

One finding of the present study is the existence of fluoroquinolone-resistant strain of *K. pneumoniae* carrying two plasmid mechanisms of resistance to quinolones: the *aac(6')-Ib-cr* variant gene harbored by two strains (S01 and S13) and the *qnrB1* gene present in three strains (S01, S03, and S13); these were also previously identified in Algeria [35]. One strain (S09) had aquired the *qnrS1* gene. This is the second report of *qnrS1* in ESBL-*K. pneumoniae* in Algeria [26]. It was previously described that *qnrB* was predominant among strains from Africa that *qnrS*. An association has been found between the production of *blaCTX-M-15, qnrB1* and/or *qnrS1* gene in all strains of *K. pneumoniae*, based on data from studies in Algeria [36] and in other countries [37] which report cases of *K. pneumoniae* harboring *blaCTX-M-15* and *aac(6')-Ib-cr* [38].

Based on studies conducted in Algeria, we report for the first time the chromosomal mechanism of resistance to quinolones in three *K. pneumoniae* strains (S01, S03, and S13) mediated by the Ser83Leu (S83I) substitution in *gyrA* gene, which is consistent with previous studies from several countries [39]. The *gyrA* (S83I) gene has been identified in strains of *K. pneumoniae* carrying plasmid mechanisms especially the *qnrB1* gene. Thus, the appearance of chromosomal mutations in the genes encoding DNA gyrase can increase the level of resistance of the bacteria to quinolones [40].

Our *qnr* positive strains show resistance to aminoglycosides, cotrimoxazole, sulfonamide trimethoprime, fosfomycin, and cycline. This could be explained by the fact that the plasmid support of quinolone resistance is present in a cassette as part of an integron carrying the other PMQR genes like *blaCTXM-15*, *qnrB* and the multiresistance genes to other antibiotics [41].

Figure 1. Phylogenetic tree of multilocus sequence typing (MLST) clinical MDR K. pneumoniae isolates.



Numbers (01, 03, 09 and 13) correspond to the number of each strain of Klebsiella pneumoniae.

Few studies in Algeria have identified the detailed expression of resistance to aminoglycosides. Our data show that all the strains were resistant to aminoglycosides but with different gene expression levels, and this includes *aph(6)-Id*, *aac(3)-IIe*, *aac(6')-Ib-cr*, *aph(3')-Ia*, and *aph(3'')-Ib* genes. One strain (S01) of *K. pneumoniae* harbored genes of the type *aadA11*, and this was first detected in Algeria from clinical strains of *K. pneumoniae*. Among these identified genes, the most concerning is the *aac(6')-Ib-cr* variant gene [42]. All of aminoglycosides resistant genes discovered in this study have not been studied in Algeria, especially since most of them have been located in mobile elements (plasmids, integron, transposon and integrative conjugative element) [43].

This study is the first investigation in Algeria analyzing resistance to sulfonamides and tetracycline in clinical strains of *K. pneumoniae*. Resistance to sulfonamides in all *K. pneumoniae* strains (n = 4) is due to *sul1* and *sul2* genes. In addition, we identified resistance to trimethoprime by *dfrA1*, *A7*, *dfrA14*, and *dfrB1*. All the strains showed resistance to fosfomycine and carried a *fosA* gene. There were three strains that carried *tetA*, *tetG*, and *tetR* genes that lead to resistance to cyclines. The acquisition of resistance to fosfomycin and tetracycline by clinical isolates of *K. pneumoniae* sharing ESBL and/or carbapenemases represents a new threat that complicates the situation and shows that *K. pneumoniae* remains a very serious causative agent of therapy failure [44].

The most similar isolates S03 and S13 (ST307) differed only by 39 SNPs and both carried the ESBLs genes: *blaTEM-1*, *blaCTX-M-15*, *blaOXA-1*, *blaSHV-28*, and *blaTEM* (PaPb). They belong to the same strain ST-307 as indicated in Figure 1 that demonstrates the phylogenetic tree of multilocus sequence typing (MLST) of *K pneumoniae*. The ST307 clone has been previously detected in Algeria by [45]. Our results show that this clone was associated with three ESBLs (*blaCTX-M-15*), *qnrB* and other genes. Several reports have showed that the origin of MDR ST307 *K. pneumoniae* was from clinical samples, as well as from other sources [57]. The WHO recently declared that the ST307 strain poses critical threat to public health It has also been concluded that ST307 *K. pneumoniae* can often carry transferable resistance-conferring genes

against carbapenems and a variety of additional resistance and virulence determinants, in addition to integrative and conjugative elements, and phages. As a result, ST307 *K. pneumoniae* is emerging globally as an important vehicle for the dissemination of AMR determinants [58], and has been responsible for several global nosocomial and long-term care center outbreaks [59]. The MLST analysis indicated that ST307 consisted of one deep-branching lineage which contained the *gyrA* S83I mutation in the quinolone resistance determinant region (QRDR) that had global distribution [60].

Conclusions

The present study reports the first analysis and identification of the bacterial resistome of MDR clinical strains belonging to *Klebsiella pneumoniae* in Oum Bouaghi, Algeria. Our data demonstrate multiple clinical *K. pneumoniae* strains resistant to most common antibiotic families. This is also the first reported detection of *K. pneumoniae* producing *blaNDM-5* gene in Algeria. Control of antimicrobial resistance requires monitoring and surveillance of the level of emergence of resistant bacteria, as well as the resistance genes and their location. These measures can limit the presence of new diverse AMR encoding genes that could be a source of emergence of new human pathogens in the future.

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References

1. Jim ON (2016) Tackling drug resistant infections globally: final report and recommendations; the review on antimicrobial resistance. Government of the United Kingdom: London, UK.
2. Goyal D, Dean N, Neill S, Jones P, Dascomb K (2019) Risk factors for community acquired extended-spectrum beta-lactamase-producing Enterobacteriaceae infections — a retrospective study of symptomatic urinary tract infections, open forum infectious diseases. Oxford University US. In Press. 357p.
3. Bush K (2010) Bench-to-bedside review: the role of β-lactamases in antibiotic-resistant gram-negative infections. Crit Care 14 Suppl 3: 224.
4. Coque TM, Baquero F, Cantón R (2008) Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 13 Suppl 47: 19044.
5. Padmini N, Ajilda AAK, Sivakumar N, Selvakumar G (2017) Extended spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: critical tools for antibiotic resistance pattern. J Basic Microbiol 57: 460–470.
6. Carattoli A (2013) Plasmids and the spread of resistance. Int J Med Microbiol 303: 298–304.
7. McInnes RS, McCallum GE, Lamberte LE, van SchaikW (2020) Horizontal transfer of antibiotic resistance genes in the human gut microbiome. Curr Opin Microbiol 53: 35–43.
8. Ranjbar R, Memariani H, Sorouri R (2017) Molecular epidemiology of extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* strains isolated from children with urinary tract infections. Arch Pediatr Infect 5 Suppl 2: e39000.
9. Clinical and Laboratory Standards Institute (2015) Performance standards for antimicrobial susceptibility testing, twenty-fifth informational supplement M100-S25. The recommendations of Antibiogram Committee French Society for Microbiology (CA-SFM, 2019). Available: http://www.sfm-microbiologie.org/. Accessed: 1 April 2020.
10. Kim D, Song L, Breitwieser FP, Salzberg SL (2016) Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res 26: 1721–1729.
11. Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13: e1005595.
12. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, Harris SR (2017) ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom 3: e000131.
13. Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12: 59–60.
14. Yoshimura D, Kajitani R, Gotoh Y, Katahira K, Okuno M, Ogura Y, Hayashi T, Itoh T (2019) Evaluation of SNP calling methods for closely related bacterial isolates and a novel high-accuracy pipeline: BactSNP. Microb Genom 5: e000261.
15. Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35: 4453–4455.
16. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR (2015) Rapid phylogenetic analysis of large samples of recombinant bacterial whole-genome sequences using Gubbins. Nucleic Acids Res 43: e15.
17. Beghain J, Bridier-Nahmias A, Le Nagard H, Denamur E, Clermont O (2018) Clermon typing: an easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping. Microb Genom. 4: e000192.
18. Rolain JM (2013) Food and human gut as reservoirs of transferable antibiotic resistance encoding genes. Front Microbiol 4 Suppl 173: 1-10.
19. Merino S, Camprubı S, Albertı S, Benedı VJ, Tomas JM (1992) Mechanisms of *Klebsiella pneumoniae* resistance to complement-mediated killing. Infect Immun 60 Suppl 6: 2529-2535.
20. Belbel Z, Chettibi H, Dekhil M, Ladjama A, Nedjai S, Rolain JM (2014) Outbreak of an armA methyl transferase-producing ST39 *Klebsiella pneumoniae* clone in a pediatric Algerian Hospital. Microb Drug Resist 20: 310-315.
21. Rodríguez-Martínez JM, Cano ME, Velasco C, Martínez-Martínez L, Pascual A (2011) Plasmid mediated quinolone resistance: an update. J Infect Chemother 17: 149-182.
22. Woodford N, Turton JF, Livermore DM (2011) Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 35 Suppl 5: 736–55.

23. Merah-Fergani O, Sebaihia M, Berrazeg M, Amraoui R, M. Diene S, Rolain JM (2022) Occurrence and diversity of extended-spectrum β-lactamases in clinical isolates of Enterobacteriaceae in a tertiary care hospital in Algeria. J Infect Dis Antimicrob Agents 39 Suppl 1: 1-14.
24. Abderrahim A, Djahmi N, Loucif L, Nedjai S, Chelaghma W, Gameci-Kirane D, Dekhil M, Lavigne JP, Pantel A (2022) Dissemination of OXA-48 and NDM-1 producing Enterobacterales isolates in an Algerian hospital. Antibiotics 11: 750.
25. Babafela B, Awosile, Michael A, Oluwawemimo A, Olugbenga K, Ezekiel O (2022) Beta-lactamase resistance genes in Enterobacteriaceae from Nigeria. Afr J Lab Med 11 Suppl 1: a1371.
26. Zemmour A, Dali-Yahia R, Maatallah M, Saidi-Ouahrani N, Rahmani B, Benhamouche N, Al-Farsi HM, Giske CG (2021) High-risk clones of extended-spectrumβ-lactamase-producing *Klebsiella pneumoniae* isolated from the University Hospital Establishment of Oran, Algeria (2011–2012). PloS One 16: e0254805.
27. Zahid SA (2015) Identification of *blaOXA-1* genes in *Klebsiella* isolated from urinary tract infections. International Journal of Advanced Research 3 Suppl 3: 947-950.
28. Mesli E, Berrazeg M, Drissi M, Bekkhoucha SN, Rolain JM (2013) Prevalence of carbapenemase-encoding genes including New Delhi metallo-lactamase in *Acinetobacter* species, Algeria. Int J Infect Dis 17: 739–743.
29. Asma S, Lotfi L, Gupta SK, Mazouz D, Houria C, Jean-Marc R (2014) NDM-5 carbapenemase-encoding gene in multidrug-resistant clinical isolates of *Escherichia coli* from Algeria. Antimicrobial Agents Chemother 58 Suppl 9: 5606-5608.
30. Nabti LZ, Sahli F, Olowo-OA, Benslama A, Harrar A, Lupande-MD, Diene SM, and Rolain JM (2022) Molecular characterization of clinical carbapenem-resistant Enterobacteriaceae isolates from Sétif, Algeria. Microb Drug Resist 28: 274-279.
31. Dagher TN, Azar E, Al-Bayssari C, Chamieh SA, and Rolain JM (2019) First detection of colistin-resistant *Klebsiella pneumoniae* in association with NDM-5 carbapenemase isolated from clinical lebanese patients. Microb Drug Resist 25: 925-930.
32. Loucif L, Chelaghma W, Cherak Z, Bendiama E, Beroual F, Rolain JM (2022) Detection of *NDM-5* and *MCR-1* antibiotic resistance encoding genes in Enterobacterales in long-distance migratory bird species *Ciconia ciconia*, Algeria. Sci Total Environ 814: 152861.
33. Yousfi M, Mairi A, Bakour S, Touati A, Hassissen L, Hadjadj L, Rolain JM (2015) First report of NDM-5-producing *Escherichia coli* ST1284 isolated from dog in Bejaia, Algeria. New Microbes New Infect 8: 17–18.
34. Hornsey M, Phee L, WarehamDW (2011) A novel variant, NDM-5, of the New Delhi metallo-b-lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. Antimicrob Agents Chemother 55: 5952–5954.
35. Meradi L, Djahoudi A, Abdi A, Bouchakour M, Perrier Gros Claude JD, Timinouni M (2011) *Qnr* and *aac (6')-Ib-cr* types quinolone resistance among Enterobacteriaceae isolated in Annaba, Algeria. Pathol Biol 59: e73-78.
36. Touati A, Brasme L, Benallaoua S, Gharout A, Madoux J, De Champs C (2008) First report of qnrB-producing *Enterobacter cloacae* and qnrA-producing *Acinetobacter baumannii* recovered from Algerian hospitals. Diagn Microbiol Infect Dis 60: 287–290.
37. Poirel L, Gutiérrez C, Leviandier C, Nordmann P, Cordeir NF, Pirez LA, Seij V, Bazet C, Rieppi G, Vignoli R (2006) prevalence and genetic analysis of plasmid mediated quinolone resistance determinants *Qnr A* and *Qnr S* in Enterobacteriaceae isolates from a French university hospital. Antimicrob Agents Chemother 50: 3992–3997.
38. Bado I, Gutierrez C, Garcıa-Fulgueiras V, Garcıa-Fulgueiras V, Cordeiro NF, Pirez LA, Veronica Seija, Bazet C, Rieppi G, Vignoli R2016) *CTX-M-15* in combination with *aac(6')-Ib-cr* is the most prevalent mechanism of resistance both in *Escherichia coli* and *Klebsiella pneumoniae*, including *K. pneumoniae* ST258, in an ICU in Uruguay. J Glob Antimicrob Resist 6: 5–9.
39. Yakout MA, Ghada HA (2022) A novel *parC* mutation potentiating fluoroquinolone resistance in *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates. J Infect Dev Ctries 16 Suppl 2: 314-319. doi: 10.3855/jidc.15142.
40. Gu Y, Zhang J, Yu Y, Zhou Z, Du X (2004) Drug-resistant mechanisms and prevalence of *Enterobacter cloacae* resistant to multi-antibiotics. Chin J Nosocomiol 14: 1321– 1324.
41. Yanat B, Machuca J, Díaz-De-Alba P, Mezhoud H, Touati A, Pascual Á (2017) Characterization of plasmid-mediated quinolone resistance determinants in high-level quinolone-resistant Enterobacteriaceae isolates from the community: first report of *qnrd* gene in Algeria. Microb Drug Resist 23: 90-97.
42. Yanat B, Rodríguez-Martínez JM, Touati A (2017) Plasmid-mediated quinolone resistance in Enterobacteriaceae: a systematic review with a focus on Mediterranean countries. Eur J Clin Microbiol Infect Dis 36: 421-435.
43. Ioannis K, Eleni V, Zoi DP, and Athanasios T (2021) *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens 10: 373.
44. Aggoune N, Tali-Maamar H, Assaous F, Benamrouche N, Naim M, Rahal K (2014) Emergence of plasmid mediated carbapenemase OXA-48 in a *Klebsiella pneumoniae* strain in Algeria. J Glob Antimicrob Resist 2: 327-329.
45. Cheng G, Hu Y, Yin Y, Yang X, Xiang C, Wang B, Chen Y, Yang F, Lei F, Wu N, Lu N, Li J, Chen Q, Li L, Zhu B (2012) Functional screening of antibiotic resistance genes from human gut microbiota reveals a novel gene fusion. FEMS Microbiol Lett 336: 11–16.
46. Habeeb MA, Haque A, Nematzadeh S, Iversen A, Giske CG (2013) High prevalence of 16S rRNA methylase *RmtB* among CTX-M extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* from Islamabad, Pakistan. Int J Antimicrob Agents 41: 524–526.
47. Castanheira M, Farrell SE, Wanger A, Rolston KV, Jones RN, Mendes RE (2013) Rapid expansion of KPC-2-producing *Klebsiella pneumoniae* isolates in two Texas hospitals due to clonal spread of ST258 and ST307 lineages. Microb Drug Resist 19: 295–297.
48. Bonura C, Giuffre M, Aleo A, Fasciana T, Di Bernardo F, Stampone T, Giammanco A, Palma DM, Mammina C, MDR-GN Working Group (2015) An update of the evolving epidemic of *blaKPC* carrying *Klebsiella pneumoniae* in Sicily, Italy, 2014: emergence of multiple non-ST258 clones. PLoS One 10: e0132936.
49. Park DJ, Yu JK, Park KG, Park YJ (2015) Genotypes of ciprofloxacin resistant *Klebsiella pneumoniae* in Korea and their characteristics according to the genetic lineages. Microb Drug Resist 21: 622–630.

50. Mansour W, Grami R, Ben Haj Khalifa A, Dahmen S, Chatre P, Haenni M, Aouni M, Madec JY (2015) Dissemination of multidrug-resistant *blaCTXM-15/IncFIIk* plasmids in *Klebsiella pneumoniae* isolates from hospital and community-acquired human infections in Tunisia. Diagn Microbiol Infect Dis 83: 298–304.
51. Ocampo AM, Chen L, Cienfuegos AV, Roncancio G, Chavda KD, Kreiswirth BN, Jimenez JN (2016) A two-year surveillance in five Colombian tertiary care hospitals reveals high frequency of non-CG258 clones of carbapenem-resistant *Klebsiella pneumoniae* with distinct clinical characteristics. Antimicrob Agents Chemother 60: 332–342.
52. Ruiz-Garbajosa P, Hernández-García M, Beatobe L, Tato M, Méndez MI, Grandal M, Aranzábal L, Alonso S, López MÁ, Astray J, Cantón R (2016) A single-day point-prevalence study of faecal carriers in long-term care hospitals in Madrid (Spain) depicts a complex clonal and polyclonal dissemination of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 71: 348–352.
53. Lazareva IV, Ageevets VA, Ershova TA, Zueva LP, Goncharov AE, Darina MG, Svetlichnaya YS, Uskov AN, Sidorenko SV (2016) Prevalence and antibiotic resistance of carbapenemase-producing Gram-negative bacteria in Saint Petersburg and some other regions of the Russian federation. Antibiot Khimioter 61: 28–38.
54. Dropa M, Lincopan N, Balsalobre LC, Oliveira DE, Moura RA, Fernandes MR, da Silva QM, Matté GR, Sato MIZ, Matté MH (2016) Genetic background of novel sequence types of CTX-M-8- and CTX-M-15-producing *Escherichia coli* and *Klebsiella pneumoniae* from public wastewater treatment plants in Sao Paulo, Brazil. Environ Sci Pollut Res Int 23: 4953– 4958.
55. Harada K, Shimizu T, Mukai Y, Kuwajima K, Sato T, Usui M, Tamura Y, Kimura Y, Miyamoto T, Tsuyuki Y, Ohki A, Kataoka Y (2016) Phenotypic and molecular characterization of antimicrobial resistance in *Klebsiella* spp. isolates from companion animals in Japan: clonal dissemination of multidrug-resistant extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*. Front Microbiol 7: 1021.
56. Schaufler K, Nowak K, Dux A, Semmler T, Villa L, Kourouma L, Bangoura K, Wieler LH, Leendertz FH and Guenther S (2018) Clinically relevant ESBL-producing *K. pneumoniae* ST307 and *E. coli* ST38 in an urban West African rat population. Front Microbiol 9: 150.
57. Loncaric I, Cabal Rosel A, Szostak MP, Licka T, Allerberger F, Ruppitsch W, Spergser J (2020) Broad-spectrum cephalosporin-resistant *Klebsiella* spp isolated from diseased horses in Austria. Animals 10: 332.
58. Pitout JDD, Finn TJ (2020) The evolutionary puzzle of *Escherichia coli* ST131. Infect Genet Evol 81: 104265.
59. Strydom KA, Chen L, Kock MM, Stoltz AC, Peirano G, Nobrega DB, Lowe M, Ehlers MM, Mbelle NM, Kreiswirth BN, Pitout JDD (2020) *Klebsiella pneumoniae* ST307 with OXA-181: threat of a high-risk clone and promiscuous plasmid in a resource-constrained healthcare setting. J Antimicrob Chemother 75: 896–902.
60. Gisele Peirano, Liang Chen, Barry N. Kreiswirth, Johann D, D Pitouta (2020) Emerging antimicrobial-resistant high-risk *Klebsiella pneumoniae* clones ST307 and ST147. Antimicrob Agents Chemother 64 Suppl 10: e01148-20.

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