One Health 19 (2024) 100904

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Exploring AMR and virulence in *Klebsiella pneumoniae* isolated from humans and pet animals: A complement of phenotype by WGS-derived profiles in a One Health study in Egypt

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ARTICLE INFO

Keywords:
K. pneumoniae
One Health
WGS
Resistomes
Virulome
Human/Pet animals interface

ABSTRACT

Klebsiella pneumoniae is a ubiquitous nosocomial pathogen associated with various types of infections in hospitalized patients and different animal species. In the current study, 49 *Klebsiella* strains isolated from humans, dogs, and cats were investigated using NGS technology. MALDI-TOF failed to identify newly discovered *K. variicola* and *K. quasipneumoniae* isolates correctly. MLST analysis revealed different sequence types among *K. pneumoniae* isolates, and the most frequent STs were ST29, ST219, and ST37. Three ST23 that are generally known as hypervirulent type were identified but they lacked major discriminatory determinants for hypervirulent *K. pneumoniae* (hvKp). *K. pneumoniae* isolates showed high diversity, and several isolates from humans and animals were assigned to the same ST and were almost identical. Isolates from humans exhibited more pronounced resistance patterns compared to the animal isolates. High levels of resistance were observed for piperacillin, trimethoprim/sulfamethoxazole, and cephalosporins, and resistance to carbapenem compounds was only found in isolates of human origin. Three strains of human origin were extensively drug-resistant (XDR). A diverse range of resistance genes primarily confer resistance to beta-lactams., phenicol/quinolone, aminoglycoside, macrolide, sulfonamides, and fosfomycin were identified in silico. However, there were inconsistencies between the phenotypic characterization of isolates and the set of resistance genes detected in silico in this set of *Klebsiella* isolates. Further research using a larger number of isolates from various sources is necessary to fully comprehend the relationship between the presence of antimicrobial resistance determinants and phenotypic data. It is also necessary to monitor the spread of *K. pneumoniae* from a One Health perspective in Egypt.

1. Introduction

The genus *Klebsiella* (*K.*) is part of the *Enterobacteriaceae* family. Currently, the genus *Klebsiella* comprises 27 species and eight subspecies, according to the List of Prokaryotic Names with Standing in Nomenclature (LPSN, accessed 28 September 2024) [1]. Among the members of the genus *Klebsiella, K. pneumoniae* is responsible for the majority of infection cases in humans. *K. pneumoniae* is a Gram-negative, usually capsular, facultatively anaerobic, non-motile bacterium and includes three subspecies (subspecies pneumoniae, ozaenae, and rhinoscleromatis) [1]. It is a common cause of antimicrobial-resistant (AMR) infections in humans and is one of the ESKAPE pathogens, threatening human and animal health globally [2]. It is ubiquitously found in humans and is usually associated with urinary tract infections, ventilator-associated pneumonia, bacteremia, and liver abscesses in hospitalized patients [3–5]. It has also been isolated from various livestock, wildlife species, and different environmental sources such as soil and water [6]. In animals, it is an important cause of epidemic metritis and cervicitis in mares, pneumonia and septicaemia in foals [7], and has been frequently associated with mastitis in bovines [8], leading to high

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https://doi.org/10.1016/j.onehlt.2024.100904
Received 9 July 2024; Received in revised form 25 September 2024; Accepted 25 September 2024
Available online 27 September 2024

losses in the dairy industry, due to decrease of milk quality and high mortalities among affected cows [9,10]. Despite good management practices (environmental hygiene and use of inorganic bedding materials), *Klebsiella* mastitis can also occur on well-managed dairy farms [11]. Companion animals, such as dogs and cats, have been found to act as reservoirs for multidrug-resistant (MDR) *K. pneumoniae*, which is associated with severe pathogenicity [12]. This resistance could potentially be transmitted to humans as the interaction between humans and their pets could plays a role in the spread and dissemination of resistance [13].

In Egyptian health care system, *K. pneumoniae* is a notorious pathogen. The incidence of MDR as well as the extensively drug-resistant (XDR) isolates has been increasing in intensive care units (ICUs) [14,15]. It is worth mentioning that strains resistant to colistin that are harboring the *mcr-1* gene [16,17], and carbapenem resistant isolates harboring several resistance genes mediating resistance to β-lactams and carbapenems [18] have been isolated from clinical samples in several hospitals. Additionally, XDR hypervirulent *K. pneumoniae* (XDR-hvKP) strains with hypermucoviscous phenotypic characteristics have been isolated from patients with ventilator-associated pneumonia in different hospitals located at a great distance from each other [15,19], highlighting the dominance and spread of this dangerous clonal lineage in Egypt. As in human healthcare, *K. pneumoniae* has also been found in several animal hosts, environmental samples, and foods, highlighting its role as a One Health pathogen in Egypt. Carbapenemase-producing strains have been isolated from broilers, drinking water, and workers at poultry farms at relatively high frequency [20]. Carbapenemase- and ESBL-producing strains have also been isolated from tomatoes, irrigation water, and fecal samples of farmworkers, revealing the transmission of *K. pneumoniae* via fresh food to consumers [21]. Several MDR and carbapenem-resistant isolates from cattle, sheep, goats, and humans share similar genetic characteristics, raising concerns about transmission of resistance between animal and human pathogens [22]. Most studies carried out, either on human or animal samples, used classical antibiotics sensitivity testing and PCR as molecular tools for the characterization of resistance genes. However, implementation of Next Generation Sequencing (NGS) technology and emerging antibiotic sensitivity testing (AST) tools to investigate resistance profiles or virulence in *K. pneumoniae* are rare in Egypt.

Therefore, the current study aimed to determine the susceptibility of *K. pneumoniae* isolated from humans, and pet animals (dogs, and cats) in Egypt to various antibiotics *in vitro* and whole genome sequencing (WGS)-based genotypic characterization of resistance and virulence associated genes as well as strain genome similarity.

2. Materials and methods

2.1. Ethical approval

The ethical committee at the Faculty of Veterinary Medicine, Benha University, has approved this study with reference number (BUVFTM 36–10-22). Nagoya approval to transfer the biological material was obtained from the Egyptian Environmental and Affair Agency under reference Nr. 00306023010800/6 obtained. *K. pneumoniae* isolates were sent to the Institute of Bacterial Infections and Zoonoses (IBIZ, Jena, Germany) of Friedrich-Loeffler-Institut for confirmation, typing, and WGS analysis.

2.2. Bacterial isolates and identification

In the current study, 49 *Klebsiella* isolates recovered from humans ($n = 29$), dogs ($n = 16$), and cats ($n = 4$) were characterised. Isolates were recovered from clinical samples collected from respiratory and urinary tract infections in the Al Qalyubia and Giza Governorates in 2021 (Table 1, Supplementary Table S1). Isolates were obtained from clinical samples of unrelated humans and pets (dogs and cats). The majority of human isolates were obtained from Benha University Hospital in Al Qalyubia governorate, while most dog and cat isolates were obtained from pet clinics in Imbaba city of Gize governorate. It's important to note that there is no close or direct contact between human and animal patients. All isolates were initially identified by classical bacteriology based on colony morphology and Gram stain, as well as by biochemical tests, i.e., oxidase, indole, citrate utilization test, and urea hydrolysis as previously described [23]. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) was used to confirm the identity of the isolates as previously described [24]. The MALDI-TOF measurements were carried out using a Microflex LT instrument (Bruker Daltonics, Bremen, Germany), following the MALDI Biotyper manufacturer's recommendation on the log score value of 0–3 for species identification. Score values between 2.300 and 3.000 were considered 'highly probable for species identification', and values between 2.000 and 2.290 were considered 'secure genus identification'. The whole-genome sequencing (WGS) data confirmed the identity of the genus and species of each isolate using Kraken2 (v2.0.7_beta) [25]. The largest percentage proportion (first match) was considered for the genus and species identification.

Table 1
Numbers, host, and source of 49 *Klebsiella* isolates investigated in the current study.

Host	Type of sample	Number (%)
Human (n = 29) 59.2 %	Urine	11 (22.4 %)
	Nasal swab	2 (4.1 %)
	Sputum	12 (24.5 %)
	Blood	4 (8.2 %)
Dog (n = 16) 32.6 %	Nasal swab	14 (28.6 %)
	Urine	1 (2 %)
	Pus	1 (2 %)
Cat (n = 4) 8.2 %	Nasal swab	4 (8.2 %)

2.3. Antibiotic susceptibility testing (AST)

The minimum inhibitory concentration (MIC) was determined *in vitro* using the automated and validated MICRONAUT-S system (Micronaut, MERLIN Diagnostics GmbH, Bornheim-Hersel, Germany) as previously described [26]. MICRONAUT-S MDR MRGN-Screening MIC plates (catalog number E1–218-040) containing a panel of 16 antibiotics

Table 2
Antibiotic classes and compounds present in the MICRONAUT-S MDR MRGN-Screening MIC plates used in the current study and their concentration ranges.

Antimicrobial group	Antibiotics	Concentration, μg/mL
Aminoglycosides	Amikacin (AMK)	4–32
Cephalosporins	Ceftazidime (CAZ)	1–128
	Cefotaxime (CTX)	1/4–8/4
	Ceftazidim/Avibactam (CAA)	1/4–16/4
	Ceftolozan/Tazobactam (CTA)	1/4–8/4
Amphenicols	Chloramphenicol (CMP)	8–18
Fluoroquinolones	Ciprofloxacin (CIP)	0,25–2
	Levofloxacin (LEV)	0,5–2
Polymyxins	Colistin (COL)	1–8
Phosphonics	Fosfomycin (FOS)	32–128
Carbapenems	Imipenem (IMP)	1–8
	Meropenem (MER)	0,125–128
Penicillin	Piperacillin (PIP)	8–16
	Piperacillin/Tazobactam (PIT)	4/4–64/4
Glycylcyclines	Tigecycline (TGC)	0,25–4
Dihydrofolate reductase inhibitor/Sulfonamide antibiotic	Trimethoprim/ Sulfamethoxazole (T/S)	1/19–4/76

belonging to ten antimicrobial groups (Table 2) were used. The results were analyzed and MIC values were determined according to the recommendations of the clinical laboratory standard institute (CLSI, 2021) guidelines for *K. pneumoniae*. The strains were automatically classified using the built-in MICRONAUT software, as susceptible, intermediate, and resistant. Three standard reference strains were used as controls: *K. pneumoniae* ATCC BAA-2452, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853.

2.4. Whole genome sequencing (WGS) analysis of the isolates

The genomic DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) following to the manufacturer's instructions. To prepare sequencing libraries, Nextera XT DNA Library Prep Kit (Illumina, Inc., San Diego, CA, USA) was used, and the paired-end sequencing on an Illumina MiSeq sequencer (Illumina, USA) was followed. The raw sequencing data was analyzed using the Linux-based pipeline WGSBAC built at FLI (v 2.0, https://gitlab.com/FLI_Bioinfo/WGSBAC) as previously described [27,28], which performs the quality control of the raw sequencing data utilizing FastQC (v. 0.11.7) [29]. The pipeline utilizes Shovill v. 1.0.4 for assembly, which is based on the SPAdes assembler [30]. ABRIcate (v 0.8.10) (https://github.com/tseemann/abricate), together with the Virulence Factor Database, is utilized by WGSBAC for predicting virulence factors, and ABRIcate, the Comprehensive Antibiotic Resistance Database (CARD) [31], and ResFinder [32] databases for determining genetic features leading to AMR. Point mutations leading to AMR were identified using, NCBI's AMRFinderPlus tool [33] with *Klebsiella*-specific parameters. Present plasmid replicons were identified using ABRIcate against the PlasmidFinder database [34]. WGSBAC performed *in-silico* multilocus sequence typing (MLST) based on the assembled genomes using the software mlst v. 2.16.1 which incorporates the species-specific scheme (*K. pneumoniae*) available on PubMLST [35] as published by Diancourt et al. 2005 [36].

Single nucleotide polymorphism (SNP) typing in the core genome region was conducted using Snippy (https://github.com/tseemann/snippy) using genomes of *K. variicola* At-22 (GCF_000025465.1), *K. pneumoniae* HS11286 (GCF_000240185.1), and *K. quasipneumoniae* 01A030T (GCF_020525925.1) as reference for the respective species. The cgSNP alignment was analyzed by Maximum likelihood analysis using RAxML v8.2.12 [37] and the resulting tree visualized by Microreact [38]. Additionally, core genome MLST (cgMLST) was carried out using SeqSphere+ v8.2.0 [39] with the scheme *K. pneumoniae* sensu lato cgMLST v1.0 available at https://www.cgmlst.org/ncs/schema/Kpneumoniae1188/ (accessed on 15.05.2023).

3. Results

3.1. Klebsiella isolate identification and WGS analysis based on MLST and cgSNP

Using MALDI-TOF MS, 20 (40.81 %) isolates (13 from humans, six from dogs, and one from a cat) exhibited score values ranging from 2.3 to 2.46 and were considered secure *K. pneumoniae*, while 29 (59.18 %) isolates demonstrated score values falling within the range of 1.860 to 2.280, affirming their affiliation to the genus *Klebsiella* spp. Using WGS data, 35 isolates (27 from humans and eight from dogs) were confirmed as *K. pneumoniae*, nine isolates (seven from dogs, one from a cat, and one from a human) were identified as *K. quasipneumoniae,* four isolates (three from cats and one from a dog) were found to be *K. variicola* and one isolate from a human was identified as *K. oxytoca* (Supplementary Table S.1). Of note, among isolates that had been identified as *K. pneumoniae* using MALDI-TOF with a score value more than 2.3, two were identified as *K. variicola* and one as *K. quasipneumoniae* using WGS.

The MLST analysis revealed diverse sequence types (ST) among the 49 *Klebsiella* spp. In total, 40 isolates were assigned to the following 18 different STs: ST16, ST20, ST23, ST29, ST34, ST37, ST45, ST101, ST111, ST151, ST189, ST219, ST353, ST437, ST985, ST1308, ST1994, and ST2502. The most frequent STs among *K. pneumoniae* were ST29 and ST219 (six isolates for each), followed by ST37 (four isolates) and ST23 (three isolates). All *K. variicola* isolates ($n = 4$) were assigned to ST1994, and the *K. oxytoca* isolate was assigned to ST151. Initially nine isolates (one *K. pneumoniae* and eight *K. quasipneumoniae*) could not be assigned to a distinct sequence type (Fig. 1).

The core genome SNP analysis of the *Klebsiella* isolates revealed that eight of the nine *K. quasipneumoniae* (six from dogs, one from a human, and one from a cat) that had not been assigned to any ST, were virtually identical (0–6 SNPs difference), while one isolate recovered from a dog was assigned to ST1308 showing over 134,000 SNPs difference to this cluster (Fig. 2A). Three of the four *K. variicola* were identical, and one isolate from a cat differed from these in 766 SNPs (Fig. 2B). *K. pneumoniae* isolates showed high diversity. Two identical isolates recovered from humans and belonging to ST437 were clustered with the reference strain HS11286, to which they exhibited 5900 different SNPs. Several isolates from humans and animals assigned to the same ST were almost identical. For example, a ST29 isolate from a dog was identical to four ST29 isolates from humans with only 2–7 SNPs variation. Additionally, a ST16 isolate from a nasal swab of a dog displayed only 4 SNP differences to a ST16 isolate obtained from a human urine sample (Fig. 3). It is noteworthy that all similar isolates were isolated from humans and dogs living in the same city despite the absence of direct or close contact between human and dog patients.

3.2. Phenotypic and genotypic characterization of resistance in Klebsiella isolates

The *Klebsiella* isolates in the current study were tested against 16 antibiotics. In general, isolates recovered from humans showed more pronounced resistance patterns than isolates obtained from pet animals, i.e., dogs and cats. More than one-third of the isolates were MDR, displaying resistance to antibiotics from at least three antimicrobial groups. High resistance was seen for piperacillin in 32 isolates and for trimethoprim/sulfamethoxazole in 30 (61 %) isolates, followed by cephalosporines (cefotaxime and ceftazidime) in 26 (53 %) isolates. A high level of non-susceptibility (resistance and susceptibility in increased dose) was also found for fluoroquinolones, i.e., ciprofloxacin and levofloxacin, in 30 (61 %) and 17 (34.6 %) isolates, respectively. On the other hand, a high level of susceptibility (98 %) was seen for tigecycline and fosfomycin, followed by chloramphenicol (88 %), and carbapenems, i.e., meropenem (90 %) and imipenem (88 %). As expected, resistance to carbapenem compounds was found only in *K. pneumoniae* of human origin. Five human isolates recovered from urine ($n = 4$), and sputum ($n = 1$) displayed resistance to imipenem and meropenem. All other *Klebsiella* spp. (non-kp i.e. *K. quasipneumoniae, K. variicola* and *K. oxytoca*) isolates and isolates from pet animals either kp or non-kp showed susceptibility to carbapenems. Most of the *K. pneumoniae* isolates in the current study showed MDR patterns, while the non—*K. pneumoniae* isolates were susceptible to almost all tested antibiotics. For instance, all *K. quasipneumoniae* isolates recovered from dogs and cats ($n = 8$), the *K. oxytoca* isolates, and three out of four *K. variicola* isolates were susceptible to almost all tested antibiotics except colistin, while only one human *K. quasipneumoniae* isolate and a *K. variicola* isolate recovered from a cat displayed resistance to antibiotics from at least three antibiotic classes (Table 3).

Three strains from humans recovered from blood and urine samples were extensively drug-resistant (XDR) and displayed resistance to aminoglycosides (amikacin), cephalosporins (ceftazidime/avibactam, cefotaxime, ceftazidime, and ceftolozan/tazobactam), fluoroquinolones (ciprofloxacin and levofloxacin), carbapenem compounds (imipenem and meropenem), penicillins (piperacillin and piperacillin/tazobactam), dihydrofolate reductase inhibitor/sulfonamide antibiotic (trimethoprim/sulfamethoxazole), and were susceptible in increased dose to

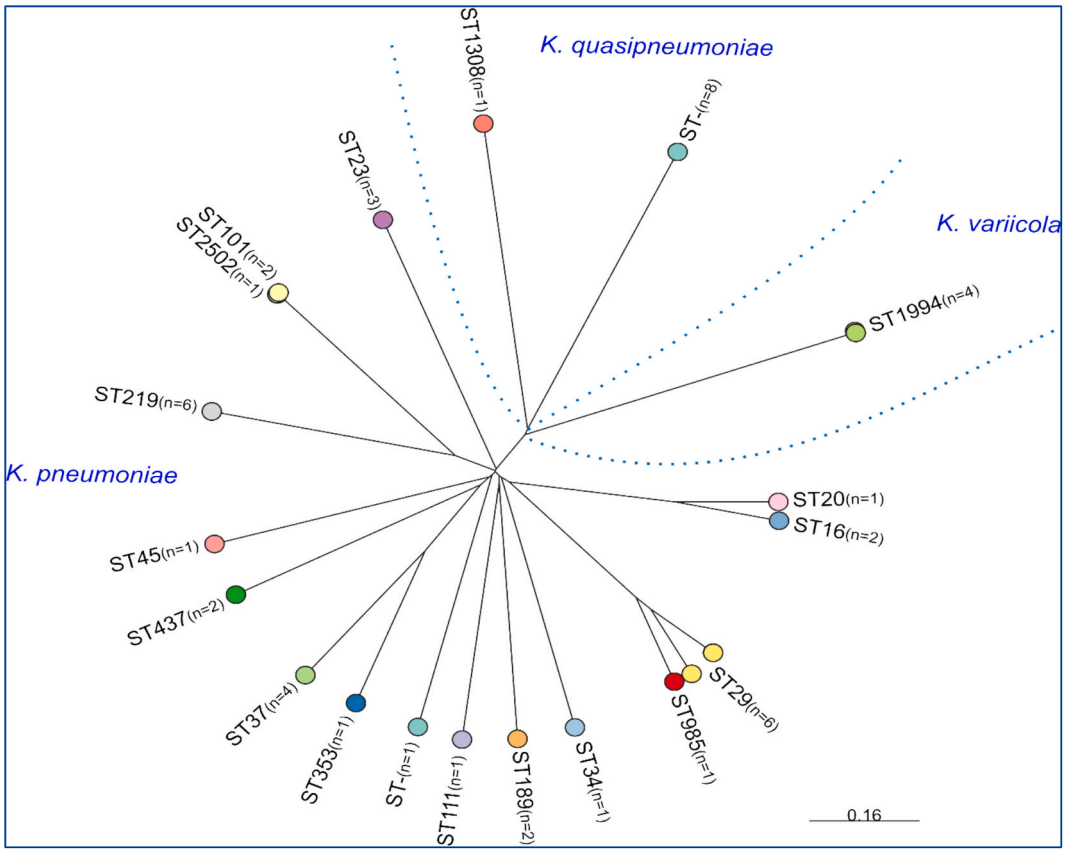

Fig. 1. Neighbor-joining tree based on cgMLST allelic distances; color and label indicate MLST sequence types with the number of corresponding isolates in brackets.

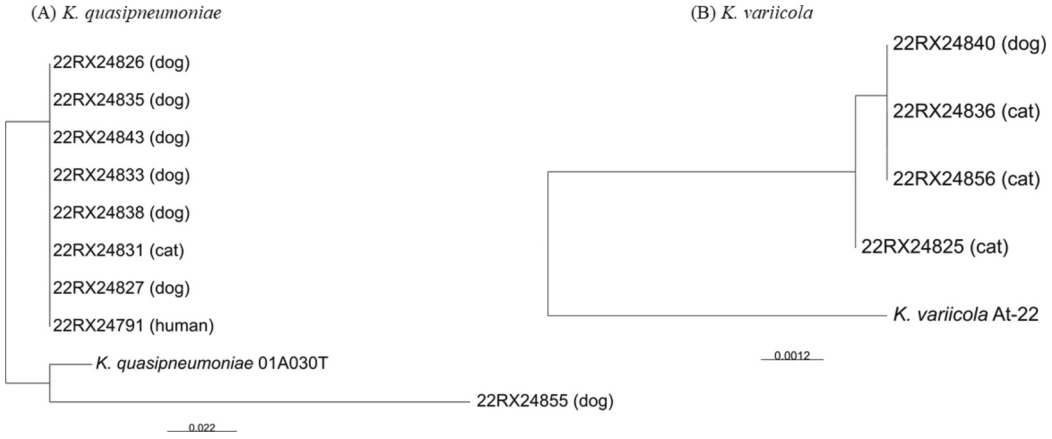

Fig. 2. Maximum likelihood tree based on cgSNPs of *K. quasipneumoniae* (A) and *K. variicola* (B) isolates. The scale bar indicates nucleotide changes per site.

chloramphenicol and colistin, while being susceptible only to phosphonic acid derivatives (fosfomycin) and glycylcyclines (tigecycline).

A wide variety of resistance genes mediating resistance mainly to beta-lactams ($n = 33$), phenicol/quinolone ($n = 19$), aminoglycoside ($n = 14$), macrolide ($n = 4$), tetracycline (*tet*A and *tet*D), sulfonamides (*sul*1 and *sul*2), and fosfomycin (*fos*A variants), were identified in silico in the current set of *Klebsiella* isolates (Supplementary Table S1). Twenty different efflux pump genes, *oqx*A and *oqx*B variants, mediating resistance to phenicol/quinolone and *fos*A variants conferring resistance to fosfomycin were identified in 100 % of isolates. However, non-susceptibility to chloramphenicol was seen only in six isolates mainly obtained from humans, and all isolates were susceptible to fosfomycin except one *K. pneumoniae* isolate recovered from a nasal swab of a dog. Likewise, all isolates showed non-susceptibility to colistin despite the absence of genes mediating resistance to colistin. The *tet*A and *tet*D genes conferring resistance to tetracyclines were present in 16 isolates, although only one of these, a *K. pneumoniae* isolate from human origin, displayed resistance to tigecycline in the sensitivity testing. Thus, an inconsistency between the phenotypic characterization of isolates and the set of in silico detected resistance genes was apparent in this set of *Klebsiella* isolates (Supplementary Table S1).

A total of 170 distinct AMR genes were identified. The distribution of these genes among the isolates ranged from 29 to 98 resistance genes per isolate. It was clear that *K. pneumoniae* isolates harboured a higher number of AMR genes than other *Klebsiella* spp. For instance, the highest number of AMR determinants ($n = 98$), was seen in a *K. pneumoniae*

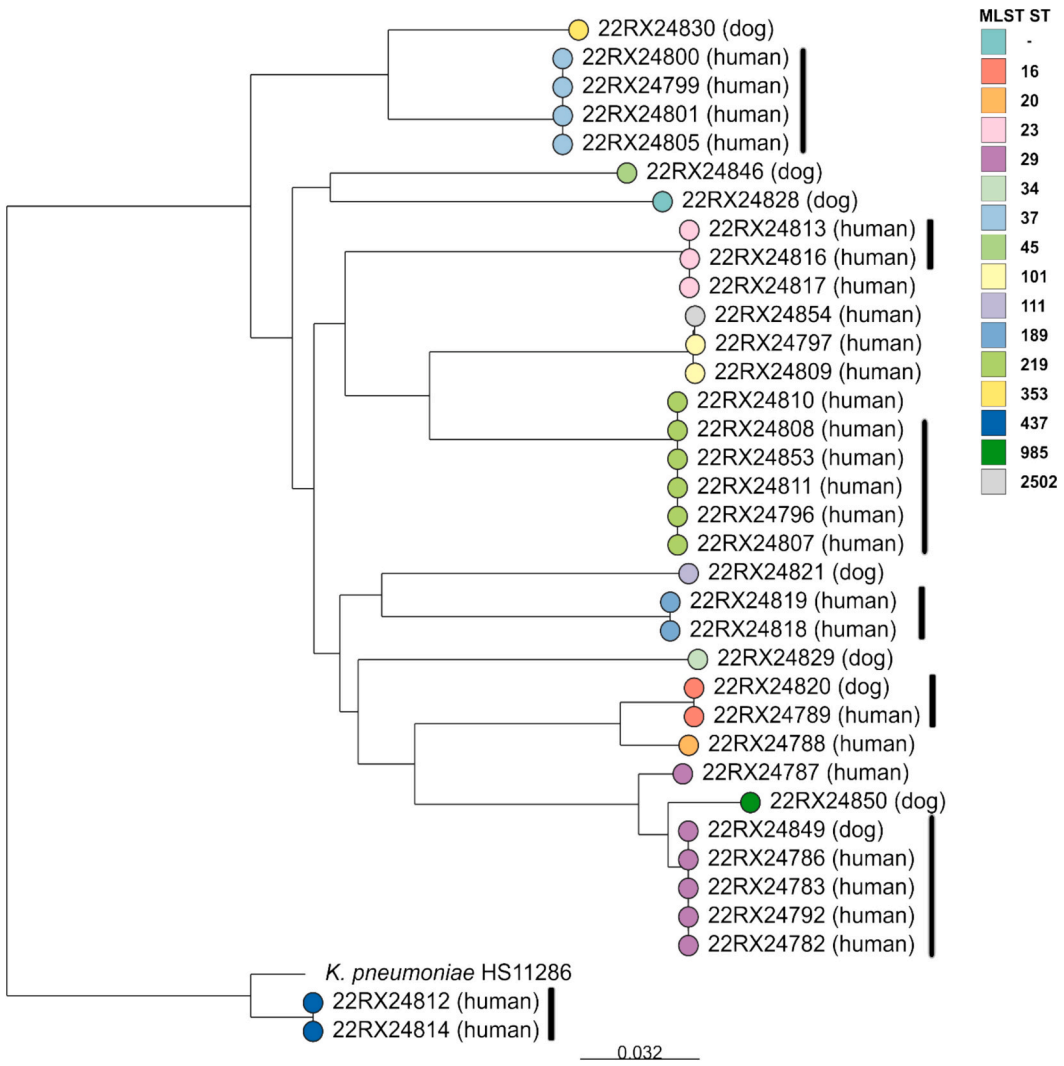

Fig. 3. Maximum likelihood tree based on cgSNPs of *K. pneumoniae*; black bars: SNP distance between strains max. 10 SNPs. The scale bar indicates nucleotide changes per site.

isolate of ST2502 isolated from a blood sample of human origin, followed by two isolates belonged to ST101, harboured 75 AMR genes isolated from urine and sputum samples of humans, and six isolates belonged to ST219 isolated from blood and sputum of human and harboured 64 AMR genes. The lowest number of AMR genes ($n = 29$) was found in *K. quasipneumoniae* isolates.

3.3. Characterization of plasmid replicons and virulence-associated genes in Klebsiella isolates

The potential plasmid replicons were identified in the assemblies using PlasmidFinder. All *K. variicola* isolates harboured one replicon (IncFIB.pENTAS01.1_pENTAS01) and the same ten virulence-associated genes. There were no replicons detected in eight of the nine *K. quasipneumoniae* isolates, but all isolates harboured the same nine virulence-associated genes. Only a strain from a dog harboured three replicons and ten virulence-associated genes. The *K. oxytoca* strain carried four replicons and 16 virulence-associated genes. This analysis also revealed that the plasmid replicons in the *35 K. pneumoniae* isolates ranged from 1 to 9, and the strains harboured a wide variety of virulence-associated genes ranging from 10 to 29 genes (Supplementary Table S1). The highest number of replicons ($n = 9$) and virulence genes ($n = 25$) was found in an isolate belonging to ST2502 recovered from a blood sample of a human. This strain was categorized as XDR and was susceptible only to fosfomycin and tigecycline and harboured the highest number of AMR genes ($n = 98$). Another two strains belonging to ST437 showed the same resistance pattern (XDR) and harboured eight replicons and 21 virulence genes. The highest number of virulence genes ($n = 29$) was found in three strains belonging to ST23, which were identified as hypervirulent strains. The three strains harboured a moderate number of AMR genes ($n = 56$) while being among the strains with the lowest number of plasmid replicons (2–3 replicons). It is noticed that four genes, i.e., *iro*B, *iro*C, *iro*D, and *iro*N, were found only in ST23 isolates, and another four genes, *iuc*A, *iuc*B, *iuc*C, and *iut*A, were also found in ST23 isolates and XDR ST2502. On the other hand, ten virulence genes were detected in all *K. pneumoniae,* i.e., *entA, entB, fepC, ompA, and yagV/ecp*E, *yagW/ecp*D, *yagX/ecp*C, *yagY/ecp*B, *yagZ/ecp*A and *ykgK/ecp*R (Supplementary Table S1).

4. Discussion

The genus *Klebsiella* possesses 27 species and eight subspecies. *K. pneumoniae* is one of the major MDR bacteria causing nosocomial infections and threatening the health care system worldwide. It is associated with a high mortality rate among patients and an extraordinary spread in the environment, and therefore justly classified as one of the most common and serious MDR pathogens [40,41]. In the current study, the resistance profiles and genomes of 49 *Klebsiella* isolates

Table 3
AST results of 49 *K. pneumoniae* isolates recovered from humans and pet animals.

Host	Klebsiella spp.	PIP	T/S	CTX	CAZ	CIP	LEV	CTA	CAA	AMK	PIT	IMP	MER	CMP	COL	TGC	FOS
Human	*K. Pneumoniae*	R	R	S	S	R	I	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	S	S	R	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	S	S	R	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	S	S	S	S	S	S	S	I	S	S
Dog	*K. Pneumoniae*	R	R	S	S	I	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	S	R	R	R	I	I	S	S	I	R	S
Human	*K. Pneumoniae*	R	R	R	R	R	I	R	R	R	I	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	I	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	I	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	I	I	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	I	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	I	S	S	S	S	S	S	S	I	S	S
Cat	*K. variicola*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Cat	*K. variicola*	R	R	S	S	R	R	S	S	S	S	S	S	R	I	S	S
Dog	*K. variicola*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Cat	*K. variicola*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	I	S	R	R	R	I	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	S	S	R	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	S	S	S	S	S	S	S	I	S	S
Dog	*K. Pneumoniae*	R	R	R	R	R	R	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	S	R	R	R	R	R	R	R	R	R	R	I	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	R	R	S	R	R	R	R	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	R	R	R	R	R	R	I	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	R	R	R	R	R	R	I	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	I	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	I	S	S	S	S	S	S	S	S	I	S	S
Human	*K. Pneumoniae*	R	R	R	R	R	R	R	R	R	R	R	R	I	I	S	S
Human	*K. oxytoca*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. Pneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. Pneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	I
Dog	*K. Pneumoniae*	R	R	R	R	R	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. Pneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. Pneumoniae*	R	R	R	R	R	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. quasipneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
Dog	*K. Pneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Human	*K. quasipneumoniae*	R	R	R	R	R	R	S	S	S	S	S	S	S	I	S	S
Dog	*K. quasipneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. quasipneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Cat	*K. quasipneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. quasipneumoniae*	R	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. quasipneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. quasipneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
Dog	*K. quasipneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S

S - Susceptible	17	19	23	23	19	32	40	41	42	41	43	44	43	0	48	48
I - Susceptible, increase	0	0	0	0	7	5	0	0	0	3	1	0	4	48	0	1
R - Resistant	32	30	26	26	23	12	9	8	7	5	5	5	2	1	1	0

Amikacin (AMK), Cefotaxime (CTX), Ceftazidime (CAZ), Ceftazidime/Avibactam (CAA), Ceftolozan/Tazobactam (CTA), Chloramphenicol (CMP), Ciprofloxacin (CIP), Levofloxacin (LEV), Colistin (COL), Fosfomycin (FOS), Imipenem (IMP), Meropenem (MER), Piperacillin (PIP), Piperacillin/Tazobactam (PIT), Tigecycline (TGC), Trimethoprim/Sulfamethoxazole (T/S). Color refer to resistance profile e.g.Susceptable (white), Susceptible, increased exposure (yellow), and resistant (red).

obtained from humans, cats, and dogs were investigated. Despite MALDI-TOF being used as the first line of diagnosis in several laboratories and the score value ≥2.3 being considered to be secure for genus and species identification [24], it has failed to correctly identify two *K. variicola* and one *K. quasipneumoniae* isolates. The inability of MALDI-TOF to discriminate between *Klebsiella* species can be due to the inherent similarity between bacteria or the lack of sufficient spectra in the database [42] for newly identified microorganisms such as *K. variicola* and *K. quasipneumoniae*. Based on WGS data, *K. pneumoniae* and *K. quasipneumoniae* were confirmed in clinical samples obtained from humans and dogs in two governorates of Egypt, while *K. variicola* was identified in samples obtained from dogs and cats and *K. oxytoca* was identified in a human sample. This result confirmed the fact that *K. pneumoniae* is a One Health pathogen in Egypt. It has been previously recovered from broilers, drinking water, and workers at poultry farms [20], tomatoes, irrigation water, fecal samples of farmworkers [21] and

from cattle, sheep, goats, and humans in the Giza governorate [22].

Klebsiella pneumoniae is a member of the ESKAPE pathogens, which are the most serious MDR agents. The existence of ESKAPE pathogens, particularly isolates with a high prevalence of resistance to β-lactam in pet animals, has been reported [43,44]. This is likely to impact treatment options and patient prognosis. Pet animals such as dogs and cats are in close contact with humans; thus, shedding such dangerous pathogens from these animals is considered an important source of infection in humans [45]. The complex route of zoonoses transmission from humans to animals also cannot be ruled out. Thus, surveillance and control of such notorious pathogens should be carried out under the strict guidance of One Health, i.e. a single health authority or close cooperation between human and animal health authorities.

It was furthermore observed that isolates obtained from human samples showed higher levels of resistance than isolates obtained from animals, and *K. pneumoniae* isolates developed resistance against most of the tested antibiotics rather than other *Klebsiella* spp. This further supports the classification of *K. pneumoniae* among ESKAPE pathogens as one of the most dangerous MDR pathogens. Almost all (25 out of 27) *K. pneumoniae* isolates from human samples in this study displayed resistance to antibiotics from at least four different antimicrobial classes and can thus be considered as MDR; of these, five isolates displayed resistance to carbapenem compounds. Isolation of MDR and XDR *K. pneumoniae* isolates from inpatients admitted to ICUs is common in Egypt [14,15]. Such isolates were found to be resistant even to last resort antibiotics such as colistin [16,17], and carbapenems [18]. Dissemination of such notorious isolates has been observed worldwide in hospitalized persons, causing a worrying situation for public health [5,46–49]. However, this development is still neglected in veterinary medicine and environmental health sectors [6].

The current study revealed that several *K. pneumoniae* isolates recovered from human and animal samples were assigned to the same MLST and were identical based on cgSNP analysis. For example, an ST29 dog isolate was identical to several ST29 isolates from human origin, and an ST16 isolate from human origin was identical to an ST16 isolate from a dog, despite absence the direct contact between sampled humans and dogs. This could be the result of the circulation of *K. pneumoniae* between animals and humans or an identical source of infection. Despite the strains were isolated from unrelated humans and dogs, the discovered similarities and differences significantly enhance our understanding of the potential widespread dissemination of resistant strains or resistance genes among humans and animals in Egypt.

On the other hand, a high level of susceptibility was observed among other *Klebsiella* spp. Only one *K. quasipneumoniae* isolate of human origin showed resistance to compounds from three antibiotic classes. *K. quasipneumoniae* isolated from a human as well as dogs and a cat were identical based on cgSNP analysis. In the same context, *K. variicola* isolated from dogs and a cat were also identical, suggesting the circulation of the same isolates in different hosts.

Numerous factors contribute to the pathogenicity and virulence of *K. pneumoniae*, including adherence factors, capsular (k) antigens, siderophore activity, and O-lipopolysaccharide (LPS) [50]. Compared to other species, *K. pneumoniae* isolates have a higher number of virulence-associated genes and plasmid replicons. Three *K. pneumoniae* isolates were assigned to ST23 and had the majority of virulence-associated genes (n = 29), particularly genes related to iron uptake, such as enterobactins (*ent*A, *ent*B and *fep*C), yersiniabactin (*ybt*A, *ybt*E, *ybt*P, *ybt*Q, *ybt*S, *ybt*T, and *ybt*U), salmochelin (*iro*B, *iro*C, *iro*D and *iro*N) and aerobactin (*iuc*A, *iuc*B, *iuc*C and *iut*A). However, these isolates showed a high level of susceptibility and lacked the discriminatory determinants for hypervirulent *K. pneumoniae* (hvKp), which include allantoin utilization genes and hypermucoid locus (*rmpA*) genes associated with severe human infections [51,52]. This is consistent with a previous study from Vietnam in which hvKP isolates were susceptible to most antibiotics tested [49].

Several antimicrobial resistance genes were found in isolates from humans and pet animals, such as *bla*SHV variants and *bla*CTX-M-15, which confer resistance to ß-lactams, *cat*B3, which confers resistance to phenicol, *oqx*A and *oqx*B, which confer resistance to phenicol/quinolone, *aac*(3)-IIe, *aph*(3″)-Ib, and *aph*(6)-Id, which confer resistance to aminoglycoside, and *fos*A variants, which confer resistance to fosfomycin. A further concerning aspect is that isolates from pet animals carry numerous antibiotic-resistance genes, regardless of whether they exhibit antibiotic resistance or not. This poses a significant public health risk, as these genes can lead to the development of multidrug-resistant strains in pets, creating a reservoir for potential human infections. In some cases, antibiotic resistance genes were present in isolates, but the isolates were not resistant to the antibiotics tested. Conversely, some isolates were resistant to antibiotics despite lacking the corresponding resistance genes. This inconsistency between the phenotypic characterization of isolates and the existence of resistance genes has been reported before [49]. In the current study, all isolates harboured *fos*A variants that could confer resistance to fosfomycin. However, only one isolate was non-susceptible to this antibiotic. Moreover, one human *K. pneumoniae* isolate displayed resistance to tigecycline despite 16 isolates harboring the *tet*A and *tet*D genes that are known to confer resistance to tetracyclines.

The only limitation of the current study is that isolates were obtained from clinical samples of humans and pet animals (dogs and cats) from different cities, and there was no close or direct contact between animal and human patients.

5. Conclusion

Klebsiella pneumoniae is a One Health pathogen that affects both humans and animals in Egypt. The isolates of *K. pneumoniae* in our current study showed high diversity. While some human and animal isolates were nearly identical and belonged to the same sequence type (ST), the human isolates displayed more significant resistance patterns compared to the animal isolates. We found discrepancies between the phenotypic characteristics observed in the lab and the antimicrobial resistance (AMR) genes identified through in silico analysis. To better understand the relationship between the presence of AMR genes and the phenotypic data, further research involving a large number of isolates from various sources (such as clinical and non-clinical samples) is necessary. It's crucial to investigate and analyze the genetic makeup of isolates collected from humans and animals in the same locations or in close/direct contact with each other, as well as from their environment. This will help us fully understand the dynamic of resistance development and the connection between the presence of AMR genes of *K. pneumoniae* in different One Health disciplines in Egypt.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100904.

Funding

This research received no external funding.

CRediT authorship contribution statement

Enas A. Soliman: Validation, Methodology, Investigation, Formal analysis, Data curation. **Alaa Saad:** Methodology, Formal analysis. **Ashraf A. Abd El Tawab:** Validation, Supervision, Formal analysis, Data curation. **Fatma I. Elhofy:** Validation, Investigation, Formal analysis. **Amira M. Rizk:** Validation, Methodology, Formal analysis, Data curation. **Manar Elkhayat:** Methodology, Formal analysis, Data curation. **Tamara Kozytska:** Methodology, Data curation. **Majdil Ilyas:** Methodology. **Marwa Bassiouny:** Methodology, Data curation. **Hanka Brangsch:** Writing – review & editing, Validation, Software, Formal analysis, Data curation. **Mathias W. Pletz:** Writing – review & editing, Validation, Conceptualization. **Heinrich Neubauer:** Writing – review & editing, Validation, Resources, Formal analysis, Conceptualization. **Lisa**

D. Sprague: Writing – review & editing, Validation, Formal analysis, Conceptualization. **Gamal Wareth:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Raw sequencing data produced in the current study have been deposited in the European Nucleotide Archive (ENA), with a project accession number PRJEB76311.

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