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RESEARCH ARTICLE

Clonal overlap and resistance profiles of multidrug-resistant *Klebsiella pneumoniae* in humans and domestic animals in Brazil: A One Health molecular epidemiology study

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ABSTRACT

Background and Aim: The global rise of multidrug-resistant (MDR) *Klebsiella pneumoniae* poses a serious threat to human and animal health. Close proximity between humans and domestic animals may facilitate zoonotic transmission of MDR strains, underscoring the need for integrated surveillance strategies. This study aimed to investigate the genetic diversity, resistance mechanisms, and virulence gene profiles of *K. pneumoniae* isolates from domestic animals and humans in Mato Grosso, Brazil, within the One Health framework.

Materials and Methods: A total of 48 clinical isolates (33 from animals and 15 from humans) were analyzed. Identification was confirmed through 16S ribosomal RNA sequencing. Antimicrobial susceptibility was tested using disk diffusion (animal isolates) and minimum inhibitory concentration (human isolates). Resistance (bla_{kpc-2} and bla_{NDM}) and virulence genes (*entB*, *fimH*, *wabG*, *ugE*, etc.) were detected through polymerase chain reaction. Multilocus sequence typing (MLST) was performed on seven housekeeping genes, and sequence types (STs) were assigned using the Pasteur Institute database (Paris, France).

Results: MDR phenotypes were found in 70.83% (34/48) of isolates – 78.78% of animal and 53% of human samples. Virulence genes were present in 77.08% of isolates; *entB* was the most prevalent (60.61%). The bla_{kpc-2} gene was found in three human isolates, and bla_{NDM} was found in one human and one bovine isolate. MLST revealed 39 STs, including 9 novel ones. Clonal complexes (CC)258 (human), CC15 (animal), and CC147 (both species) indicated potential interspecies transmission.

Conclusion: This study provides the first comprehensive molecular epidemiological snapshot of *K. pneumoniae* in domestic animals and humans in Mato Grosso. The discovery of shared clonal complexes and high MDR rates demands urgent cross-sectoral surveillance and control strategies under the One Health approach.

Keywords: bla_{KPC-2}, bla_{NDM}, Brazil, multidrug-resistant *Klebsiella pneumonia,* multilocus sequence typing, one health, virulence genes, zoonotic transmission.

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INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen commonly isolated from the mucosal surfaces of humans and animals, as well as from environmental sources such as water, soil, vegetation, and food [1]. Multidrug-resistant (MDR) strains of *K. pneumoniae* pose a significant public health concern, particularly under the One Health paradigm, due to their ability to circulate across human, animal, and environmental interfaces [2]. The pathogenicity of *K. pneumoniae* is driven by a range of virulence factors that facilitate adherence to host tissues, iron acquisition, and immune evasion. These virulence traits are primarily mediated by structural components such as capsules, lipopolysaccharides, and fimbriae, which not only enhance adaptability but also contribute to antimicrobial resistance by supporting enzyme production that neutralizes therapeutic agents [3, 4].

Antimicrobial resistance further amplifies the threat posed by *K. pneumoniae*, frequently leading to difficult-to-treat infections and hospital outbreaks. The severity of infection is often associated with the multidrug resistance profile of the isolate, a problem exacerbated by the overuse and misuse of antibiotics in both clinical and veterinary settings [5]. Of significant concern is the emergence of carbapenem-resistant strains, particularly those producing *K. pneumoniae* carbapenemase (KPC), which have been strongly linked to increased mortality rates [6, 7]. Another resistance mechanism of growing concern is the bla_{NDM} gene, which encodes New Delhi metallo-β-lactamase and contributes to the rapid global dissemination of resistant clones [7].

To monitor and control the spread of these high-risk strains, molecular epidemiological tools such as multilocus sequence typing (MLST) have become indispensable. MLST offers high-resolution genotyping by sequencing conserved regions of multiple housekeeping genes, enabling precise characterization of clonal lineages. This technique is particularly useful for identifying zoonotic transmission pathways and tracing interspecies dissemination of resistant clones. Furthermore, MLST data contribute to international databases, thereby strengthening global surveillance efforts and informing public health interventions at both regional and international levels [8].

Despite the growing recognition of *K. pneumoniae* as a critical MDR pathogen with zoonotic potential, there remains a significant lack of data on its molecular epidemiology in underrepresented regions such as Mato Grosso, Brazil. Most available studies have concentrated on human clinical settings, with limited integration of data from veterinary and environmental sources, thereby hindering a comprehensive understanding of transmission dynamics. Furthermore, while global MLST databases continue to expand, there is still a paucity of genotypic data linking animal and human isolates from the same geographical area. This hampers our ability to detect interspecies clonal dissemination and the emergence of high-risk clones, such as those carrying carbapenemase genes such as bla_{KPC-2} and bla_{NDM}. In addition, few studies in Brazil have concurrently evaluated both virulence gene profiles and antimicrobial resistance mechanisms within a One Health framework. The lack of such integrated surveillance limits the development of effective, locally tailored intervention strategies. Therefore, filling this gap is essential to inform public health policies, antimicrobial stewardship programs, and veterinary practices in both regional and global contexts.

In response to these gaps, the present study aimed to conduct a comprehensive molecular characterization of *K. pneumoniae* isolates collected from both domestic animals and humans in the state of Mato Grosso, Brazil. Specifically, the study sought to (i) assess the prevalence of multidrug resistance and identify phenotypic resistance profiles, (ii) detect the presence of key resistance determinants, particularly bla_{KPC-2} and bla_{NDM}, (iii) investigate the distribution of virulence-associated genes, and (iv) characterize the clonal structure of the isolates using MLST. By comparing genotypic patterns and resistance markers across animal and human isolates, the study aimed to uncover potential interspecies transmission pathways and identify emerging high-risk clones. Ultimately, these findings are intended to provide a foundational dataset for regional epidemiological monitoring and contribute to the broader global surveillance of MDR *K. pneumoniae* within the One Health framework.

MATERIALS AND METHODS

Ethical approval and Informed consent

Ethical approval was obtained from the institutional review boards of both participating hospitals. The study was approved by the Ethics and Research Committee on the Use of Animals of the Universidade Federal de Mato Grosso (UFMT) (protocol number: 23108.236834/2017-13) and by the Research Ethics Committee of Plataforma Brasil at the Hospital Universitário Júlio Muller, also affiliated with UFMT (protocol number: 82549918.1.0000.5541). Verbal informed consent was obtained from all human participants as well as from the owners of the animals included in the study.

Study period and location

Clinical isolates were obtained from specimens collected between January 2016 and December 2017 at the participating hospitals, including the Veterinary Hospital at the Federal University of Mato Grosso and the Júlio Muller University Hospital.

Sample collection and bacterial isolation

Clinical isolates were obtained from specimens collected at veterinary and human hospitals and

processed in the microbiology laboratories of the Veterinary Hospital of the Federal University of Mato Grosso and the Júlio Muller University Hospital. Clinical specimens were systematically collected from defined anatomical sites in domestic animals and human patients admitted to veterinary and medical hospitals across the state of Mato Grosso. Although the samples were collected during an earlier period, the scarcity of data from this region underscores the significance of publishing these findings to bridge existing knowledge gaps and inform integrated One Health public health strategies.

Collection, bacterial isolation, and identification

Animal clinical specimens were plated on 5% sheep blood agar (Sigma-Aldrich, Darmstadt, Germany) and MacConkey agar (Neogen Corporation, São Paulo, Brazil) and incubated aerobically at 37°C for 24–48 h. Suspected *K. pneumoniae* colonies were confirmed using biochemical tests, following the protocol outlined by Quinn *et al*. [9]. Human isolates were identified as *K. pneumoniae* using the VITEK 2 automated system (bioMérieux, Marcy l'Étoile, France).

Antimicrobial susceptibility testing (AST)

AST was conducted using the Kirby–Bauer disk diffusion method [9], Inhibition zone diameters were interpreted using CLSI and Brazilian Committee on AST (BrCAST) standards [10, 11]. The American Type Culture Collection *K. pneumoniae* 13883 strain was used as a positive control to ensure assay accuracy and data reliability. Resistance profiles were assessed using 12 antibiotics spanning key antimicrobial classes: Penicillins (amoxicillin–clavulanate), cephalosporins (cephalexin), carbapenems (imipenem, meropenem), aminoglycosides (amikacin, gentamicin), quinolones (ciprofloxacin), phenicols (chloramphenicol), tetracyclines (doxycycline), nitrofurans (nitrofurantoin), and sulfonamides (with and without trimethoprim) (Bio-Rad Brazil, Rio de Janeiro, Brazil). Resistance classification followed CLSI and BrCAST standards [11–13].

Minimum inhibitory concentrations (MICs) for human isolates were determined using the BacT/ALERT 3D (bioMérieux) and VITEK 2 Compact systems (bioMérieux) as per the manufacturer's guidelines. The use of different AST methodologies reflected disparities in laboratory resources and infrastructure between the veterinary and human hospitals. All MIC interpretations strictly adhered to CLSI [12] criteria. Testing encompassed seven antimicrobial classes comprising 16 antibiotics (BD BBL Sensi-Disc, Becton Dickinson, USA), including penicillins (ampicillin with sulbactam and piperacillin with tazobactam), cephalosporins (cefepime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, and cefuroxime axetil), carbapenems (ertapenem, imipenem, and meropenem), aminoglycosides (amikacin and gentamicin), quinolones (ciprofloxacin), polymyxin (colistin), and glycylcycline (tigecycline) [12]. Isolates were classified based on the MDR criteria defined by Magiorakos *et al*. [14], where resistance to one or more agents in at least three antimicrobial categories constitutes MDR status.

DNA extraction and molecular identification

Genomic DNA was extracted by inoculating bacterial colonies into brain–heart infusion broth and then incubating them overnight at 37°C with agitation. After centrifugation, the resulting pellet was resuspended in 1 mL of lysis buffer and processed through the phenol-chloroform method described by Sambrook and Russell [15]. The extracted DNA was resuspended in 50 µL of ultrapure water and stored at −20°C for further analysis. DNA integrity and purity were verified through agarose gel electrophoresis. The extracted DNA underwent 16S *ribosomal RNA* gene amplification through polymerase chain reaction (PCR) using primers 27F (AGAGTTTGATCCTGGCTCAG) [16] and 1492R (GGTTACCTTGTTACGACT) [17]. The PCR products were purified using the illustra ExoProStar 1-STEP Kit (GE Healthcare Life Sciences, Cytiva, Marlborough, MA, USA) and then sequenced with the BigDye Terminator Ready Reaction Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3500 Genetic Analyzer (Applied Biosystems, a brand of Thermo Fisher Scientific headquartered in Waltham, Massachusetts, USA). Sequences were compared with GenBank (National Center for Biotechnology Information – NCBI, a division of the U.S. National Library of Medicine at the National Institutes of Health [NIH], headquartered in Bethesda, Maryland, USA) entries using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) (National Center for Biotechnology Information (EUA). BLAST: Basic Local Alignment Search Tool [software online]. Bethesda [MD]: U.S. National Library of Medicine), and when applicable, deposited. Accession numbers and isolate metadata are available in the supplementary table. Chimeric sequences were screened and removed using DECIPHER software (Biomatters Ltd. (New Zealand). DECIPHER: sequence analysis software [software online]. Auckland [NZ]: Biomatters) to ensure data quality.

Detection of virulence and resistance genes

PCR was used to detect seven virulence genes and the resistance genes bla_{KPC-2} and bla_{NDM}, using primer sequences listed in Table 1 [18–23]. Previously characterized positive control samples harboring the target resistance genes were used to validate the PCR assays. These controls were also sequenced to confirm the presence of primer-specific nucleotide regions. PCR was conducted on a MyCycler™ Thermal Cycler (Bio-Rad, Hercules, CA, USA) in 25 µL reactions containing 25 ng of DNA, 1 U Taq polymerase (Sigma, MilliporeSigma, St. Louis, MO, USA), 1 mM deoxynucleoside triphosphates, 15 mM $MgCl_2$, 1× PCR buffer (comprising 200 mM Tris-HCl, pH 8.4, and 500 mM KCl), and 20 pmol of each primer. The amplification protocol included

Table 1: *Primers* used to detect virulence genes and the bla_{KPC-2} resistance gene in *K. pneumoniae* isolates.

Gene	Function/location	Sequence (5'–3')	Product (pb)	Reference
fimH	Production of fimbrial adhesions	GCT CTG GCC GAT AC YAC S AC GG GC RWA R TAA CG YGCC TGG AAC GG	423	[18]
mrkD	Production of fimbrial adhesions	TATYGKCTT AAT GGC GCT GG TAA TCG TAC GTC AGG TTA AAG AYC	945	[18]
wabG	Lipopolysaccharide Biosynthesis	ACC ATC GGC CAT TTG ATA GA CGG ACT GGC AGA TCC ATA TC	683	[18]
ugE	Lipopolysaccharide Biosynthesis	TCT TCA CGC CTT CCT TCA CT GAT CAT CCG GTC TCC CTG TA	535	[19]
entB	Transport of siderophore	CGC CCA GCC GAA AGA GCA GA CAT CGG CAC CGA ATC CAG AC	508	[20]
KfuBC	Iron transportation	GAA GTG ACG CTG TTT CTG GC TTT CGT GTG GCC AGT GAC TC	797	[21]
bla_{KPC2}	Carbapenemase enzyme	TGTCACTGTATCGCCGTC CTCAGTGCTCTACAGAAAAACC	1011	[22]
NDM	New Delhi metallo-β-lactamase	CAGCAACCGCGCCCAACTTTGGCCCGCTCAAGG TTGATCAGGCAGCCACCAAAAGCGATGTCGG FAM-TTTTACCCCGGCCCCGGCCACACCAGTGACAA-BHQ1	121	[23]

an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 2 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, concluding with a final extension at 72°C for 5 min. Amplification of bla_{KPC-2} and bla_{NDM} genes followed similar cycling conditions with primer-specific annealing and extension settings. PCR products were visualized on 1.5% agarose gels stained with GelRed (Biotium, Fremont, CA, USA), and images were captured using the ChemiDoc XRS system and Image Lab software (Bio-Rad Laboratories, Hercules, CA, USA). Real-time PCR for bla_{NDM} detection was carried out on the QuantStudio 5 system (Thermo Fisher Scientific, USA) using TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific), with thermal cycling consisting of an initial denaturation at 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s, as described by Feng *et al*. [23].

MLST genotyping and sequence analysis

MLST was performed by amplifying seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*), as specified by the Pasteur Institute's MLST protocol. The resulting sequences were compared to global MLST databases to determine sequence types (STs), and the GoeBURST algorithm was used to group STs into clonal complexes (CCs). Novel alleles and STs were submitted to the Pasteur Institute MLST database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) [24].

Statistical and bioinformatics analysis

Bioinformatic analysis, including multiple sequence alignment and polymorphism evaluation, was conducted using Clustal Omega (European Molecular Biology Laboratory, United Kingdom). Population genetic metrics such as nucleotide diversity (π) were calculated to assess genetic variation among isolates. Descriptive statistical analysis was carried out using R: A language and environment for statistical computing (version 4.3.1) [software online] (Vienna, Austria). Data were organized into tables and visualized using graphs to facilitate interpretation. Although no inferential statistical tests were applied, a significance threshold of $p < 0.05$ was maintained. Data were further standardized and reanalyzed using the Pasteur Institute MLST database to ensure consistency. Isolates identified as members of the *K. pneumoniae* complex but not specifically as *K. pneumoniae* were excluded, maintaining data integrity for global comparison and epidemiological interpretation.

RESULTS

Origin of isolates

Of the 48 *K. pneumoniae* isolates analyzed, 69% (33/48) originated from domestic animals and 31% (15/48) from human sources (Table 2).

Anatomical distribution of samples

Samples were obtained from 15 different anatomical sites. The most frequent sources included urine (34%, 16/48), rectal swabs (15%, 7/48), ear swabs (11%, 5/48), and lung lavage fluid (11%, 5/48) (Table 3).

Antimicrobial resistance profiles

Disk diffusion testing revealed high antimicrobial resistance rates, consistent with CLSI and BrCAST standards [11–13]. Among animal isolates, nitrofurantoin and sulfonamides showed the highest resistance, each affecting 88% (29/33) of samples. The lowest resistance rates were noted for imipenem (0%, 0/33), meropenem (1%, 1/33), and amikacin (2%, 1/33) (Table 4). MDR phenotypes were identified in 78.78% (26/33) of animal isolates and 53% (8/15) of human isolates, yielding an overall MDR rate of 70.83% (34/48).

Virulence and resistance gene distribution

Virulence gene screening showed that 77.08% (37/48) of isolates harbored at least one virulence factor. In animal isolates, *entB* was the most prevalent (60.61%, 20/33), followed by *wabG* and *fimH* (both

Table 2: Distribution of *Klebsiella pneumoniae* isolates by host category—domestic animals versus humans—in Mato Grosso (Midwest Brazil), 2016–2017.

Host category	Host	Total
Domestic animals (n = 33)	Canine	15
	Equine	8
	Feline	5
	Bovines	4
	Pig	1
Humans (n = 15)	Human	15
Total		48

Table 3: Absolute frequency and percentage of isolates site of 48 *Klebsiella pneumoniae*, Mato Grosso.

Sites/samples	Absolute frequency	Percentage
Urine	16	34
Rectal swab	7	15
Ear swab	5	11
Tracheobronchial lavage	3	6
Gastric contents	2	4
Eye swab	2	4
Lung	2	4
Liver	2	4
Catheter point	2	4
Blood	2	4
Interdigital secretion swab	1	2
Milk	1	2
Chest fluid	1	2
Abdominal cavity fluid	1	2
Gastric ulcer secretion	1	2
Total	48	100

48.48%, 16/33). Among human isolates, the prevalence of virulence genes was as follows: *ugE* (60%, 9/15), *entB* (46.67%, 7/15), *fimH* (33.33%, 5/15), and *wabG* (33.33%, 5/15), regardless of sample site. Regarding resistance genes, bla_{KPC-2} was detected in 3 out of 15 (20%) human isolates but was absent in all animal-derived samples. The bla_{NDM} gene was found in one human rectal swab isolate (6.66%, 1/15) and one bovine ocular swab isolate (3.03%, 1/33) (Supplementary material).

MLST results

MLST analysis identified 39 distinct STs, including 9 novel ones (ST4527–ST4535). These STs were grouped into 32 CCs and six singletons (Table 5). Clonal complex CC258 was the most frequent in human isolates (27%, 4/15), while CC15 appeared in 9% (3/33) of animal isolates. CC147 was the only clonal complex found in both humans (ST392) and animals (ST4530, canine origin), indicating potential interspecies clonal overlap.

Genetic diversity analysis

The concatenated gene sequence analysis revealed no insertions, deletions, or tetra-allelic single-nucleotide polymorphisms. Pairwise sequence identity ranged from 95.62% to 100%. Among the 3,012 nucleotide positions assessed, 188 sites (6.24%) were polymorphic. The nucleotide diversity index (π) was calculated to be 0.01843 (Figure 1).

Table 4: Antimicrobial resistance profile of *Klebsiella pneumoniae* isolates using agar disk diffusion in animal isolates and the automated VITEK2 method (broth microdilution) in human isolates.

Antimicrobial categories	Resistance* (%)		
	Agent	Domestic animals (%) (n = 33)	Humans (%) (n = 15)
Penicillins	AMC	42 (14/33)	NR
β-lactams	PIT	NR	60 (9/15)
	AMP/SUB	NR	60 (9/15)
Cephalosporins	CFE	31 (12/33)	NR
β-lactams	CPM	NR	60 (9/15)
	CFO	NR	53 (08/15)
	CAZ	NR	60 (09/15)
	CRO	NR	60 (9/15)
	CRX	NR	60 (9/15)
	CRX axetil	NR	60 (9/15)
Carbapenems	MPM	1 (4/33)	53 (08/15)
β-lactams	IPM	0 (0/33)	53 (08/15)
	ERT	NR	53 (08/15)
Aminoglycosides	AMI	21 (7/33)	13 (02/15)
	GEN	48 (16/33)	47 (07/15)
Quinolones	CIP	48 (16/33)	47 (07/15)
Fenicol	CLO	45 (15/33)	NR
Polymyxin	COL	NR	33 (05/15)
Tetracyclines	DOX	45 (15/33)	NR
Nitrofurans	NIT	88 (29/33)	NR
Sulfonamides	SUL	88 (29/33)	NR
	SUT	42 (14/33)	NR
Glycylcyclines	TIG	NR	60 (9/15)

Legend: AMC=Amoxicillin+clavulanic acid, AMI=Amikacin, AMP/SUB=Ampicillin with sulbactam, CAZ=Ceftazidime, CFE=Cephalexin, CFO=Cefoxitin, CIP=Ciprofloxacin, CLO=Chloramphenicol, COL=Colistin (polymyxin E), CTF=Ceftiofur, CPM=Cefepime, CRO=Ceftriaxone, CRX=Cefuroxime, DOX=Doxycycline, ENO=Enrofloxacin, ERT=Ertapenem, GEN=Gentamicin, IPM=Imipenem, MBF=Marbofloxacin, MTZ=Metronidazole, MPM=Meropenem, NEO=Neomycin, NIT=Nitrofurantoin, PIT=Piperacillin/tazobactam, SUL=Sulfonamide, SUT=Sulfonamide+trimethoprim, TIG=Tigecycline, NR=Not realized.
*Samples classified as intermediately resistant were regrouped with those classified as resistant
Human isolates exhibited lower resistance to amikacin (13%, 02/15) and colistin (33%, 05/15), with 50% of the isolates classified as MDR. Overall, 86% (41/48) of the isolates were MDR

DISCUSSION

Overview of antimicrobial resistance and virulence in *K. pneumoniae*

This study presents evidence of high antimicrobial resistance and prevalence of virulence genes in *K. pneumoniae* isolates from both domestic animals and humans in the state of Mato Grosso, Brazil. The detection of MDR strains – particularly those harboring clinically relevant STs such as ST15, ST11, and ST437 – highlights the risk of zoonotic transmission and the convergence of resistance and virulence traits. Our findings underscore the need for integrated surveillance strategies within the One Health framework, particularly in regions where data are scarce.

Table 5: *Klebsiella pneumoniae* isolates with the species, isolation site, ST and CC of the 49 isolates.

Species	Isolation site	ST	CC
Canis lupus familiaris	Ear swab	35	35
C. lupus familiaris	Ear swab	4529	993
C. lupus familiaris	Ear swab	1393	1393
C. lupus familiaris	Ear swab	4546	*Singletons*
C. lupus familiaris	Urine	983	983
C. lupus familiaris	Urine	15	15
C. lupus familiaris	Urine	4530	147
C. lupus familiaris	Urine	15	15
C. lupus familiaris	Urine	873	873
C. lupus familiaris	Urine	1824	1059
C. lupus familiaris	Urine	307	307
C. lupus familiaris	Interdigital secretion swab	788	788
C. lupus familiaris	Eye swab	2474	2474
C. lupus familiaris	Chest fluid	889	889
C. lupus familiaris	Rectal swab	4527	*Singletons*
Felis catus	Urine	307	307
F. catus	Urine	1110	152
F. catus	Ear swab	20	20
F. catus	Abdominal cavity fluid	4531	101
F. catus	Rectal swab	567	567
Sus scrofa domesticus	Lung	188	188
Bos taurus	Milk	231	231
B. taurus	Rectal swab	289	289
B. taurus	Eye swab	3494	*Singletons*
B. taurus	Liver	266	1401
Equus caballus	Rectal swab	1089	*Singletons*
E. caballus	Rectal swab	1089	*Singletons*
E. caballus	Gastric contents	534	534
E. caballus	Gastric contents	534	534
E. caballus	Tracheobronchial lavage	3883	*Singletons*
E. caballus	Liver	2035	3849
E. caballus	Urine	15	15
E. caballus	Lung	534	534
Homo sapiens sapiens	Blood	11	258
H. sapiens sapiens	Blood	634	1373
H. sapiens sapiens	Rectal swab	392	147
H. sapiens sapiens	Rectal swab	392	147
H. sapiens sapiens	Urine	437	258
H. sapiens sapiens	Tracheobronchial lavage	11	258
H. sapiens sapiens	Urine	12	12
H. sapiens sapiens	Catheter tip	11	258
H. sapiens sapiens	Urine	4532	585
H. sapiens sapiens	Catheter tip	323	323
H. sapiens sapiens	Urine	4533	524
H. sapiens sapiens	Urine	4534	17
H. sapiens sapiens	Urine	70	70
H. sapiens sapiens	Sputum	60	60
H. sapiens sapiens	Urine	12	12
H. sapiens sapiens	Ulcer secretion	4535	70

CC=Clonal complexes, ST=Sequence type

MDR prevalence and infection types

The MDR *K. pneumoniae* primarily causes urinary, enteric, and respiratory infections, consistent with findings reported in previous studies by Diancourt *et al*. [24] and Cardoso Almeida *et al*. [25]. In this study, MDR *K. pneumoniae* was detected in 100% of animal isolates and 53% of human isolates. This highlights a major challenge for One Health initiatives – the transmission of MDR pathogens through direct human–animal contact and through contaminated environments [1, 26]. Animals are of particular concern as reservoirs and vectors for the dissemination of antimicrobial resistance genes. This issue is especially pertinent in veterinary medicine, as prior research by Ewers *et al*. [27] has underscored the significant role of *K. pneumoniae* in nosocomial infections.

Resistance to β-lactams and carbapenems

K. pneumoniae exhibited the highest resistance to β-lactam antibiotics [28, 29], with intrinsic resistance to ampicillin and penicillin confirmed in isolates from sheep and goats in Egypt. Carbapenem resistance remains relatively rare in veterinary medicine, largely due to the limited and judicious use of this antibiotic class, which is typically reserved for severe infections in companion animals. Nevertheless, reports from other countries indicate a rising trend in carbapenem resistance among animal-derived isolates [30–32]. Although bla_{KPC-2} has been identified in *K. quasipneumoniae* isolates in Brazil, no such findings have been reported in animal-derived *K. pneumoniae* isolates to date [30–32]. Human isolates often exhibit elevated resistance levels due to the expression of carbapenemase enzymes, particularly KPC [33, 34]. Additionally, a study from Egypt revealed high resistance to penicillins, cephalosporins, and sulfonamides among *K. pneumoniae* isolates from sheep and goats [35].

Virulence determinants and their functions

To establish infection, *K. pneumoniae* must circumvent mechanical and chemical barriers and evade innate immune responses [36]. The virulence of *K. pneumoniae* is multifactorial, involving various genetic and structural determinants. Fimbrial adhesins, such as *fimH* and *mrkD*, facilitate bacterial adherence to the urinary epithelium, thereby enhancing colonization [36]. Other virulence factors, such as *ugE* and *wabG*, aid in evasion of the complement system, whereas *entB* and *kfu* contribute to iron acquisition and bacterial proliferation [37–39].

Prevalence of virulence genes in human and animal isolates

The prevalence of virulence genes in this study aligns with findings by Kot *et al*. [37] and Kus *et al*. [40], who reported high detection rates of *entB, mrkD,* and *fimH* in human isolates. Numerous veterinary studies have characterized virulence genes in MDR *K. pneumoniae* isolates from animals [41–43]. Consistent with our findings, these investigations also reported frequent detection of *wabG, entB,* and *fimH* among animal isolates. In the current study, *entB* was the most prevalent virulence gene detected in both animal and human isolates. This observation is consistent with Davoudabadi *et al*. [44], who reported 100% prevalence

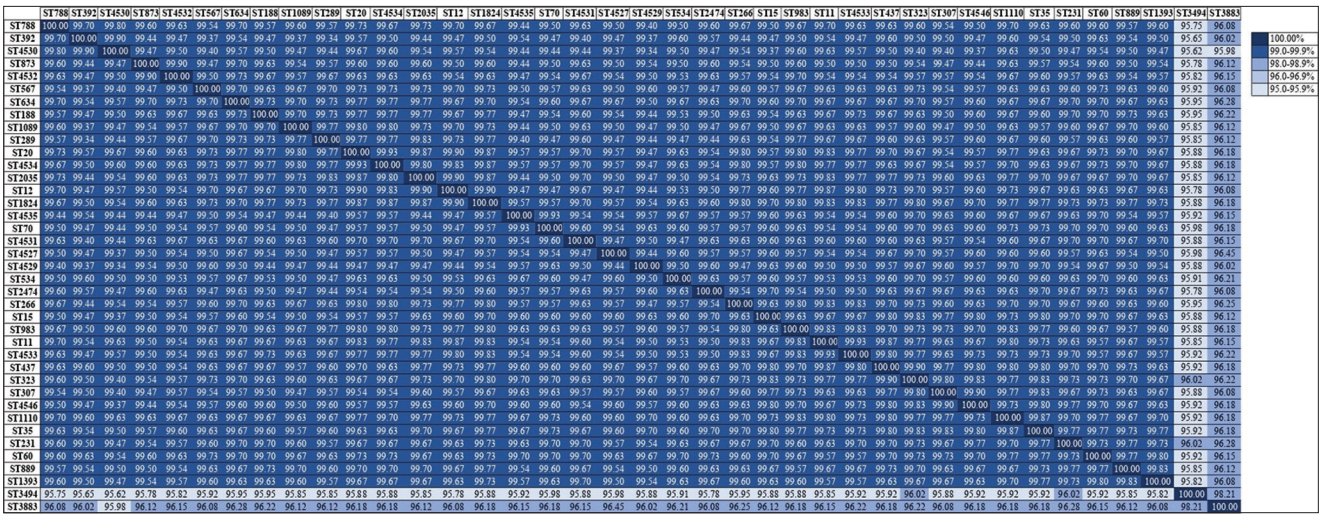

Figure 1: Percent identity and divergence score matrix of the seven concatenated gene nucleotide sequence type of *Klebsiella pneumoniae* from animals and humans.

of *entB* in 52 human *K. pneumoniae* isolates. The findings confirm the presence of multiple virulence factors, including *entB, ugE, fimH, wabG,* and *mrkD*, in fecal samples from diverse host species. This is in agreement with Amaretti *et al*. [45], who also reported high prevalence of *entB* and *mrkD* in *K. pneumoniae* isolates from human fecal samples. Contrary to Osman *et al*. [41], who detected high prevalence of *kfu* and *ugE* genes in buffalo milk samples with mastitis, this study found no virulence genes in bovine milk-derived isolates. Nonetheless, these findings should be interpreted cautiously due to the limited number of samples associated with mastitis.

Role of *fimH* in urinary infections

The *fimH* gene, encoding fimbrial adhesins, was identified in 43.75% (21/48) of total isolates and in 23.80% (5/21) of those from urine samples. Previous studies by Jiang *et al*. [46] and Sarshar *et al*. [47] have highlighted the critical role of *fimH* in urinary tract infections caused by *Enterobacteriaceae*.

Genetic diversity and ST distribution

Genetic analysis revealed substantial population diversity among *K. pneumoniae* isolates in this study. The isolates demonstrated broad ST variability, including several newly identified STs and singletons. Despite ST diversity, high nucleotide identity among isolates indicated potential clonal similarity between animal- and human-derived strains [26, 48–51]. Notably, ST15 and ST307 identified here are epidemiologically significant due to their established association with nosocomial infections. These STs have been described in Japan as public health concerns due to their widespread association with hospitals [49]. In Portugal, ST15 has been implicated in both community- and hospital-acquired infections, including those in intensive care settings [52]. ST15 has also been reported in domestic animals across multiple European countries – including Portugal, Germany, France, and others – where it is associated with diverse clinical manifestations [27]. In this study, ST15 isolated from canine urine was found to express multiple virulence genes, including *fimH*, *mrkD*, *entB*, *ugE*, and *kfu*. In Brazil, ST15 has also been identified in polluted urban waterways, with genomic similarity to ST15 strains from humans and animals, including shared virulence factors [53]. ST15 harbors genomic traits that may enhance its dissemination and is increasingly recognized as a biomarker for the surveillance of high-risk *K. pneumoniae* clones [54].

High-risk CCs and global spread

Additional human isolates included ST11 and ST437, both members of the high-risk clonal complex CC258. The same STs were observed in human isolates from Brazil [33]. A 4-year retrospective study in China found ST11 to be the predominant strain among patients with carbapenem-resistant *K. pneumoniae*, and it was associated with a high 30-day mortality rate [55]. The ST11 lineage poses a substantial global health threat due to its strong association with hospital outbreaks and extensive dissemination [55, 56]. Furthermore, ST11 has been reported in animal hosts across Germany, Italy, Switzerland, Taiwan, and Japan [28, 57–59]. ST35, isolated from a dog with otitis, has been previously identified in various sources, including pork meat, nosocomial infections, environmental infections, bovine milk samples, and wild animals [60–62]. Animal isolates raise great concern because they can colonize humans and contaminate the environment [30]. Hence, measures must be adopted to prevent the spread of high-risk clonal lineages within the population [31].

Zoonotic potential of CC147 and cross-species transmission

As observed in the human and canine isolates, the expression of CC147 was similar to that observed in northern Brazil. This clonal complex is commonly found in hospital outbreaks, having been reported in Greece and Italy [7, 63, 64]. This finding highlights the

possibility of clonal dispersal between different species, as reported by Marques *et al*. [31], who documented fecal colonization by *K. pneumoniae* in companion animals and healthy humans sharing similar clonal lineages [31].

Drivers of resistance and need for control measures

The increase in resistance to antibiotics is mainly due to their intensive and inappropriate use, combined with the inherent ability of bacteria to mutate, which favors the selection of resistant strains [2]. Consequently, antibiotics lose their effectiveness, leading to therapeutic failure, increased healthcare costs, and even death in extreme cases [5]. Healthcare professionals, producers, health authorities, and pharmaceutical companies must implement appropriate measures to reduce the transmission of resistance to mitigate this problem. This effort requires careful consideration of bacterial epidemiology, human–animal interactions, the appropriate use of antimicrobials in all species, adherence to general infection control principles, and the implementation of appropriate public health hygiene measures to reduce transmission. To control antimicrobial resistance, modern animal husbandry and slaughter practices should be adopted, and proper handling and preparation of food should be ensured [64, 65].

Study limitations and regional relevance

Although our study has limitations, such as the relatively small sample size (48 isolates) and the unequal distribution between domestic animals (69%) and humans (31%), the data obtained are unprecedented for the state of Mato Grosso. This contribution becomes even more relevant given the scarcity of research on the resistance and virulence profiles of *K. pneumoniae* in this regional context. While the predominance of samples from hospital and clinical environments may reflect more severe cases, the findings provide valuable insights and establish a crucial foundation for future, more comprehensive and targeted investigations, thereby strengthening control and prevention strategies within the One Health framework. In this context, it is important to highlight that the samples were collected between 2016 and 2017. Despite the temporal gap, the data remain epidemiologically relevant due to the absence of similar regional studies. Thus, this work fills a critical knowledge gap and offers a strategic baseline for future monitoring and intervention efforts in both human and animal health.

CONCLUSION

This study provides the first integrated molecular epidemiological assessment of *K. pneumoniae* isolates from domestic animals and humans in the state of Mato Grosso, Brazil, reinforcing the importance of One Health-based surveillance. Among the 48 isolates analyzed, a high overall prevalence of multidrug resistance was observed, at 78.78% in animal isolates and 53% in human isolates, resulting in a total MDR rate of 70.83%. Notably, the bla_{KPC-2} gene was exclusively detected in human isolates (20%), while bla_{NDM} was found in both human (6.66%) and bovine (3.03%) isolates, reflecting potential interspecies exchange of critical resistance determinants. Virulence gene screening revealed that 77.08% of isolates harbored at least one virulence factor, with *entB*, *fimH*, and *wabG* being the most frequent, suggesting a convergence of resistance and pathogenicity traits in both hosts.

MLST analysis unveiled substantial genetic diversity, with 39 distinct STs, including nine novel STs, and the presence of CCs such as CC258 (human), CC15 (animal), and the interspecies-shared CC147. These findings underscore the presence of high-risk clones capable of crossing species barriers, highlighting the zoonotic threat posed by companion and production animals in close contact with humans. The results call for immediate implementation of integrated antimicrobial stewardship and biosecurity policies across veterinary and human healthcare sectors. Diagnostic laboratories should prioritize the use of molecular tools, such as MLST and resistance gene screening, for rapid detection of high-risk *K. pneumoniae* strains. Furthermore, veterinary practitioners should be cautious with empirical antimicrobial usage, especially with β-lactams and sulfonamides, given their high resistance rates in animal isolates.

A major strength of this study is the dual-host, dual-environment design that bridges human and veterinary microbiology under the One Health paradigm. The use of comprehensive molecular typing and resistance profiling tools provides robust genotypic evidence to support surveillance and control strategies. The identification of novel STs contributes new data to global MLST databases, enhancing the scientific community's capacity to trace transmission pathways. Given the limited regional data and small sample size, future studies should expand longitudinal sampling across diverse geographic areas and ecological settings. Whole-genome sequencing and plasmid analysis will further clarify horizontal gene transfer events and reveal additional mechanisms of resistance. Environmental sampling from shared human-animal ecosystems (e.g., water sources, animal shelters, and farms) would also enrich our understanding of reservoirs and transmission dynamics. In summary, the study highlights the critical need for harmonized antimicrobial resistance monitoring across the animal–human interface in Brazil. The identification of shared virulence traits and MDR phenotypes in genetically related *K. pneumoniae* clones emphasizes the importance of coordinated surveillance, timely diagnostics, and intersectoral collaboration to mitigate the public health threat posed by high-risk clones. These findings contribute a foundational dataset for regional and national policymakers aiming to strengthen One Health-informed AMR control efforts.

AUTHORS' CONTRIBUTIONS

ATHIS, LN, and VD: Conceptualized and designed the study and drafted the manuscript. HM, MTSC, SLC, KLTG, CSC, MAP, FKSFA, FJDS, LN, and VD: Conducted research, collected samples, and performed laboratory work. ATHIS, HM, MTSC, SLC, KLTG, CSC, MAP, FKSFA, FJDS, LN, and VD: Analyzed and interpreted the data. ABPFA, VRFS, LN, and VD: Supervised the project and revised the manuscript. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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