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Short Communication

Multidrug-resistant *Klebsiella quasipneumoniae* subsp. *similipneumoniae* carrying bla_{NDM}-$bla_{CTX-M15}$ isolated from flies in Rio de Janeiro, Brazil

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ABSTRACT

Objectives: Flies have been implicated in the dispersal of medically important bacteria including members of the genus *Klebsiella* between different environmental compartments. The aim of this study was to retrieve and characterize antibiotic-resistant bacteria from flies collected near to hospitals.
Methods: Flies were collected in the vicinity of medical facilities and examined for bacteria demonstrating phenotypic resistance to ceftriaxone, followed by determination of phenotypic and genotypic resistance profiles. In addition, whole genome sequencing followed by phylogenetic analysis and resistance genotyping were performed with the multidrug-resistant (MDR) strain Lemef23, identified as *Klebsiella quasipneumoniae* subsp. *similipneumoniae*.
Results: The strain Lemef23, classified by multiple locus sequence typing as novel ST 3397, harboured numerous resistance genes. The bla_{NDM} was located on a Tn*3000* element, a common genetic platform for the carriage of this gene in Brazil. Inference of phylogenetic orthology of strain Lemef23 and other clinical isolates suggested an anthropogenic origin.
Conclusions: The findings of this study support the role of flies as vectors of MDR bacteria of clinical importance and provide the first record of bla_{NDM-1} and $bla_{CTXM-15}$ in a Brazilian isolate of *K. quasipneumoniae* subsp. *similipneumoniae*, demonstrating the value of surveying insects as reservoirs of antibiotic resistance.

1. Introduction

Since 1940, a diverse array of antimicrobial substances have been synthesized and employed as treatments and/or prophylactically for bacterial diseases in humans, animals and plants. As a result, these substances have been disseminated into virtually all ecosystems of the planet promoting enormous pressure for the selection and maintenance of populations of resistant bacteria [1]. The frequency of isolation of multidrug-resistant (MDR) Gram-negative strains has increased globally at an alarming rate during the last 10 years [1]. Strains of *Klebsiella pneumoniae* pertaining to phylogenetic groups Kp1 to Kp7 were classified as *K. pneumoniae sensu stricto*, *K. quasipneumoniae* subsp. *quasipneumoniae*, *K. variicola* subsp. *variicola*, *K. quasipneumoniae* subsp. *similipneumoniae*, *K. variicola*

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subsp. *tropicalensis*, *K. quasivariicola* and *K. africanensis*, respectively [2,3].

Antimicrobial resistance is clearly connected to the objectives of the One Health concept and insects may act as agents of dispersion of such resistance between all three pillars of the One Health triad (people, environment and animals) [1]. It is now clear, that resistance problems in clinical settings often have their origins in environments outwith hospitals and as such, surveillance of antimicrobial determinants in environmental bacteria is as just as important as characterization of resistance in nosocomial isolates [4].

Given the great importance in terms of medical and environmental health of *Klebsiella* spp., the aim of the present study was to retrieve antibiotic-resistant *Klebsiella* spp. from flies collected near to hospitals, in Rio de Janeiro, Brazil, and to investigate the presence of the most commonly disseminated antibiotic resistance genes therein. The present work sought to contribute towards the understanding of the sentinel role of flies in microbial resistance and mostly with the One Health concept which links different environments, in this specific case hospitals and their surroundings.

2. Materials and methods

2.1. Collection and identification of flies

Collection of muscoid dipterans was carried using entomological nets in different locations within the municipality of Rio de Janeiro, in 2014, 2015 and 2016, at different points in Rio de Janeiro City: a) a garbage dumpster in Amorim community (22°52'32.2"S 43°15'01.9"W); b) in traps containing rotten meat in a municipal park namely, Quinta da Boa Vista (22°54'26.8"S 43°13'19.8"W); and c) a garbage dumpster in the grounds of the Hospital da Piedade (22°53'32.4"S 43°18'35.3"W). It should be highlighted that all collection points were in the proximity of hospitals. Samples were collected at ca. 11:00 hours on dry days with average temperatures of 29 °C. Captured specimens were transported live to the Lemef/IOC/Fiocruz, Rio de Janeiro and identified using dichotomous keys as previously reported [5].

2.2. Bacterial isolation and identification

Bacterial isolation and identification were performed as reported by Carramaschi et al. [5]. Briefly, flies were macerated in PBS buffer, diluted and used to inoculate plates of Nutrient Agar with and without ceftriaxone (1 mg/L). Representatives of the different colonies were subcultured in the same medium to obtain pure cultures with long-term storage in Brain Heart Infusion Broth (BHI - Merck) supplemented with 20% glycerol (Sigma) at −20 °C. Pure cultures of the isolates were initially identified by MALDI-TOF/MS (Bruker Daltonics).

2.3. Antimicrobial sensitivity testing (AST)

Representative colony types were reactivated from glycerol stocks on plates of Nutrient Agar and examined for resistance to the following antibiotics: cefepime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), meropenem (10 μg), gentamicin (10 μg), tetracycline (10 μg), ciprofloxacin (5 μg), trimethoprim/sulphamethoxazole (1.25 + 23.75 μg) and chloramphenicol (30 μg) (Sensifar) by the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (Becton, USA) based on the Clinical Laboratory Standard Institute guidelines [5].

2.4. Determination of antimicrobial resistance profiles using the BD Phoenix Automated Microbiology System

An isolate (Lemef23) which presented a MDR profile by AST analysis was submitted to analysis using the BD Phoenix Automated Microbiology System (BD Diagnostics).

2.5. Modified carbapenem inactivation test (mCIM) and EDTA-modified carbapenem inactivation method (eCIM)

Modified carbapenem inactivation methods without EDTA (mCIM) and with EDTA (eCIM) for phenotypic detection of carbapenemase were evaluated with the Lemef23 strain as reported previously [6].

2.6. Characterization of resistance determinants and molecular typing

PCR was used to screen for resistance determinants conferring resistance to a range of β-lactams (bla_{CTX-M}, bla_{SPM}, bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{GES}, bla_{OXA-23}, $bla_{OXA-143}$, $bla_{OXA-48\ LIKE}$, bla_{SHV}, bla_{SPM} and bla_{TEM}), aminoglycosides (*aac*(6`)-Ib) and colistin (*mcr-1* and *mcr-2*) as previously described [5]; multiple locus sequence typing, MLST-PCR was performed according to standard protocols (https://bigsdb.pasteur.fr/). For MLST analysis and phylogenetic inference it was used the PHYLOViZ platform (available at http://www.phyloviz.net). The primers designed to detect the IS*3000* were forward 5′-GCCTGGCACAACATTAACCT-3′ and reverse 5′-CGGCATGTCGAGATAGGAAG-3′ (predicted product size 749 bp). The cycling conditions used were: one cycle of 94 °C for 5 min, 30 cycles of 94 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min. The assays for IS*Aba125* and ble_{MBL} were performed as reported by Nordman et al. [7].

2.7. Genome sequencing and analysis

Strain Lemef23 was cultured overnight in Nutrient Broth at 37 °C and genomic DNA was extracted by Wizard® Genomic DNA Purification Kit (Promega Corporation, Wisconsin, USA). Libraries were prepared using the ION Plus Fragment Library Kit (Cat. 4471252 - ThermoFisher Scientific USA), and differentiated using the Ion Xpress™ Barcode Adapters kit (Cat. 4471250, 4474009- ThermoFisher Scientific USA). Template preparation was performed using ION PGM Hi-Q OT2 kit (Cat. 4471252 - ThermoFisher Scientific USA). Sequencing was performed on the ION Personal Genome Platform Machine (ThermoFisher Scientific USA) using ION 316™ Chip V2 and sequencing reaction was performed with Ion PGM ™ Hi-Q ™ Sequencing (Cat. A30004 - ThermoFisher Scientific USA). Following quality control, the generated reads were de novo assembled by SPAdes v.3.12.0 using the reads sequenced in Ion Torrent. The Center for Genomic Epidemiology tools were used to identify acquired antimicrobial resistance genes through ResFinder 3.2 (https://cge.cbs.dtu.dk/services/ResFinder/) and KmerResistance 2.2 (https://cge.cbs.dtu.dk/services/KmerResistance/), to confirm MLST alleles (https://cge.cbs.dtu.dk/services/MLST/) and taxonomy (https://cge.cbs.dtu.dk/services/SpeciesFinder/ and (https://cge.cbs.dtu.dk/services/KmerFinder/). A draft genome of Lemef23 was annotated using RAST (http://rast.nmpdr.org/). The BLASTn and the Artemis Comparison Tool were used to perform the pairwise alignment of Lemef23 contigs and MDCA01000045 contig of the *K. quasipneumoniae* CCBH16302 strain [8]. Orthofinder 2 [9] was used for phylogenetic orthology inference to assess the evolutionary affiliation of Lemef23 strain with related species deposited in the genome database of NCBI.

3. Results

3.1. Identification of flies and bacterial isolates

During 2014–2016, a total of 117 flies was captured and from those, eight strains of *Klebsiella* spp. were identified: *Chrysomya megacephala* (three strains of *K. pneumoniae*, one strain of *K. oxytoca*), *Chrysomya putoria* (two strains of *K. pneumoniae* and one strain of *K. varicola*) and *Musca domestica* (one strain of *K.*

quasipneumoniae subsp. *similipneumoniae*, initially identified as *K. pneumoniae* by MALDI-TOF/MS).

3.2. Phenotypic and genotypic resistance to antibiotics

Strain Lemef23 demonstrated resistance as assessed by AST to meropenem, cefepime, ceftazidime, gentamicin, cefoxitinetrimethoprim/sulphamethoxazole. In relation to the Phoenix BD system, resistance was detected to all the antibiotics tested with the exceptions of amikacin and colistin. The Lemef23 strain was positive in both the mCIM and eCIM tests suggesting the presence of metallo-β-lactamase. Amplicons were generated by PCR for fragments of the bla_{NDM}, bla_{TEM}, $bla_{CTX\text{-}M15}$, *aac(6')-Ib* and *int*1 genes. Each PCR was repeated in triplicate, using DNA from different colonies of the same isolate to discard false-positive results.

3.3. MLST-PCR

MLST analysis of strain Lemef23 revealed a novel combination of alelles: *gapA* – 18, *infB* – 22, *mdh* – 64, *pgi* – 22, *phoE* – 11, *rpoB* – 13, *tonB* – 227, which placed it in the new ST 3997, not related to any clonal complex.

3.4. Genome sequencing and analysis

The draft genome of strain Lemef23 consisted of a single 5 663 872 base pairs with a GC content of 57.1% GC, 6097 coding sequences, N50 of 69 087 bp, and 62 RNAs. This whole genome sequencing (WGS) project was deposited at DDBJ/ENA/GenBank under the accession WNLB00000000. The version described in this paper is version WNLB01000000.

The PlasmidFinder tool was used to detect the presence of incompatibility groups IncY, IncFII and IncR, while ResFinder and KmerResistance analyses identified genes conferring resistance to trimethoprim (*dfrA14*); sulphonamide (*sul1* and *sul2*); tetracycline (*tet*(A)); fluoroquinolone (*qnrB1*), fosfomycin (*fosA*), β-lactams ($bla_{NDM\text{-}1}$, $bla_{TEM\text{-}1B}$, $bla_{OKP\text{-}B\text{-}5}$, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}1}$) and aminoglycosides ([*aac*(3)-IIa, *aph*(6)-Id, *aph*(3")-Ib and *aac*(6')-Ib]), all with levels of nucleotide similarity > 94%.

KmerFinder identified this strain as *K. quasipneumoniae* and the phylogenetic orthology inference demonstrated its phylogenetic affinity *K. quasipneumoniae* subsp. *similipneumoniae* (Fig. 1). The contigs WNLB01000100 (containing $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1B}$) and WNLB01000153 ($bla_{OXA\text{-}1}$, acc6'-Ib-cr and *cat*B3 genes) showed sequencing coverages proximal to plasmidial contigs (WNLB01000067, WNLB01000094 and WNLB01000123) of IncY, IncR and IncFII genes.

The carbapenemase produced by Lemef23 is encoded by the bla_{NDM} gene and carried by the Tn*3000* transposon, including ble_{MBL}, *trpF*, tat(*dsbD*), *cutA*, *groES* and *groEL* genes (Fig. 2). The Tn *3000* of Lemef23 is similar to that found in CCBH16302 [8]. The Tn*3000* regions showed ca. 99% of nucleotide similarity between the Lemef23 scaffold and the CCBH16302 contig (MDCA01000045). The similarity between these syntenic regions also indicated the presence of genes encoding for a SDR family oxireductase, quinolone resistance protein (*qnrB*) and an operon transcriptional activator (*pspF*) upstream of the Tn*3000* (Fig. 2). Additionally, the WNLB01000157 and WNLB01000189 contigs were flanked by IS6 family transposase genes (contig WNLB01000187 not shown).

4. Discussion

Finding resistant bacteria in urban-collected flies can be as difficult as finding a needle in a haystack given the short generation time, elevated reproduction rates and population sizes and a constant state of flux in fly populations. However, the presence of MDR bacteria in a fly indicates environmental contamination. It is important to note that most of the literature on this topic focused on *Escherichia coli* and largely overlooked important nosocomial pathogens including the members of the complex *Klebsiella*. In the present study, eight strains identified by MALDI-TOF/MS as members of the *Klebsiella* complex (specifically *K. pneumoniae*, *K. variicola* and *K. oxytoca*) were isolated from different species of flies captured in garbage in close proximity to hospitals.

A single isolate (Lemef23)belonging to a previously unreported MLST designated ST3997, with no link to any clonal complex, presented a MDR phenotype, showing resistance to virtually all the different classes of antibiotic examined. Considering the importance of accurate identification and genetic characteristics of MDR isolates, as a component of predictive surveillance and antibiotic resistance of emerging pathogens, we performed WGS of this strain. The strain Lemef23, putatively identified as *K. pneumoniae* by MALDI-TOF/MS, was definitively identified as *K. quasipneumoniae* subsp. *similipneumoniae* by WGS. The true prevalence of *K. quasipneumoniae* and its possible clinical significance remains to be elucidated, as there have been only a few reports following its official recognition as a species in 2014 [10,11]. Moreover, this underestimation may be associated with the challenges to

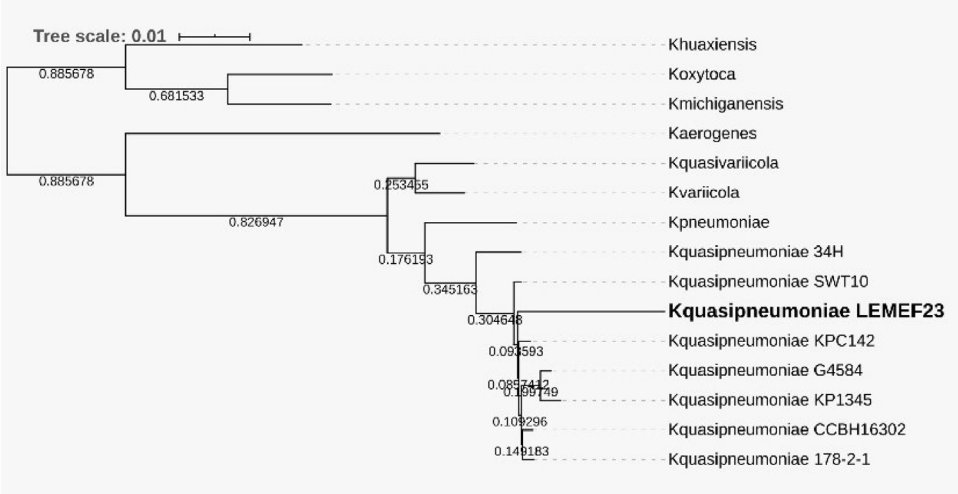

Fig. 1. Phylogenetic orthology inference obtained with Orthofinder2.

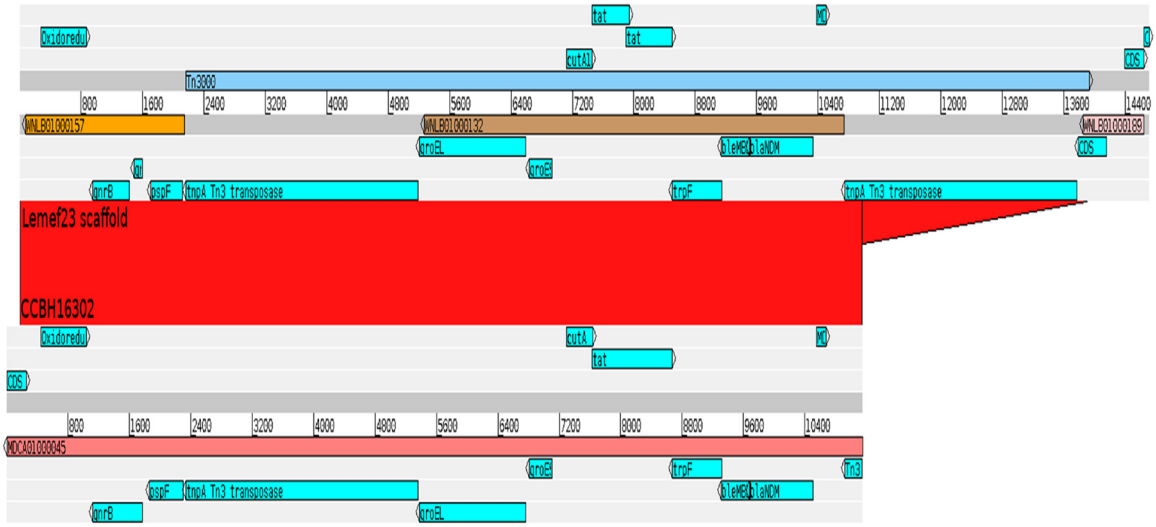

Fig. 2. Conservation of synteny of genes in Tn*3000* transposon.
Conservation of synteny of genes (light blue), including bla_{NDM}, in Tn*3000* transposon (dark blue) and 99% nucleotide similarity (red) between the Lemef23 scaffold and the MDCA01000045 contig of strain CCBH16302. In these genomic regions are found the genes encoding for SDR family oxireductase, *qnrB* (quinolone resistance protein), *pspF* (operon transcriptional activator), *tnpA* (Tn3 family transposase), *groEL*(heat shock protein 60 kDa family chaperone), *groES* (heat shock protein 10 kDa family chaperone), *cutA1* (periplasmic divalent cation tolerance protein), *tat* (twin-arginine translocation pathway signal protein), *trpF* (N-5'-phosphoribosyl anthranilate isomerase), ble_{MBL}(bleomycin resistance) and bla_{NDM} (New Delhi Metallo-β-lactamase gene).

execution of accurate differential diagnosis from *K. pneumoniae* when based on current clinical microbiology techniques, both phenotypic and molecular [10,11]. Phylogenetic analysis based on data previously published for carbapenem-resistant strains of *K. quasipneumoniae* from distinct sources and different platforms of resistance genes (Fig. 1), placed Lemef23 within a cluster of *K. quasipneumoniae* subsp. *similipneumoniae*, reinforcing its classification by WGS. Previous reports of this species in Brazil were restricted to clinical samples that showed resistance genotypes distinct from that of Lemef23 [8,12–14].

Strain Lemef23 presents the coproduction of bla_{NDM-1} with other β-lactamase genes and genetic determinants related to aminoglycoside and quinolone resistance. The results from WGS combined with the fact that no Lemef23 transconjugants were obtained in conjugation experiments (data not shown)and that attempts to detect hybridization between a bla_{NDM} probe with DNA from plasmid preparations were negative (data not shown), suggest the probable chromosomal localization of this gene. The first description of NDM in Brazil was in a *Providencia rettgeri* where the bla_{NDM} was chromosomally located [8]. In contrast, the bla_{TEM-1B}, bla_{OXA-1}, $bla_{CTX-M-15}$ genes were each detected using the same platforms and bla_{TEM-1B} and $bla_{CTX-M-15}$ were detected in the same contig, suggesting a likely association within the same plasmid. The $bla_{CTX-M-15}$ determinant has been reported in IncF, IncH12, IncI, IncK, IncL/M and IncN plasmid types [15]. In the current study, the platform PlasmidFinder detected the incompatibility groups IncY, IncFII and IncR, while PCR-based plasmid typing detected only IncFII. This observation suggests the presence of IncY and IncR molecules as low copy number plasmids and the possible presence of $bla_{CTX-M-15}$ on a novel IncY, IncFII or IncR plasmid.

The bla_{NDM} gene has been described in different genetic contexts in members of the order Enterobacterales, *Acinetobacter* spp. and *Pseudomonas aeruginosa* [16]. In Brazil, India, Morocco and Nepal, the bla_{NDM-1} in enterobacteria is associated with a mobile structure, namely Tn*3000* [16,17]. This transposon carries a conserved whole or truncated structure called IS*Aba125* upstream to the bla_{NDM-1} and the ble_{MBL} sequence downstream [17]. These elements play an important role in the rapidly evolving epidemiology of carbapenem-resistant enterobacterial strains [17]. In addition, it has been suggested that the bla_{NDM} gene has its origin in *Acinetobacter* from an unknown environmental source, in which it was associated with IS*Aba125*, then subsequent genetic events helped promote its dissemination [17]. However, the mechanisms by which it was transferred from *Acinetobacter* to Enterobacteriaceae remain unclear [17]. The Tn*3000* of Lemef23 was similar to that of *K. quasipneumoniae* subsp. *similipneumoniae* CCBH16302 isolated from surveillance swab in Rio de Janeiro State (Fig. 2) [8].

The presence of a *K. quasipneumoniae* subsp. *similipneumoniae* carrying a functional bla_{NDM-1}, on a Tn*3000* in a fly, highlights the importance of continued predictive screening of these insects as environmental reservoirs of clinically relevant resistance genes. The metallo-NDM enzyme confers resistance to carbapenem antibiotics, which, together with colistin, are among those of last recourse for the treatment of many MDR bacterial infections. Furthermore, it should be noted that infections caused by strains producing NDM are not effectively treated by ceftazidime-avibactam [18].

The presence of Tn*3000* in different STs, including Lemef23, corroborates previous hypotheses [8,17] that the spread of NDM-1-producing *Klebsiella pneumoniae*, and now closely related species, is not associated with clonal expansion but rather with this transposon. The level of virulence of this strain was not investigated in detail. However, WGS indicated the absence of the mucoviscosity-associated gene A (*magA*) [19] and the strain did not display a hypermucoviscosity phenotype by the string test (data not shown).

The phylogenomic analyses (Fig. 1) showed that Lemef23 was closely related to *K. quasipneumoniae* subsp. *similipneumoniae* isolates with anthropogenic origins. In contrast, environmental isolates, namely strains SWT10 (unpublished data, GenBank accession number VOIK00000000.1) and 178-2-1 [20], isolated from water and migratory birds, respectively, were recorded as more distant.

The association of MDR strains of the *K. pneumoniae* complex with infections in nosocomial settings is well established, but knowledge concerning both the presence and the molecular basis of MDR strains in the environment is still deficient. The importance of flies as an important mechanical vector in the spread of carbapenem resistance was emphasized by Wang et al. [4]. In a

search for resistant bacteria in flies, the present study detected MDR *K. quasipneumoniae* subsp. *similipneumoniae* carrying a pair of clinically important resistance genes ($bla_{\text{CTX-M-15}}$ and $bla_{\text{NDM-1}}$). The present data represent a meaningful contribution to knowledge on population genomics of the *K. pneumoniae* complex and provide support for the notion that surveillance of resistance in environmental sources should be actively adopted as an integral component of global public health policies.

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Competing interests

None declared.

Contributions

INC collected samples. INC, VFSB, LLC, TPGC, RJ and FFM, performed the experiments and data analysis. RCP, MMCQ and VZ conceived and designed the experiments. INC, TPGC, FFM and VZ wrote the manuscript.

Ethical approval

Not required.

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References

[1] Hernando-Amado S, Coque TM, Baquero F, Martínez JL. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nat Microbiol 2019;4:1432–42.
[2] Blin C, Passet V, Touchon M, Rocha EPC, Brisse S. Metabolic diversity of the emerging pathogenic lineages of *Klebsiella pneumoniae*. Environ Microbiol 2017;19:1881–98.
[3] DeLeo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, et al. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. Proc Natl Acad Sci U S A 2014;111:4988–93.
[4] Wang Y, Zhang R, Li J, Wu Z, Yin W, Schwarz S, et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat Microbiol 2017;6:16260.
[5] Carramaschi IN, de Castro EAR, Leite JA, Queiroz MMC, Villas Boas MHS, Rangel K, et al. First report of *Raoultella ornithinolytica* carrying $bla_{\text{KPC-2}}$ isolated from a dipteran muscoid collected in a garbage from a public hospital in Rio de Janeiro, Brazil. Rev Inst Med Trop Sao Paulo 2019;61:e32.
[6] Van Der Zwaluw K, De Haan A, Pluister GN, Bootsma HJ, De Neeling AJ, Schouls LM. The Carbapenem Inactivation Method (CIM), a simple and low-cost alternative for the carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods. PLoS One 2015;10:1–13.
[7] Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. Trends Microbiol 2011;19:588–95.
[8] Aires CA, Pereira PS, de Araujo CF, Chagas TPG, Oliveira JCR, Buonora SN, et al. Multiclonal expansion of *Klebsiella pneumoniae* isolates producing NDM-1 in Rio de Janeiro, Brazil. Antimicrob Agents Chemother 2017;61: e01048–16.
[9] Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol 2019;20:238.
[10] Rodrigues C, Passet V, Rakotondrasoa A, Brisse S. Identification of *Klebsiella pneumoniae, Klebsiella quasipneumoniae, Klebsiella variicola* and related phylogroups by MALDI-TOF Mass Spectrometry. Front Microbiol 2018;9:3000.
[11] Long SW, Linson SE, Ojeda Saavedra M, Cantu C, Davis JJ, Brettin T, et al. Whole-genome sequencing of human clinical *Klebsiella pneumoniae* isolates reveals misidentification and misunderstandings of *Klebsiella pneumoniae*, *Klebsiella variicola*and *Klebsiella quasipneumoniae*. mSphere 2017;2: e00290–17.
[12] Nicolás MF, Ramos PIP, Marques de Carvalho F, Camargo DRA, de Fátima MAC, Loss de Morais G, et al. Comparative genomic analysis of a clinical isolate of *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, a KPC-2 and OKP-B-6 beta-lactamases producer harboring two drug-resistance plasmids from Southeast Brazil. Front Microbiol 2018;9:220.
[13] Ferreira ML, Araújo BF, Cerdeira LT, Toshio C, Ribas RM. Genomic features of a clinical hypermucoviscous *K. quasipneumoniae subsp. similipneumoniae* of a clinical ESBL-producing and colistin-resistant from Brazil. Braz J Infect Dis 2019;23:207–9.
[14] Fuga B, Cerdeira L, Andrade F, Zaccariotto T, Esposito F, Cardoso B, et al. Genome sequences of clinical isolates of NDM-1-producing *Klebsiella quasipneumoniae* subsp. *similipneumoniae* and KPC-2-producing *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* from Brazil. Microbiol Resour Announc 2020;9: e00089-20.
[15] Zhang L, Lu X, Zong Z. The emergence of *bla*CTX-M-15-carrying *Escherichia coli* of ST131 and new sequence types in Western China. Ann Clin Microbiol Antimicrob 2013;12:35.
[16] Da Silva IR, Aires CAM, Conceição-Neto OC, De Oliveira Santos IC, Ferreira PN, Moreno SJP, et al. Distribution of clinical NDM-1-producing Gram-negative bacteria in Brazil. Microb Drug Resist 2019;25:394–9.
[17] Campos J, da Silva M, dos Santos P, Barros E, Pereira M, Seco B, et al. Characterization of Tn*3000*, a Transposon Responsible for $bla_{\text{NDM-1}}$ Dissemination among Enterobacteriaceae in Brazil, Nepal, Morocco, and India. Antimicrob 2015;59:7387–95.
[18] Mendes RE, Castanheira M, Woosley LN, Stone GG, Bradford PA, Flamm RK. Characterization of beta-Lactamase content of ceftazidime-resistant pathogens recovered during the pathogen-directed phase 3 REPRISE trial for ceftazidime-avibactam: correlation of efficacy against beta-Lactamase producers. Antimicrob Agents Chemother 2019;63:e02655–18.
[19] Shon AS, Bajwa RP, Russo TA. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence 2013;4:107–18.
[20] Liao X, Yang R-S, Xia J, Chen L, Zhang R, Fang L-X, et al. High colonization rate of a novel carbapenem-resistant *Klebsiella* lineage among migratory birds at Qinghai Lake, China. J Antimicrob Chemother 2019;74:2895–903.