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Short Communication

Molecular characterisation of multidrug-resistant *Klebsiella pneumoniae* belonging to CC258 isolated from outpatients with urinary tract infection in Brazil

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ABSTRACT

Objectives: This study characterised 48 *Klebsiella pneumoniae* isolates from outpatients with urinary tract infection in the micro-region of Ribeirão Preto, located in southeastern Brazil.
Methods: The isolates were identified by conventional biochemical and phenotypic tests and were confirmed as *K. pneumoniae* using a MALDI-TOF VITEK® MS system. Antimicrobial susceptibility testing was performed by the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) using 38 different antibiotic discs. Fifteen β-lactamase and ten virulence genes were investigated by PCR. Clonal relationships among the isolates were determined by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and multilocus sequence typing (MLST).
Results: Of the 48 isolates, 29 (60.4%) were classified as multidrug-resistant. A total of 46 β-lactamase genes were found in 27 (56.3%) of the isolates, with bla_{KPC} being the most prevalent distributed in 18 isolates (37.5%). Moreover, 73 virulence genes were found in 30 isolates (62.5%). ERIC-PCR results showed high genetic diversity among the isolates. Twelve different sequence types (STs) were found by MLST (ST14, ST17, ST101, ST200, ST334, ST433, ST437, ST442, ST449, ST502, ST1246 and ST2729), with ST2729 being described for the first time in this study. Seven STs were grouped in clonal complex 258 (CC258) frequently associated with various resistance and virulence genes.
Conclusions: These results raise concern about epidemiological surveillance related to colonisation of patients discharged from hospitals in order to prevent both the occurrence and spread of resistant bacterial infections in the community.

1. Introduction

The major causative pathogens associated with urinary tract infection (UTI) are *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus* [1]. *K. pneumoniae* is an important opportunistic pathogen that is frequently associated with nosocomial infections but also with community-acquired UTIs. Carbapenemase-producing *K. pneumoniae* has been associated with a significant increase in morbidity and mortality owing to limited therapeutic options [2]. Several virulence factors contribute to the pathogenicity of *K. pneumoniae*, which allow the bacteria to overcome the host's immune response and cause infections [3].

The emergence of multidrug-resistant (MDR) *K. pneumoniae* associated with several virulence factors causing nosocomial and community-acquired UTIs is a global concern, and their early identification is essential for the appropriate treatment of infected patients as well as for epidemiological control of these bacteria. This study characterised 48 *K. pneumoniae* isolates from outpatients with UTI in the micro-region of Ribeirão Preto, a city located in the Southeast Region of Brazil.

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Table 1
Patient characteristics, antimicrobial resistance, and virulence and β-lactamases genes found among the 48 *Klebsiella pneumoniae* isolates studied.

Isolate	Age (years)	Sex	Resistance profile		β-Lactamase genes	Virulence genes
			Non-susceptible to[a]:	Definition		
Kp10C	69	F	NAL, NIT, TZP, SUL, TMP	NC	None	None
Kp16C	NI	F	CTL, NAL, NIT, SUL, TMP	NC	None	None
Kp17C	NB	M	LOM, MNO, MEM, NAL, NIT, SUL, SXT, TMP	MDR	bla_{PER}	None
Kp22C	57	F	AMC, SAM, ATM, CAZ, CEC, CFM, FOX, CZO, CIP, FEP, CRO, CXM, CTL, CTX, DOX, ETP, IPM, LVX, LOM, MNO, MEM, NAL, NIT, NOR, OFX, TZP, SUL, SXT, TCC, TET, TOB, TMP	MDR	bla_{VEB}	*kfu*, *entB*, *mrkD*
Kp23C	29	F	CTL, NAL, NIT, SUL, TMP	NC	None	None
Kp25C	11	M	NIT, SUL, SXT, TMP	NC	bla_{PER}	*mrkD*
Kp26C	58	M	AMC, SAM, ATM, CAZ, CEC, CEF, CFM, CZO, CIP, FEP, CRO, CXM, CTL, CTX, DOX, STR, LVX, LOM, MNO, NAL, NIT, NOR, OFX, SUL, SXT, TCC, TET, TMP	MDR	$bla_{CTX\text{-}M\text{-}Gp1}$, bla_{VEB}	*kfu*, *entB*, *ybtS*, *mrkD*, *fimH*
Kp28C	NI	F	CHL, DOR, ETP, NIT, SUL, SXT, TMP	NC	bla_{VEB}	None
Kp29C	64	F	ATM, CAZ, CEC, CEF, CFM, CZO, FEP, CRO, CXM, CTL, CTX, STR, NAL, NIT, NOR, TZP, SUL, SXT, TCC, TMP	MDR	None	*entB*, *ybtS*, *mrkD*, *fimH*
Kp30C	59	M	AMC, SAM, CEC, CEF, CFM, CTL, DOX, STR, LOM, MNO, NAL, TZP, SUL, TCC, TMP	MDR	None	*fimH*
Kp31C	18	F	CEC, CFM, CTL, DOX, STR, LOM, MNO, NAL, NIT, SUL, TMP	MDR	None	None
Kp32C	34	F	CHL, DOR, LOM, NAL, NIT, TZP, SUL, SXT, TMP	MDR	bla_{KPC}	*mrkD*, *fimH*
Kp39C	21	F	LOM, DOR, NAL, NIT, SUL, SXT, TCC, TMP	MDR	bla_{KPC}, bla_{PER}, bla_{VEB}	*kfu*, *entB*, *k2*, *ybtS*, *mrkD*, *fimH*
Kp40C	18	F	FOX, CTL, MNO, NAL, NOR, SUL, TMP	MDR	None	None
Kp41C	35	F	CFM, CTL, DOX, STR, NAL, NOR, SUL, TMP	MDR	None	*mrkD*, *fimH*
Kp43C	66	F	AMK, AMC, CEC, CEF, CIP, DOR, STR, LVX, LOM, NAL, NIT, NOR, OFX, SUL, TOB, TMP	MDR	bla_{KPC}, bla_{PER}	*mrkD*
Kp44C	43	F	NAL, NIT, TZP, SUL, SXT, TMP	NC	bla_{PER}, bla_{VEB}	*mrkD*, *fimH*
Kp45C	60	M	AMK, IPM, NAL, NIT, SUL, SXT, TMP	MDR	bla_{KPC}	*entB*, *mrkD*
Kp47C	45	F	AMK, ATM, CAZ, CEC, CEF, CFM, FOX, CZO, FEP, ETP, STR, MNO, MEM, NAL, OFX, SUL, TOB, TMP	MDR	bla_{KPC}, bla_{VEB}	*kfu*, *mrkD*
Kp50C	53	F	AMC, SAM, CEF, FOX, CIP, CHL, CRO, CXM, CTL, CTX, DOR, DOX, LVX, LOM, MNO, NAL, NIT, NOR, OFX, TZP, SUL, SXT, TCC,TET, TMP	MDR	$bla_{CTX\text{-}M\text{-}Gp2}$	*entB*, *mrkD*
Kp57C	65	F	AMC, SAM, CHL, DOR, IPM, NAL, NIT, SUL, SXT, TCC, TOB, TMP	MDR	$bla_{CTX\text{-}M\text{-}Gp2}$	*entB*, *ybtS*, *mrkD*
Kp58C	NI	F	AMC, AMK, SAM, ATM, CEC, CEF, CFM, CZO, CIP, FEP, CRO, CXM, CTL, CTX, DOX, ETP, GEN, LOM, MNO, MEM, NAL, NIT, TZP, SUL, TCC, TOB, TMP	MDR	bla_{KPC}	*mrkD*
Kp62C	84	M	AMC, SAM, ATM, CAZ, CEC, CEF, CFM, FOX, CZO, CIP, FEP, CRO, CXM, CTL, CTX, DOX, ETP, STR, IPM, LVX, LOM, MNO, MEM, NAL, NITNOR, OFX, TZP, SUL, SXT, TCC, TET, TOB, TMP	MDR	bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp1}$, $bla_{CTX\text{-}M\text{-}Gp2}$	*allS*, *entB*, *mrkD*, *fimH*
Kp63C	4	F	NAL, NIT, SUL, TMP	NC	None	*fimH*
Kp66C	30	F	DOX, ETP, IPM, GEN, LOM, MEM, TZP, NAL, NIT, SUL, SXT, TOB, TMP	MDR	bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp1}$, $bla_{CTX\text{-}M\text{-}Gp2}$	*entB*, *mrkD*
Kp67C	63	F	CHL, DOX, IPM, NAL, NIT, TZP, SUL, SXT, TMP	MDR	None	None
Kp68C	30	F	CEC, DOX, MNO, NAL, NIT, TMP	NC	bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp1}$	*entB*, *mrkD*, *fimH*
Kp70C	94	F	AMC, AMK, SAM, ATM, CAZ, CEC, CEF, CFM, CZO, CIP, CHL, FEP, CRO, CXM, CTL, CTX, STR, LVX, LOM, MNO, NAL, NIT, NOR, OFX, TZP, SUL, SXT, TCC, TOB, TMP	MDR	None	*ybtS*
Kp91C	NI	NI	AMC, SAM, ATM, CAZ, CEC, CEF, CFM, FOX, CZO, CIP, CHL, FEP, CRO, CXM, CTL, CTX, STR, GEN, LVX, LOM, NAL, NIT, NOR, OFX, TZP, SUL, TCC, TOB, TMP	MDR	bla_{KPC}, bla_{VEB}	*entB*, *mrkD*
Kp92C	54	M	CHL, DOR, DOX, ETP, IPM, LOM, MNO, MEM, NAL, NIT, SUL, SXT, TOB, TMP	MDR	bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp1}$, bla_{VEB}	*entB*, *mrkD*
Kp93C	78	F	CEC, DOX, MNO, NAL, SUL, TMP	NC	None	None
Kp94C	15	F	STR, SUL, TMP	NC	None	None
Kp95C	1	M	CHL, DOX, ETP, IPM, LOM, MNO, MEM, NAL, NIT, NOR, TZP, SUL, SXT, TCC, TET, TMP	MDR	bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp2}$	*entB*, *mrkD*
Kp101C	20	F	DOX, STR, NAL, NIT, SUL, SXT, TMP	NC	None	None
Kp104C	7	M	DOX, SUL, TMP	NC	None	None
Kp107C	88	F	AMK, ATM, CAZ, CEC, CEF, CFM, FOX, CZO, FEP, CRO, ETP, STR, GEN, LOM, MEM, NAL, NIT, SUL, TOB, TMP	MDR	bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp2}$, bla_{VEB}	*kfu*, *entB*, *ybtS*, *mrkD*
Kp108C	NI	F	SAM, DOX, STR, NAL, NIT, SUL, TMP	NC	None	None
Kp109C	67	F	DOX, MNO, NIT, SUL, SXT, TET, TMP	NC	None	None
Kp114C	NI	F	DOX, NAL, NIT, TZP, SUL, SXT, TMP	MDR	bla_{VEB}	None
Kp115C	64	F	SAM, NAL, NIT, SUL, SXT, TOB, TMP	MDR	bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp2}$, bla_{VEB}	*allS*, *entB*, *mrkD*
Kp120C	46	M	AMC, SAM, CEC, DOX, MNO, NAL, NIT, TZP, SUL, TCC, TET, TMP	MDR	None	*mrkD*
Kp121C	30	F	NAL, NIT, SUL, TMP	NC	None	None
Kp126C	26	F	AMK, DOX, NAL, NIT, SUL, TMP	NC	None	None
Kp130C	65	F	CEC, CFM, DOX, STR, LOM, MNO, NAL, SUL, TMP	NC	None	None
Kp133C	11	F	AMK, NAL, NIT, SUL, SXT, TMP	NC	bla_{KPC}	*kfu*, *entB*, *mrkD*
Kp134C	70	M	CHL, DOX, STR, GEN, NAL, NIT, SUL, SXT, TCC, TET, TOB, TMP	MDR	bla_{KPC}	*entB*, *mrkD*
Kp136C	26	F	SAM, DOX, MNO, NAL, NIT, TZP, SUL, SXT, TCC, TMP	MDR	bla_{KPC}	*kfu*, *entB*, *ybtS*, *mrkD*
Kp139C	70	F	IPM, NAL, NIT, SUL, SXT, TMP	NC	bla_{KPC}	*entB*, *mrkD*

NI, not identified; NB, newborn; NC, not classified; MDR, multidrug-resistant.

[a] Antibiotics tested and their concentrations: AMK, amikacin (30 μg); AMC, amoxicillin/clavulanic acid (20/10 μg); SAM, ampicillin/sulbactam (10/10 μg); ATM, aztreonam (30 μg); CEC, cefaclor (30 μg); CZO, cefazolin (30 μg); CFM, cefixime (30 μg); FEP, cefepime (30 μg); CTX, cefotaxime (30 μg); FOX, cefoxitin (30 μg); CTL, ceftaroline (30 μg); CAZ, ceftazidime (30 μg); CRO, ceftriaxone (30 μg); CXM, cefuroxime (30 μg); CEF, cefalotin (30 μg); CHL, chloramphenicol (30 μg); CIP, ciprofloxacin (5 μg); DOR, doripenem (10 μg); DOX, doxycycline (30 μg); ETP, ertapenem (10 μg); GEN, gentamicin (10 μg); IPM, imipenem (10 μg); LVX, levofloxacin (5 μg); LOM, lomefloxacin (10 μg); MEM, meropenem (10 μg); MNO, minocycline (30 μg); NAL, nalidixic acid (30 μg); NIT, nitrofurantoin (300 μg); NOR, norfloxacin (10 μg); OFX, ofloxacin (5 μg); TZP, piperacillin/tazobactam (100/10 μg); STR, streptomycin (10 μg); SUL, sulfonamides (300 μg); TET, tetracycline (30 μg); TCC, ticarcillin/clavulanic acid (75/10 μg); TOB, tobramycin (10 μg); TMP, trimethoprim (5 μg); SXT, trimethoprim/sulfamethoxazole (1.25/23.75 μg).

2. Materials and methods

2.1. Bacterial isolates

A total of 48 *K. pneumoniae* were isolated in a clinical reference laboratory of a tertiary hospital located in Ribeirão Preto City (approximately 700 000 inhabitants), in São Paulo State, Brazil. This laboratory has service stations in five cities from the Ribeirão Preto micro-region, reaching more than one million inhabitants. In this study, all *K. pneumoniae* isolates were selected consecutively between March–May 2013 from positive urine cultures (≥100 000 CFU/mL) of outpatients with symptoms of UTI. The isolates were identified by conventional biochemical and phenotypic tests and were subsequently confirmed as *K. pneumoniae* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using a MALDI-TOF VITEK® MS system (bioMérieux Inc., Durham, NC) following the manufacturer's instructions.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the *K. pneumoniae* isolates was performed by the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [4]. Thirty-eight different antibiotic disks (Oxoid Ltd., Basingstoke, UK) were tested (Table 1). After determining the resistance profile, each bacterial isolate was classified into different categories including multidrug-resistant (MDR), extensively drug-resistant and pan-drug-resistant according to Magiorakos et al. [5]. When isolates did not fit the previous definitions, even if they were non-susceptible to one or more antibiotics, they were not classified.

2.3. DNA extraction

Genomic DNA from all of the isolates was extracted using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.4. Identification of β-lactamase genes

Detection of β-lactamase genes was performed by PCR using specific primers as previously described [6–8]. The following β-lactamase genes were investigated: $bla_{\text{CTX-M}}$ groups 1, 2, 8 and 9; bla_{PER}; bla_{VEB}; bla_{GES}; bla_{KPC}; bla_{VIM}; $bla_{\text{OXA-48-like}}$; $bla_{\text{OXA-1-like}}$; bla_{IMP}; bla_{SPM}; bla_{SIM}; bla_{GIM}; bla_{CMY}; bla_{MOX}; and bla_{NDM}.

2.5. Detection of virulence genes

Virulence gene detection was performed by PCR using specific primers and protocols as described previously [3,9,10]. The following virulence genes were investigated: *kfu*; *mrkD*; *allS*; *magA*; *rmpA*; *k2A*; *entB*; *ybtS*; *fimH*; and *iutA*.

2.6. Sequencing of PCR products

Following visualisation of the amplified genes, one of each amplicon was randomly selected for confirmation of identity by DNA sequencing using an automated sequencer (ABI 3500xL Genetic Analyzer; Applied Biosystems, Foster City, CA). The obtained sequences were compared with those available in GenBank using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.7. Hypermucoviscosity test

The hypermucoviscous phenotype was investigated by inoculation on Mueller–Hinton agar (Oxoid Ltd.) followed by incubation for 18–24 h at 37 °C. Following bacterial growth, a bacteriological loop was used to touch and lift vertically an isolated colony. Formation of a mucus filament of >5 mm was considered as a positive phenotype for hypermucoviscosity [11].

2.8. Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR)

The genetic similarity profile among the isolates was determined by ERIC-PCR analysis as previously described [12]. A genetic similarity dendrogram was constructed using BioNumerics v.5.1 (Applied Maths, Keistraat, Belgium) with the unweighted pair-group method with arithmetic mean (UPGMA) and the Dice similarity coefficient for cluster analysis. Only bands between 300 bp and 3000 bp were included in the analysis.

2.9. Multilocus sequence typing (MLST) and clonal analysis

MLST analyses were performed by amplifying and sequencing internal fragments of seven housekeeping genes. The protocols, primers and conditions are available in the *K. pneumoniae* MLST database (http://bigsdb.pasteur.fr/klebsiella/primers_used.html). Clonal relationships as well as the formation of clonal complexes (CCs) were determined using the eBURST v.3 algorithm (http://eburst.mlst.net/) [13].

3. Results

3.1. Isolates

A total of 48 *K. pneumoniae* isolates from urine culture of different outpatients with UTI from the micro-region of Ribeirão Preto were evaluated. Among the 48 patients, 15 (31.3%) were elderly (age ≥60 years), 13 (27.1%) were adults aged 30–59 years, 7 (14.6%) were young people aged 16–29 years and 6 (12.5%) were children aged 1–15 years. Moreover, there was one newborn and six patients did not have an identified age. Regarding patient sex, 36 (75.0%) were female and 11 (22.9%) were male; 1 patient did not have their sex reported (Table 1).

3.2. Resistance profile

Among the 48 *K. pneumoniae* isolates, all of them showed non-susceptibility (i.e. either intermediate or resistant) to four or more antibiotics tested. All isolates (100%) were non-susceptible to trimethoprim, 47 (97.9%) to sulfonamides, 43 (89.6%) to nalidixic acid, 40 (83.3%) to nitrofurantoin, 26 (54.2%) to trimethoprim/sulfamethoxazole, 24 (50.0%) to doxycycline, 19 (39.6%) to minocycline, 18 (37.5%) to lomefloxacin, 17 (35.4%) to piperacillin/tazobactam, 16 (33.3%) each to streptomycin and cefaclor, 15 (31.3%) to ticarcillin/clavulanic acid, 14 (29.2%) to ceftaroline, 13 (27.1%) each to ampicillin/sulbactam, cefixime and tobramycin, 11 (22.9%) each to amoxicillin/clavulanic acid, cefalotin and norfloxacin, 10 (20.8%) to chloramphenicol, 9 (18.8%) each to aztreonam, cefazolin, cefepime, ceftriaxone, ertapenem, imipenem and meropenem, 8 (16.7%) each to amikacin, cefotaxime, cefuroxime, ciprofloxacin, ceftazidime, tetracycline and ofloxacin, 7 (14.6%) each to doripenem, cefoxitin and levofloxacin and 5 (10.4%) to gentamicin. According to the criteria established by Magiorakos et al. [5], 29 isolates (60.4%) were classified as MDR (i.e. non-susceptible to at least one antibiotic in three or more antimicrobial categories). Although the 19 (39.6%) remaining isolates were non-susceptible to four or more antibiotics, they were not classified as MDR (Table 1; Fig. 1).

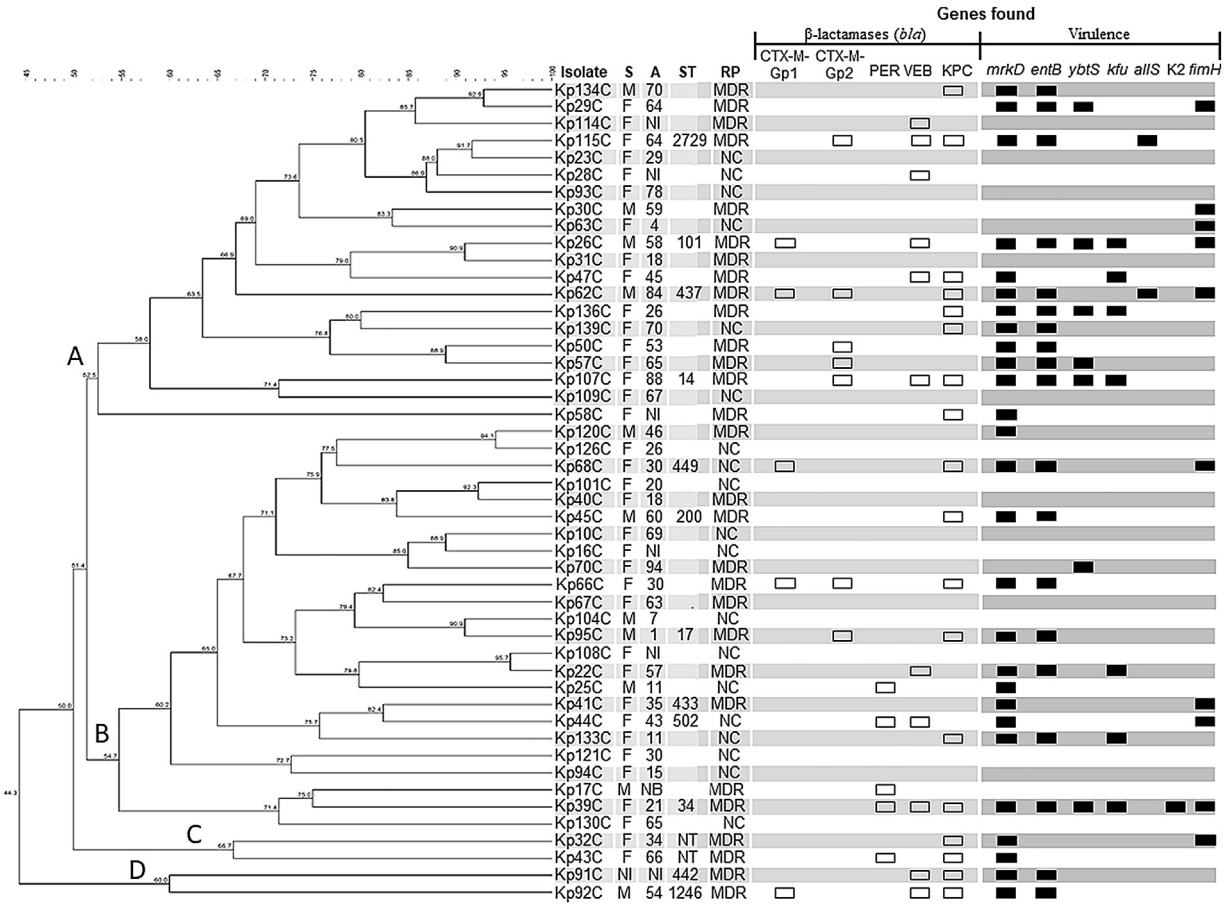

Fig. 1. Dendrogram obtained by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) analysis of 48 community-acquired *Klebsiella pneumoniae* urinary tract infection isolates. Clusters were determined using the unweighted pair-group method with arithmetic mean (UPGMA) and the Dice similarity coefficient. Similarity (%) among patterns is represented by the numbers beside the nodes. For each isolate, the resistance profile (RP) classification and the β-lactamase and virulence genes are represented. For each isolate typed by multilocus sequence tying (MLST), its respective sequence type (ST) is also shown in the figure. NT, non-typeable; NI, not identified; F, female; M, male; NB, newborn; MDR, multidrug-resistant; NC, not classified.

3.3. Detection of β-lactamase genes

Among the 48 studied isolates, 27 (56.3%) harboured one or more β-lactamase genes. A total of 46 β-lactamase genes were found, with bla_{KPC} being the most prevalent distributed in 18 isolates (37.5%), followed by bla_{VEB} in 11 isolates (22.9%), $bla_{CTX\text{-}M\text{-}Gp2}$ in 7 isolates (14.6%), and bla_{PER} and $bla_{CTX\text{-}M\text{-}Gp1}$ each in 5 isolates (10.4%) (Table 1; Fig. 1). Some isolates harbouring the bla_{KPC} gene showed no resistance to carbapenems, including isolates Kp68C, Kp91C, Kp115C, Kp133C, Kp134C and Kp136C (Table 1). For these isolates, bla_{KPC} may only confer reduced susceptibility to carbapenems.

3.4. Detection of virulence genes and hypermucoviscous phenotype

Ten virulence genes were investigated by PCR and a total of 73 virulence genes were found in 30 (62.5%) of the 48 studied isolates. The most prevalent virulence gene was *mrkD* (27 isolates; 56.3%), followed by *entB* (19 isolates; 39.6%), *fimH* (10 isolates; 20.8%), *kfu* and *ybtS* (7 isolates each; 14.6%), *allS* (2 isolates; 4.2%) and *k2* (1 isolate; 2.1%). The K1 and K2 capsular serotypes and the remaining virulence genes (*magA*, *rmpA* and *iutA*) were not found (Table 1; Fig. 1), and only two isolates (Kp58C and Kp62C) showed a hypermucoviscous phenotype. The analysed sequences were deposited in the GenBank database with accession nos. **MF417533**–**MF417545**.

3.5. ERIC-PCR and MLST data

Genetic similarity among the isolates was evaluated by ERIC-PCR and the results showed that the profile of bands obtained among the isolates was very different, indicating high genetic diversity. The dendrogram of genetic similarity showed the presence of two large clusters, designated A (20 isolates) and B (24 isolates), with 52.5% and 54.7% genetic similarity, respectively. Two smaller clusters designated C and D, comprising two isolates each, were also observed with genetic similarity of 66.7% and 60%, respectively. In all clusters, a large subdivision into smaller clusters was observed, and the maximum genetic similarity observed among the isolates was approximately 90%. None of the isolates presented 100% identity with each other.

For MLST analysis, the 4 isolates of the two smaller clusters (C and D) as well as another 10 isolates containing virulence and/or β-lactamase genes distributed in the larger clusters (A and B) were selected. Among them, two isolates (Kp32C and Kp43C) belonging to cluster C were non-typeable by MLST because it was not possible to amplify one or more housekeeping genes. Therefore, 12 isolates were analysed by MLST.

Among the 12 isolates analysed by MLST, 12 different sequence types (STs) were found, including ST14, ST17, ST101, ST200, ST334, ST433, ST437, ST442, ST449, ST502, ST1246 and ST2729, with ST2729 being described for the first time in this study (Fig. 2).

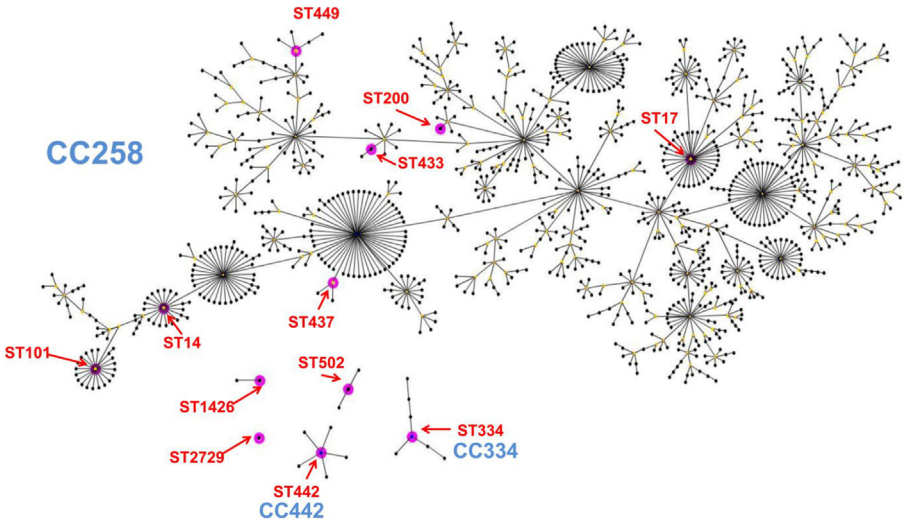

Fig. 2. eBURST diagram generated with the multilocus sequence tying (MLST) data, representing the 12 different sequence types (STs) obtained in this study (indicated by arrows). The three clonal complexes (CCs) found in this study are also indicated (blue), including CC258 (largest), CC442 and CC334. Each dot represents a ST. In pink, STs found in the *Klebsiella pneumoniae* isolates of this study; blue, primary ST founder of CCs; yellow, ST subgroup founders of the CCs; black, other STs.

4. Discussion

Community-acquired *K. pneumoniae* infections are less common than hospital infections and the main reports are of pneumonia and UTI [1]. For many years, several studies have demonstrated the high incidence of resistance to sulfonamides in Gram-negative bacteria isolated from community-acquired UTIs from many parts of the world. Therefore, sulfonamides are no longer indicated as the first-choice therapy for these infections; however, trimethoprim and trimethoprim/sulfamethoxazole are among the preferred agents for the treatment of acute uncomplicated cystitis [14]. For the other antimicrobials tested in the current study, a non-susceptibility rate ranging from 10.4% (gentamicin) to 100.0% (trimethoprim) was found. Moreover, the isolates presented indices above 50% non-susceptibility for nitrofurantoin (83.3%) (Table 1).

Among the β-lactamase genes found in the isolates, the bla_{KPC} gene was the most prevalent, distributed in 18 isolates (37.5%). These results corroborate those of Pinto et al. who found a prevalence of 68% for the bla_{KPC} gene in *K. pneumoniae* isolated from four hospitals in Porto Alegre, a city in southern Brazil [15]. These results are also worrying since the management of extended-spectrum β-lactamases (ESBLs) in UTIs is challenging due to the limited therapeutic options available outside of the hospital setting. The emergence of community-acquired ESBL-producing Enterobacteriaceae has been increasingly reported in Brazil and around the world [16].

Recently, great concern has been noted regarding patients discharged from hospitals colonised with MDR bacteria since many infections may be related to the hospital although they are isolated from outpatients [17,18]. The worrying level of antimicrobial resistance found among the isolates in the current study was surprising since bacterial isolates coming from the community are theoretically away from the constant selective pressure of the hospital environment. These findings associated with the presence of the bla_{KPC} gene suggest that the patients included in this study have a strong link with healthcare institutions and are not truly outpatients. Therefore, although there is no information, it is possible that many patients may have a history of hospitalisation, acquiring these bacteria in hospital and thus being colonised by them.

Although the isolates showed different STs, indicating great clonal diversity, seven STs (ST14, ST17, ST101, ST200, ST433, ST437 and ST449) were grouped in the large CC known as CC258 (also called CC258/11), which has been described in several countries and has been associated with the dissemination of bla_{KPC}, especially in Latin America (Fig. 2) [19]. This diversity was expected since the bacterial isolates were obtained from the community, with a very heterogeneous population coming from 16 cities close to Ribeirão Preto.

K. pneumoniae belonging to STs of CC258 are frequently associated with various resistance and virulence genes. In Brazil, the STs belonging to CC258 have been described as the most frequently found among KPC-producing *K. pneumoniae* in hospitals [19]. Nowadays, one of the major public-health concerns is the dissemination of MDR *K. pneumoniae* harbouring virulence and resistance genes associated with the hypermucoviscous phenotype, also called MDR hypervirulent *K. pneumoniae*, which is associated with a significant mortality rate [20]. In the present study, two isolates (Kp58C and Kp62C) with a hypermucoviscous phenotype harbouring ESBL and virulence genes were observed. Isolate Kp62C, besides presenting a hypermucoviscous phenotype, also presented ST437 belonging to CC258, was MDR and harboured β-lactamase genes (bla_{KPC}, $bla_{CTX\text{-}M\text{-}Gp1}$ and $bla_{CTX\text{-}M\text{-}Gp2}$) and virulence genes (*allS*, *entB*, *mrkD* and *fimH*) (Table 1; Fig. 1).

In conclusion, this study describes MDR *K. pneumoniae* isolates harbouring a large number of resistance and virulence genes, including high-risk international clones belonging to CC258, causing UTI in outpatients from five cities in Ribeirão Preto micro-region located in the Southeast Region of Brazil. It should be pointed out that these patients may be colonised by MDR *K. pneumoniae* acquired in the hospital environment. However, lack of information on the patients' clinical history does not allow us to make this correlation. These results raise concern about epidemiological surveillance related to colonisation of patients discharged from hospitals in order to prevent both the occurrence and spread of resistant bacterial infections in the community.

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Competing interests

None declared.

Ethical approval

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