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Misidentification of *Raoultella* spp. (*R. terrigena*, *R. planticola*) and *Klebsiella* spp. (*K. variicola*, *K. grimontii*) as *Klebsiella pneumoniae*: Retrospective study of a necropsy-associated bacterial collection from horses

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ABSTRACT

Misidentifications as *Klebsiella pneumoniae* were observed during a French retrospective study of a necropsy-associated *K. pneumoniae* bacterial collection from horses. Accordingly, the present study aimed to further characterise the 12 *Raoultella* spp. and *Klebsiella* spp. strains involved in these misidentifications. The strains were identified and characterised using the Api 20E system, *K. pneumoniae* PCR detection, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry and whole-genome sequencing. Antimicrobial susceptibilities were tested by the disc diffusion method with a panel of 40 antibiotics. Thus, misidentifications as *K. pneumoniae* mainly concerned *Raoultella* spp. (*R. terrigena*, *R. planticola*) rather than *Klebsiella* spp. (*K. variicola*, *K. grimontii*), with a dominance of *R. terrigena*. Among the 12 strains, only *K. grimontii* was multi-drug resistant and none were considered hypervirulent. MALDI-TOF was sufficient to avoid misidentification but commercial spectra databases should be expanded with *K. grimontii* and *R. terrigena* reference spectra to improve identification accuracy. This is probably (i) the first report of *K. grimontii* and *R. planticola* isolations from horses and (ii) the second report of *R. terrigena* and *K. variicola* isolations from horses.

1. Introduction

Klebsiella pneumoniae is an important pathogen affecting both humans and animals and is a major source of hospital-acquired infections associated with high morbidity and mortality worldwide. The World Health Organization (WHO) considers it a serious global threat, as multidrug-resistant (MDR) strains belong to the critical group of bacterial pathogens of public health importance (World Health Organization, 2024). As part of the ESKAPE (acronym representing a group of highly virulent and antibiotic-resistant bacterial pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) group (Rice, 2008), *K. pneumoniae* poses a challenge in clinical settings.

Numerous *Klebsiella* spp. have been previously misidentified (Long

et al., 2017; Saxenborn et al., 2021), most often as *K. pneumoniae*, due to shortcomings in species identification technologies available in clinical microbiology laboratories. This has led to an underestimation of the clinical relevance of other *Klebsiella* and *Raoultella* species, as well as potential discrepancies in epidemiological data. The development of more reliable identification methods, e.g. matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) and whole-genome sequencing (WGS), has refined the *K. pneumoniae* taxonomy in recent years. As a result, two species complexes have expanded: (i) the *K. pneumoniae* species complex (KpSC) now contains *K. pneumoniae*, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*, *K. quasipneumoniae* subsp. *similipneumoniae*, *Klebsiella variicola* subsp. *variicola* (*K. variicola*), *K. variicola* subsp. *tropicalensis*, *Klebsiella africana* and *Klebsiella quasivariicola* (Wyres et al., 2020); (ii) the *Klebsiella*

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oxytoca species complex (KoSC) now contains *Klebsiella grimontii*, *Klebsiella huaxiensis*, *Klebsiella michiganensis*, *K. oxytoca*, *Klebsiella pasteurii*, *Klebsiella spallanzanii* and three unnamed species (Yang et al., 2022). Additionally, the *Raoultella* genus (including *Raoultella ornithinolytica*, *Raoultella planticola*, *Raoultella terrigena* and *Raoultella electrica*) was separated from the *Klebsiella* genus in 2001 (Drancourt et al., 2001).

Appel et al. (2021). suggested that *Raoultella* spp. should be considered an underdiagnosed pathogen due to misidentifications. This is an important issue, as a recent study found that 14 % of strains initially identified as *K. pneumoniae* by the API 20E system were misidentified; these included eight *Raoultella* spp., four *Klebsiella* spp., two *Escherichia coli* and one *Citrobacter freundii* among 109 cultivable strains of equine origin isolated between 1996 and 2020 from 108 necropsies and one analysis of foetal appendages (Gravey et al., 2024). Such misidentifications could have serious consequences in clinical settings. Accurate species identification is crucial for effective patient management and essential for monitoring infections and detecting potential outbreaks.

The present study aims to use phenotypic and genomic approaches to further characterise the strains newly identified as *Raoultella* spp. and *Klebsiella* spp., which were excluded from the study on *K. pneumoniae* by Gravey et al. (2024). This work highlights the importance of accurate species identification in improving clinical decision-making and controlling the spread of precisely-identified bacteria, especially when they are antimicrobial-resistant.

2. Materials and methods

2.1. Bacterial strains

A retrospective investigation of *K. pneumoniae* (Gravey et al., 2024) identified 12 *Raoultella* spp. and *Klebsiella* spp. strains, all of equine origin (Table 1), which had been misidentified as *K. pneumoniae* based on the API 20E system (bioMérieux) during routine bacteriological diagnosis at the time of strain isolation. These strains were isolated between 2001 and 2020 at ANSES's Normandy Laboratory for Animal Health from 11 necropsies of foetuses and foals (<1 year), and one analysis of foetal appendages (Table 1). Strains were maintained using CryoBeads™ (bioMérieux) at a temperature ≤ -65 °C. They were cultivated on ready-to-use 5 % sheep blood agar (bioMérieux) incubated at 35 ± 2 °C for 24 h to obtain single colonies.

2.2. Bacterial identification

Several bacterial identification techniques were applied to the strains, including: (i) the API 20E system (bioMérieux); (ii) the

ADIAVET™ CEMO KLEB/PSEUDO real-time PCR kit (BioX Diagnostics) on DNA extracted using the Macherey-Nagel NucleoSpin Tissue kit; (iii) MALDI-TOF MS analysis using an MBT Smart mass spectrometer with MBT Compass and FlexControl software (Bruker Daltonics), applying the BDAL 8468 MSPs database and the direct smear method. All the techniques were implemented according to the manufacturer's instructions. Finally, (iv) WGS was performed on single colonies to identify the bacterial species of the 12 strains misidentified as *K. pneumoniae* using the rMLST tool (Jolley et al., 2012). In cases of discrepancies between identification methods, WGS was considered the gold standard.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar plates according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline (<http://www.eucast.org>) with a panel of 40 antibiotics (Bio-Rad) (Table 2). Inhibition zone diameters were interpreted as susceptible, susceptible dose dependent or resistant using the breakpoints for Enterobacterales available in the 2023 veterinary recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM, 2023) or, failing that, those available for Enterobacterales strains of human origin (CASFM/EUCAST, 2024; CASFM, 2013) (Table 2). The MDR classification, defined as antimicrobial resistance to at least one antimicrobial drug from three or more antimicrobial categories, was determined taking into account the classes of the 35 antibiotics remaining after excluding ampicillin, azithromycin, erythromycin, rifampicin and ticarcillin because of natural resistances observed for *Klebsiella* spp. and *Raoultella* spp (CASFM/EUCAST, 2024).

2.4. Whole-genome sequencing

Isolates were subcultured overnight in Luria-Bertani broth at 35 ± 2 °C. Then 1 ml was centrifuged for 10 min at 4000 rpm and the pellet was suspended in 100 μ l PBS 1X. Cells were lysed using Magna Pure Lysis Buffer (Roche) and Proteinase K recombinant PCR Grade (Roche). DNA was extracted using the Magna-Pure system (Roche).

Dual-indexed Illumina sequencing libraries were constructed from each DNA sample using the Nextera XT DNA library preparation kit (Illumina), pooled, and sequenced on the Illumina NextSeq 500 platform (Plateforme de Microbiologie Mutualisée P2M, Institut Pasteur). Sequencing was performed according to the manufacturer's instructions and using a 2×150 -bp paired-end configuration with a minimum coverage of 30x. FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and MultiQC (Ewels et al., 2016) were used for raw read quality control. Both adapters and reads with a median Phred

Table 1
Origin of strains.

Strain	Age	Sex	Breed	French region	Year	Context	Co-microbial identification
007	Foal	Male	Thoroughbred	Normandy	2005	Necropsy (endo/enterotoxaemia)	<i>Escherichia coli</i> , <i>Streptococcus zooepidemicus</i> , <i>Clostridium perfringens</i>
015	Foal	Female	Thoroughbred	Maine-et-Loire	2003	Necropsy (<i>Rhodococcus equi</i> infection)	<i>Rhodococcus equi</i>
081	Foetus	Female	Thoroughbred	Normandy	2016	Aerobic culture of foetal appendages of living foal (placentitis)	<i>Escherichia coli</i> , <i>Staphylococcus xylosum</i>
089	Foetus	Male	Trotter	Normandy	2001	Necropsy (infectious abortion)	<i>Escherichia coli</i> , <i>Staphylococcus xylosum</i> , <i>Streptococcus zooepidemicus</i>
093	Foetus	Female	Thoroughbred	Normandy	2005	Necropsy (infectious abortion)	<i>Escherichia coli</i> , <i>Staphylococcus xylosum</i>
096	Foetus	Female	Pony	Normandy	2012	Necropsy (infectious abortion)	<i>Staphylococcus xylosum</i>
097	Foetus	Male	Thoroughbred	Normandy	2014	Necropsy (infectious abortion)	<i>Escherichia coli</i> , <i>Staphylococcus xylosum</i>
104	Foetus	Female	Thoroughbred	Normandy	2018	Necropsy (infectious abortion)	-
106	Foal	Male	Thoroughbred	Normandy	2019	Necropsy (sepsis)	<i>Escherichia coli</i> , <i>Staphylococcus xylosum</i>
108	Foetus	Male	Thoroughbred	Normandy	2015	Necropsy (abortion - placentitis)	<i>Vibrio parahaemolyticus</i>
109	Foetus	Female	Thoroughbred	Normandy	2015	Necropsy (infectious abortion)	<i>Streptococcus equisimilis</i>
144	Foetus	Female	Thoroughbred	Normandy	2020	Necropsy (infectious abortion)	<i>Escherichia coli</i> , <i>Staphylococcus xylosum</i>

Table 2
Antimicrobial susceptibility test.

Antibiotic class	Antibiotic	Concentration (disc/ μ g)	Breakpoints (mm) S \geq / R <	Strain												
				007	015	081	089	093	096	097	104	106	108	109	144	
β -lactams	Ertapenem	10	23 / 23 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Imipenem	10	22 / 19 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Meropenem	10	22 / 16 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Ticarcillin	75	23 / 23 ^b	R	R	R	R	R	R	R	R	R	R	R	R	R
	Cefalexin	30	18 / 12 ^a	S	S	S	S	S	S	S	S	S	S	S	S	S
	Cefamandole	30	22 / 15 ^c	S	S	S	S	S	S	S	S	S	S	S	S	S
	Cefoxitin	30	22 / 15 ^a	S	S	S	S	S	S	S	SDD	S	S	S	S	S
	Ceftazidime	10	22 / 19 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Ceftriaxone	30	25 / 22 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Ceftiofur	30	21 / 18 ^a	S	S	S	S	S	S	S	S	S	S	S	S	S
	Cefepime	30	27 / 24 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Ceftolozane-tazobactam	30–10	22 / 22 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Aztreonam	30	26 / 21 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Ampicillin	10	14 / 14 ^b	S	R	S	S	R	S	S	S	S	S	S	S	S
	Mecillinam	10	15 / 15 ^b	S	R	S	S	S	S	S	S	S	S	S	S	S
	Temocillin	30	50 / 17 ^b	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD
	Amoxicillin-clavulanate	20–10	21 / 14 ^a	S	SDD	S	S	SDD	S	S	S	S	S	S	S	S
	Ticarcillin-clavulanate	75–10	23 / 20 ^b	S	S	S	S	SDD	S	S	S	S	S	S	S	S
	Piperacillin-tazobactam	30–6	20 / 20 ^b	S	S	S	S	S	S	S	S	S	S	S	S	S
	Aminoglycosides	Streptomycin	10	15 / 13 ^a	S	S	S	S	R	S	S	R	R	S	S	S
Gentamicin		15	18 / 16 ^a	S	S	S	S	R	S	S	S	S	S	S	S	
Kanamycin		30	17 / 15 ^a	S	S	S	S	S	S	S	S	S	S	S	S	
Tobramycin		10	16 / 16 ^b	S	S	S	S	R	S	S	S	S	S	S	S	
Netilmicin		30	21 / 19 ^c	S	S	S	S	SDD	S	S	S	S	S	S	S	
Amikacin		30	18 / 18 ^b	S	S	S	S	S	S	S	S	S	S	S	S	
Spectinomycin		100	20 / 20 ^c	S	S	S	R	R	S	S	S	R	R	S	S	
Nalidixic acid		30	20 / 15 ^a	S	S	S	S	S	S	S	S	S	S	S	S	
Quinolones	Ciprofloxacin	5	25 / 22 ^b	S	S	S	SDD	S	S	S	S	S	S	R	S	
	Norfloxacin	10	25 / 22 ^c	S	S	S	S	S	S	S	S	S	S	S	S	
	Levofloxacin	5	23 / 19 ^b	S	S	S	S	S	S	S	S	S	S	S	S	
	Marbofloxacin	5	19 / 19 ^a	S	S	S	S	S	S	S	S	S	S	S	S	
	Erythromycin	15 UI	22 / 17 ^c	R	R	R	R	R	R	R	R	R	R	R	R	
	Azithromycin	15	22 / 17 ^c	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	SDD	
Phosphoric acid	Fosfomycin	200	21 / 21 ^b	S	S	S	S	S	R	S	S	R	S	S		
	Chloramphenicol	30	22 / 19 ^a	S	S	S	S	S	S	S	S	S	S	S		
Phenicol	Rifampicin	30	19 / 14 ^c	R	R	R	R	R	R	R	R	R	R	R		
	Tetracycline	30	19 / 17 ^a	S	R	S	S	R	S	S	S	S	S	S		
Tetracyclines	Tigecycline	15	18 / 18 ^b	S	S	S	R	S	S	S	S	S	S	S		
	Trimethoprim	5	16 / 12 ^a	S	S	S	S	R	S	S	R	R	S	S		
Pyrimidines	Trimethoprim-sulphamethoxazole	1.25–23.75	16 / 10 ^a	S	S	S	S	R	S	S	R	R	S	S		
	MDR			No	No	No	No	Yes	No	No	No	No	No	No		

MLS, macrolide-lincosamide-streptogramin; MDR, multi-drug resistance; S, susceptible; SDD, susceptible dose dependent; R, resistant. The MDR classification was assessed after exclusion of ampicillin, azithromycin, erythromycin, rifampicin and ticarcillin because of natural resistances observed for *Klebsiella* spp. and *Raoultella* spp (CASFM/EUCAST, 2024).

^a Breakpoints for *Enterobacteriales* (CASFM, 2023).

^b Breakpoints for *Enterobacteriales* (CASFM/EUCAST, 2024).

^c Breakpoints for *Enterobacteriales* or for various classes of antibiotics (CASFM, 2013).

score lower than 25 and/or shorter than 70 pb were removed from the fastq files using fqCleanER (<https://gitlab.pasteur.fr/GIPhy/fqCleanER>). Genomes were assembled using the SPAdes Genome Assembler v.3.12.0 with parameters recommended for 150 pb paired-end reads (k-mer lengths of 21, 33, 55, 77) (Bankevich et al., 2012). The quality of the assemblies was assessed in Quast (Gurevich et al., 2013). Genomes are available in the NCBI database using bioproject accession number PRJNA1135923.

2.5. In silico analysis

Several *in silico* analyses were performed for each genome: (i) the species was checked using the rMLST software (Jolley et al., 2012); (ii) sequence type (ST) was attributed according to the Multilocus Sequence Typing (MLST) scheme previously described using the BIGSdb-Kp database (Diancourt et al., 2005) for *K. variicola* and <https://pubmlst.org/organisms/klebsiella-oxytoca/> for *K. grimontii*; (iii) whole virulence encoding genes were looked for in the BIGSdb-Kp database with a 90 % identity threshold using the KMA software (Bialek-Davenet et al., 2014; Clausen et al., 2018); (iv) acquired antibiotic resistance genes and chromosomal mutations were determined using both ResFinder and RGI databases with 90 % identity and coverage thresholds, respectively (Zankari et al., 2012; Alcock et al., 2020); (v) families of plasmids carried by the strains were looked for using the PlasmidFinder tool (Carattoli et al., 2014).

org/organisms/klebsiella-oxytoca/ for *K. grimontii*; (iii) whole virulence encoding genes were looked for in the BIGSdb-Kp database with a 90 % identity threshold using the KMA software (Bialek-Davenet et al., 2014; Clausen et al., 2018); (iv) acquired antibiotic resistance genes and chromosomal mutations were determined using both ResFinder and RGI databases with 90 % identity and coverage thresholds, respectively (Zankari et al., 2012; Alcock et al., 2020); (v) families of plasmids carried by the strains were looked for using the PlasmidFinder tool (Carattoli et al., 2014).

3. Results

3.1. Identification of strains

The strains studied were isolated from 11 necropsies of eight fetuses and three foals, and the foetal appendages of one living foal. Eleven of

the 12 animals were located in Normandy, with an overrepresentation of Thoroughbreds (n = 10) skewed by the necropsy recruitment, and a sex (male:female) ratio of 0.7. The context of strain isolations concerned mostly abortions (n = 8) and one placentitis with a living foal, but also one sepsis, one endo/enterotoxaemia and one *Rhodococcus equi* infection. All the isolates revealed co-infections with other bacteria except for strain #104 (Table 1). These strains were initially identified as *K. pneumoniae* using the API 20E system at the time of their isolation, between 2001 and 2020. In our study, a second analysis with the API 20E system showed equivalent results, except for strain #093. Additionally, the ADIAVET™ CEMO KLEB/PSEUDO real-time PCR yielded positive results for *K. pneumoniae* in all strains, with Ct values ranging from 30.57 to 35.98 (Table 3). However, MALDI-TOF and WGS revealed *K. pneumoniae* misidentifications that were not detected by the API 20E system or real-time PCR. Discordant identification results were resolved using WGS as the gold standard method. Specifically, (i) strains #007 and #015 were identified as *K. variicola*, and strain #109 as *R. planticola* by both methods; (ii) strains #081, #089, #096, #097, #106, #108 and #144 were identified as *R. terrigena* by WGS while MALDI-TOF accurately identified both *R. terrigena* and *R. ornithinolytica*, leading to classification at the genus level. Similarly, strain #104 was identified as *R. terrigena* by WGS and as *R. ornithinolytica* by MALDI-TOF; (iii) strain #093 was identified as *K. grimontii* by WGS and as *K. oxytoca* by MALDI-TOF (Table 3). Moreover, following WGS analysis, STs were assigned to *K. variicola* #007 (ST394), #015 (ST710) and *K. grimontii* #093 (ST394) (Table 4).

3.2. Phenotypic and genotypic characterisation of antibiotic resistance

The antimicrobial susceptibilities are presented in Table 2. Setting aside the natural resistances to ampicillin, azithromycin, erythromycin, rifampicin and ticarcillin, resistances were observed to 11/35 antibiotics from six classes: β -lactams (mecillinam), aminoglycosides (streptomycin, gentamicin, tobramycin and spectinomycin), quinolones (ciprofloxacin), phosphoric acid (fosfomycin), tetracyclines (tetracycline and tigecycline), and pyrimidines (trimethoprim and trimethoprim-sulphamethoxazole), with one to three resistant strains out of the 12 studied. A total of 10 different antimicrobial resistance profiles were

identified, including one MDR profile (*K. grimontii* #093 isolated in 2001 from a Thoroughbred foetus) (Table 2).

Two to nine resistance genes were identified per strain (Table 4), with a median of 3.5 genes when all strains were considered. All 12 strains harboured at least one β -lactam resistance gene with a dominance of the *bla*TER-2 gene (8/12 strains) among the *R. terrigena* strains, which corresponds to the chromosomal beta lactamase of the species. *K. variicola* #015 and *K. grimontii* #093 — both of which were resistant to ampicillin — and strain #015, resistant to mecillinam, also harboured a *bla*TEM gene. Four of the five aminoglycoside-resistant strains harboured *aph*(3'')-Ib and *aph*(6)-Id genes, including *K. grimontii* #093 that also harboured *aac*(3)-Iia and *ant*(3'')-Ia, which is in agreement with the fact that this strain was resistant to four aminoglycosides while the other strains were only resistant to streptomycin or spectinomycin; no aminoglycoside-resistant gene was identified in the spectinomycin-resistant *R. terrigena* #108. All three pyrimidine-resistant strains (*R. terrigena* #104 and #106, and *K. grimontii* #093) harboured the *dfr*A1 pyrimidine resistance gene. No strain harboured a phenicol resistance gene, in concordance with the phenotypic results. Concerning the tetracycline class, only the tetracycline-resistant *K. variicola* #015 and *K. grimontii* #093 harboured the *tet*(A) tetracycline resistance gene, while the tigecycline-resistant *R. terrigena* #89 did not. Despite the presence of the *fosA* gene in 11/12 genomes, only *R. terrigena* #097 and #108 were fosfomycin-resistant (Table 4).

3.3. Identification of plasmids and virulence-related genes

Six strains harboured one to three plasmids grouped under four different profiles (Table 4). Col(pHAD28) and Col440I were identified in *R. terrigena* #89, #104 and #106; Col(pHAD28), IncB/O/K/Z and IncFII (pHN7A8) were identified in *R. terrigena* #144; and IncN and IncM1, respectively, in *K. variicola* #015 and *K. grimontii* #093.

Five strains harboured two to 12 virulence genes grouped under four different profiles (Table 4). In more detail, ferric uptake system *kfuABC*, type-3 fimbriae cluster *mrk* and aerobactin receptor *iutA* were detected in *K. variicola* #007 and #015; it should be noted that *R. terrigena* #097 harboured only the *mrkJ* gene and no strain harboured both *iutA* and aerobactin cluster *iucABCD*. The *ybt-fyuA-irp* yersiniabactin cluster was

Table 3
Species identification by the Api 20E system, *K. pneumoniae* PCR detection, MALDI-TOF and WGS.

Strain	Api 20E system (% ID)	<i>K. pneumoniae</i> PCR (Ct) ^a	MALDI-TOF (score) ^b	WGS analysis
007	<i>Klebsiella pneumoniae</i> (98.2)	Detected (31.17)	<i>Klebsiella variicola</i> (2.30), <i>Klebsiella variicola</i> (3.20)	<i>Klebsiella variicola</i>
015	<i>Klebsiella pneumoniae</i> (98.9)	Detected (30.57)	<i>Klebsiella variicola</i> (3.34), <i>Klebsiella variicola</i> (3.33)	<i>Klebsiella variicola</i>
081	<i>Klebsiella pneumoniae</i> (95.0), possibility of <i>Raoultella terrigena</i>	Detected (34.26)	<i>Raoultella ornithinolytica</i> (2.31), <i>Raoultella terrigena</i> (2.30)	<i>Raoultella terrigena</i>
089	<i>Klebsiella pneumoniae</i> (96.0), possibility of <i>Raoultella terrigena</i>	Detected (35.86)	<i>Raoultella ornithinolytica</i> (2.15), <i>Raoultella terrigena</i> (2.14)	<i>Raoultella terrigena</i>
093	<i>Klebsiella oxytoca</i> (97.8), possibility of <i>Raoultella planticola</i>	Detected (33.83)	<i>Klebsiella oxytoca</i> (2.43), <i>Klebsiella oxytoca</i> (2.33)	<i>Klebsiella grimontii</i>
096	<i>Klebsiella pneumoniae</i> (95.0), possibility of <i>Raoultella terrigena</i>	Detected (34.95)	<i>Raoultella terrigena</i> (2.36), <i>Raoultella ornithinolytica</i> (2.33)	<i>Raoultella terrigena</i>
097	<i>Klebsiella pneumoniae</i> (95.0), possibility of <i>Raoultella terrigena</i>	Detected (35.54)	<i>Raoultella terrigena</i> (2.43), <i>Raoultella ornithinolytica</i> (2.36)	<i>Raoultella terrigena</i>
104	<i>Klebsiella pneumoniae</i> (96.0), possibility of <i>Raoultella terrigena</i>	Detected (31.98)	<i>Raoultella ornithinolytica</i> (2.23), <i>Raoultella ornithinolytica</i> (2.19)	<i>Raoultella terrigena</i>
106	<i>Klebsiella pneumoniae</i> (95.0), possibility of <i>Raoultella terrigena</i>	Detected (35.23)	<i>Raoultella terrigena</i> (2.29), <i>Raoultella ornithinolytica</i> (2.21)	<i>Raoultella terrigena</i>
108	<i>Klebsiella pneumoniae</i> (96.0), possibility of <i>Raoultella terrigena</i>	Detected (35.19)	<i>Raoultella ornithinolytica</i> (2.30), <i>Raoultella terrigena</i> (2.28)	<i>Raoultella terrigena</i>
109	<i>Klebsiella pneumoniae</i> (97.3), possibility of <i>Raoultella planticola</i>	Detected (35.98)	<i>Raoultella planticola</i> (2.50), <i>Raoultella planticola</i> (2.45)	<i>Raoultella planticola</i>
144	<i>Klebsiella pneumoniae</i> (81.8), possibility of <i>Raoultella terrigena</i>	Detected (35.05)	<i>Raoultella ornithinolytica</i> (2.22), <i>Raoultella terrigena</i> (2.19)	<i>Raoultella terrigena</i>

^a ADIAVET™ CEMO KLEB/PSEUDO real-time PCR.

^b The first and second best matches are indicated. All scores were ≥ 2.00 .

Table 4
WGS analysis.

Strain	007	015	081	089	093	096	097	104	106	108	109	144
Species	<i>K. variicola</i>	<i>K. variicola</i>	<i>R. terrigena</i>	<i>R. terrigena</i>	<i>K. grimontii</i>	<i>R. terrigena</i>	<i>R. terrigena</i>	<i>R. terrigena</i>	<i>R. terrigena</i>	<i>R. terrigena</i>	<i>R. planticola</i>	<i>R. terrigena</i>
MLST, ST ^a	394	710	n.a.	n.a.	394	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Plasmid	-	IncN	-	Col (pHAD28), Col440I	IncM1	-	-	Col (pHAD28), Col440I	Col (pHAD28), Col440I	-	-	Col(pHAD28), IncB/O/K/Z, IncFII(pHN7A8)
β-lactam gene	<i>blaLEN2</i>	<i>blaLEN13</i> , <i>blaTEM-1C</i>	<i>blaTER-2</i>	<i>blaTER-2</i>	<i>blaOXY-6-4</i> , <i>blaTEM-1B</i>	<i>blaTER-2</i>	<i>blaTER-2</i>	<i>blaTER-2</i>	<i>blaTER-2</i>	<i>blaTER-2</i>	<i>blaPLA-4A</i>	<i>blaTER-2</i>
Aminoglycoside gene	-	-	-	<i>aph(3'')-Ib</i> , <i>aph(6)-Id</i>	<i>aac(3)-IIa</i> , <i>ant</i> (3'')-Ia, <i>aph(3'')-Ib</i> , <i>aph(6)-Id</i>	-	-	<i>aph(3'')-Ib</i> , <i>aph(6)-Id</i>	<i>aph(3'')-Ib</i> , <i>aph(6)-Id</i>	-	-	-
Quinolone gene	<i>oqxAB</i>	<i>oqxAB</i>	-	-	<i>oqxB</i>	<i>oqxB</i>	-	-	-	-	-	-
Phosphoric acid gene	<i>fosA</i>	<i>fosA</i>	<i>fosA</i>	<i>fosA</i>	-	<i>fosA</i>	<i>fosA</i>	<i>fosA</i>	<i>fosA</i>	<i>fosA</i>	<i>fosA</i>	<i>fosA</i>
Phenicol gene	-	-	-	-	-	-	-	-	-	-	-	-
Tetracycline gene	-	<i>tetA</i>	-	-	<i>tetA</i>	-	-	-	-	-	-	-
Pyrimidine gene	-	-	-	-	<i>dfrA1</i>	-	-	<i>dfrA1</i>	<i>dfrA1</i>	-	-	-
<i>allABCDRS</i> , <i>arcC</i> , <i>fdrA</i> , <i>gcl</i> , <i>glxKR</i> , <i>hyi</i> , <i>KP1_1364</i> , <i>KP1_1371</i> , <i>ybbWY</i> , <i>yIbEF</i>	-	-	-	-	<i>allR</i> , <i>yIbE</i>	-	-	-	-	-	-	-
<i>clbABCDEFGHIJKLMNQP</i> ^b QR	-	-	-	-	-	-	-	-	-	-	-	-
<i>kfuABC</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>kvgAS</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>mceABCDEFGHIJ</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>mrkABCFHIJ</i> ^b	+	+	-	-	-	<i>mrkJ</i>	-	-	-	-	-	-
<i>fyuA</i>	-	-	-	-	+	-	-	-	-	-	-	-
<i>irp1</i> , <i>irp2</i> ^b	-	-	-	-	<i>irp1</i>	-	-	-	-	-	-	-
<i>ybtAEPQSTUX</i> ^b	-	-	-	-	<i>ybtAQSTX</i>	-	-	-	-	-	-	<i>ybtEU</i>
<i>iutA</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>iucABCD</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>iroBCDN</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>rmpA</i> , <i>rmpA2</i>	-	-	-	-	-	-	-	-	-	-	-	-

+ , positive/presence; -, negative/absence; n.a., not applicable.

^a MLST allelic profiles correspond to *gapA-infB-mdh-pgi-phoE-rpoB-tonB* loci according to Diancourt et al (Diancourt et al., 2005). for *K. variicola* and <https://pubmlst.org/organisms/klebsiella-oxytoca> for *K. grimontii*.

^b The partial presence of virulence clusters is highlighted by the precision of the gene(s) present.

partially detected in *K. grimontii* #093 and only two genes of cluster *ybt* were detected in *R. terrigena* #144. No genes involved in the allantoin metabolism (*allABCDRS*, *arcC*, *fdRA*, *gcl*, *glxKR*, *hyi*, *ybbWY*, *ylibEF*) as well as the KP1_1364 and KP1_1371 genes, were detected, except for the *allB* and *ylibE* genes in *K. grimontii* #093. Likewise, colibactin cluster *clb*, two-component system *kvgAS*, microcin E492 cluster *mce*, salmochelin cluster *iro* or mucoid phenotype regulators *rmpA* and *rmpA2* were not detected in any of the strains studied. Overall, no strains were considered hypervirulent.

4. Discussion

The Gravey et al. retrospective study (Gravey et al., 2024) revealed 12 *Raoultella* spp. and *Klebsiella* spp. strains misidentified as *K. pneumoniae* by the API 20E system. These strains, all of equine origin, were among the 14 % of the misidentified *K. pneumoniae* cases among 109 cultivable strains isolated in France between 1996 and 2020. Initially, all strains were identified as *K. pneumoniae* from 108 necropsies and one analysis of foetal appendages (the remaining three misidentifications were two *E. coli* strains and one *C. freundii* strain not included in the present study). The combined use of MALDI-TOF and WGS enabled the identification of four distinct bacterial species, with *R. terrigena* being the most prevalent (8/12), followed by *K. variicola* (2/12), *K. grimontii* (1/12) and *R. planticola* (1/12). The *K. pneumoniae* PCR detection using the ADIAVET™ CEMO KLEB/PSEUDO real-time PCR gave false positive results with exceptionally high Ct values (30.57–35.98), suggesting a non-optimal sensitivity of the PCR scheme. It can be assumed that PCR amplifications performed on field swabs would have potentially shown negative results. The use of MALDI-TOF successfully prevented all misidentifications as *K. pneumoniae*, offering accurate species-level identification for *R. planticola* and *K. variicola*, and genus-level identification for *R. terrigena*, whose species was identified by WGS. *K. grimontii* was identified exclusively by WGS.

Members of the KpSC are often misidentified as *K. pneumoniae* (Long et al., 2017; Saxenborn et al., 2021; Rodríguez-Medina et al., 2019) but in this study, misidentifications mainly concerned *Raoultella* spp. (eight *R. terrigena* and one *R. planticola* strain), corresponding to 60 % of the misidentifications in the Gravey et al. retrospective study (Gravey et al., 2024). Our study highlighted bacterial species that are rarely isolated from horses. To the best of our knowledge, this is the first description of *R. planticola* and the second description of *R. terrigena* that had previously been reported from raw horsemeat intended for human consumption in Japan (Furuhata et al., 2015). None of the nine *Raoultella* spp. strains were considered virulent and none were MDR. It is difficult to express the potential role of these strains in equine mortalities, with the exception of *R. terrigena* #104, where the absence of co-microbial identification supports its involvement in the abortion case, and *R. terrigena* #96, where co-culture was observed only with *Staphylococcus xylosum* also in an abortion case.

Misidentifications of members of the KpSC and KoSC as *K. pneumoniae* concerned only 25 % of the studied strains: *K. variicola* #007 and #015 and *K. grimontii* #093. Neither *K. variicola* #007 nor #015 were MDR, and while they were the most virulent (detection of ferric uptake system *kfuABC*, type-3 fimbriae cluster *mrk* and aerobactin receptor *iutA*), they were not considered hypervirulent. *K. grimontii* #093 was the only MDR strain, but it did not exhibit resistance to third-generation cephalosporins and carbapenems. *K. variicola* is considered an emerging human pathogen (Rodríguez-Medina et al., 2019; Mondo et al., 2021), with the first isolation of equine origin recently reported following identification using MALDI-TOF. Our study may be the first to report the isolation of *K. grimontii* from a horse.

To conclude this study, misidentifications as *K. pneumoniae* mainly concerned *Raoultella* spp. rather than *Klebsiella* spp. *R. terrigena* was the dominant species among the 14 % of misidentifications observed in the Gravey et al. retrospective study (Gravey et al., 2024), which aimed to analyse an equine strain collection isolated in France between 1996 and

2020 from 108 necropsies and one analysis of foetal appendages. Initially, these strains were misidentified as *K. pneumoniae* by the API 20E system. This finding supports the hypothesis that *Raoultella* spp. should be considered underdiagnosed due to misidentifications (Appel et al., 2021). Routine bacteriological diagnosis in clinical microbiology laboratories must be updated with more efficient identification tools like MALDI-TOF. In this study, MALDI-TOF avoided all misidentifications as *K. pneumoniae*, although commercial spectra databases need to be expanded to include *K. grimontii* and *R. terrigena* reference spectra to ensure optimal performance. WGS is also a reliable identification tool. Although the strains investigated in this study are not *K. pneumoniae*, the *K. grimontii* strain poses a risk due to its MDR status. Better identification of these bacterial species will provide a clearer picture of their incidence in equine infections.

Ethical approval

No ethical approval was needed for this study.

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CRediT authorship contribution statement

Sévin Corinne: Writing – review & editing, Validation, Investigation. **Maillard Karine:** Writing – review & editing, Investigation. **Lan-glois Bénédicte:** Writing – review & editing, Software, Investigation. **Duquesne Fabien:** Writing – review & editing, Investigation. **Foucher Nathalie:** Writing – review & editing, Resources. **Le Hello Simon:** Writing – review & editing, Supervision, Resources, Project administration, Methodology. **Léon Albertine:** Writing – review & editing, Resources. **Petry Sandrine:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Gravey Francois:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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