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Abstract

Urinary tract infections (UTIs) in cats are increasingly recognized as clinically significant, often involving multidrug-resistant (MDR) bacteria with zoonotic potential. Despite the growing number

of reports describing resistant bacteria in feline populations, genomic data on feline uropathogens remain scarce in Tunisia. This study aimed to characterize the resistome and sequence types of bacterial isolates recovered from cats with clinical signs of UTI using whole-genome sequencing (WGS). Urine samples were collected from eleven diseased domestic cats, and six isolates were identified and sequenced using the Illumina iSeq100 platform. The analysis revealed multiple multi-drug resistant (MDR) species, including *Mammaliicoccus lentus* (formerly *Staphylococcus lentus*, n=2), *Staphylococcus schleiferi* subsp. *coagulans* (n=1), *Mammaliicoccus sciuri* (formerly *Staphylococcus sciuri*, n=1), *Enterococcus faecalis* (n=1), *Enterococcus casseliflavus* (n=1), and *Klebsiella aerogenes* (n=1). Genomic profiling uncovered diverse antimicrobial resistance determinants, notably genes conferring acquired resistance to beta-lactams, methicillin, macrolides, tetracyclines, and colistin, underscoring their clinical and zoonotic relevance. This work represents the first WGS-based characterization of feline UTI pathogens in Tunisia and emphasizes the necessity of integrating companion animals into genomic surveillance programs under the One Health approach to monitor and mitigate antimicrobial resistance dissemination.

1 Introduction

Feline urolithiasis and urinary tract infections (UTIs) are among the most prevalent urinary disorders in domestic cats, particularly in males. While once considered a consequence of urolith formation, bacterial infection is increasingly acknowledged as a central factor in the pathogenesis and recurrence of feline lower urinary tract disease (FLUTD) (Dorsch et al., 2019 ; Sakauchi et al., 2025; Van Bohemen et al., 2025). These infections are commonly caused by uropathogenic *Escherichia coli* (UPEC), along with a variety of other Gram-negative and Gram-positive bacteria. In feline UTIs, pathogens such as *Enterococcus faecalis*, Staphylococcus schleiferi, Mammaliicoccus lentus (formerly *Staphylococcus lentus*), *Enterococcus casseliflavus*, *Mammaliicoccus sciuri* (formerly *Staphylococcus sciuri*), and *Klebsiella aerogenes* have been increasingly reported (Gharsa et al., 2015; Ben Said et al., 2017; Hansen-Jones et al., 2018; Jousserand et al., 2025). Globally, these species frequently harbor clinically significant antimicrobial resistance determinants, including genes encoding extended-spectrum β-lactamases (ESBLs), vancomycin resistance, and aminoglycoside-modifying enzymes, raising growing concern about their zoonotic potential and the risk of transmission to humans (Bhat, 2021). Indeed, during the last two decades, the spread of antimicrobial resistance (AMR) among companion animal pathogens has raised alarm, particularly due to the

zoonotic potential of multidrug-resistant (MDR) lineages (figure 1) (Jousserand et al., 2025; Guardabassi et al., 2004, Jin et al., 2025).

Whole-genome sequencing (WGS) has revolutionized microbial epidemiology, enabling high-resolution characterization of resistance determinants, virulence factors, mobile genetic elements, and phylogenetic relationships across hosts and environments (Collineau et al., 2019; Oniciuc et al. 2025). Investigations so far have relied primarily on phenotypic and molecular typing, without employing WGS. Given the increasing emphasis on the One Health framework, WGS offers a powerful tool to explore the genetic determinants of resistance and virulence, as well as to trace potential zoonotic transmission routes (Collineau et al., 2019; Kekre et al., 2021; Oniciuc et al. 2025). Despite the increasing recognition of antimicrobial resistance in feline pathogens, no Tunisian study has yet utilized whole-genome sequencing (WGS) to comprehensively investigate UTI isolates, thereby limiting our understanding of their resistance mechanisms, virulence attributes, and potential for zoonotic transmission.

This work constitutes the first WGS investigation of feline UTI pathogens in Tunisia. By characterizing the resistome and sequence types of bacterial isolates from infected cats, it contributes essential genomic data to strengthen veterinary antimicrobial resistance monitoring within the One Health framework.

2. Methods

2.1 Case and Sample Collection

Eleven diseased domestic cats presenting clinical signs of lower urinary tract infection (LUTI) were collected from private veterinary clinic located in Tunis. Clinical data such as age, sex, breed, and urinary tract signs were recorded. Urine samples were obtained by free catch or voiding. Urine samples were refrigerated (4°C) immediately until processing within 6 hours of collection. Urine analysis included evaluation of specific gravity, and a dipstick and sediment examination. Cats were classified as having LUTI if they presented one or more of the following clinical or physical examination findings: stranguria, dysuria, hematuria, periuria, pollakiuria, inappropriate urination,

87 vulvar discharge, malodorous urine, owner-reported signs of “UTI” or pain and voiding during
88 bladder palpation.

89 **2.2 Isolation and storage of collected isolates**

90 Since enterococci, staphylococci, and Enterobacterales are among the primary bacterial causes of
91 feline UTIs, this study focused exclusively on these groups. Enterococci were isolated from urine
92 samples using Slanetz-Bartley agar (Biokar Diagnostics) and incubated at 37 °C for 24–48 h.
93 Colonies exhibiting characteristic morphology (small, pink, round, and smooth) were re-streaked to
94 ensure purity. *Staphylococcus* spp. were recovered on Chapman agar (Micoxpress, Málaga, Spain)
95 under the same incubation conditions. For Enterobacterales, urine samples were first suspended in
96 trypticase soy broth (Difco, USA) and then inoculated onto MacConkey agar (Difco, USA) at 37 °C
97 for 24 h. Colonies displaying typical morphology were selected for sub-culture: lactose-fermenting
98 colonies appeared pink to red with smooth, round edges, whereas non-lactose-fermenting colonies
99 were colorless and round. Pure cultures were obtained by re-streaking these colonies on MacConkey
100 agar. Pure isolates of each species were subsequently conserved in Brain Heart Infusion Broth
101 (Difco, USA) supplemented with 20% glycerol and stored at –80 °C until further analysis.

102 **2.3. Identification of Species** and **Antimicrobial Susceptibility Testing**

103 The bacterial identification was determined by Gram staining, catalase, coagulase, and the VITEK2
104 system (BioMérieux, Marcy-l'Étoile, France). Phenotypic antimicrobial resistance assessment was
105 assayed using the disk-diffusion method into Mueller-Hinton agar or by minimum inhibitory
106 concentration (MIC) determination using the E-test method, according to the performance standards
107 of CA-SFM guidelines (CA-SFM 2023). For *Enterococcus* spp. isolates, the disk diffusion (Kirby–
108 Bauer) method was employed using ampicillin (10 µg), erythromycin (15 µg), gentamicin (500 µg),
109 streptomycin (300 µg), linezolid (30 µg), vancomycin (30 µg), tetracycline (30 µg), and teicoplanin
110 (30 µg) (Bio-Rad, France). Isolates resistant to vancomycin and linezolid were further subjected to

MIC determination. The tested antibiotics for *Staphylococcus* spp. isolates were: penicillin (10 µg), oxacillin (10 µg), cefoxitin (30 µg), vancomycin (30 µg), gentamicin (10 µg), kanamycin (30 µg), tobramycin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), amikacin (10 µg), fusidic acid (10 µg), teicoplanin (30 µg), fosfomycin (200 µg), chloramphenicol (30 µg), and ampicillin (30 µg). For enterobacteria isolates, tested antibiotics were: ampicillin, cefazolin, cephalothin, cefoxitin, cefpodoxime, ceftriaxone, cefotaxime, ceftazidime, cefepime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, imipenem, meropenem, ciprofloxacin, and gentamicin. *E. coli* ATCC 25922, *Enterococcus feacalis* ATCC 29212, *Staphylococcus aureus* ATCC29213 were used as control strains.

2.4. DNA extraction, WGS and analysis, assembly and annotation

DNA was extracted by using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The quantity and quality of extracted DNA were measured using a Qubit™ 4 and Fluorometer (Invitrogen, USA). Libraries were prepared by the Illumina DNA Prep (M) Tagmentation kit (Illumina), according to the manufacturer's instructions. The prepared libraries were diluted to a final loading concentration of 100 pM and sequenced on the iSeq 100 System. De novo genome assembly of the raw sequenced reads was performed by Spades software (version 3), available at the Proksee platform (https://proksee.ca/). The assembled genomes were annotated and mapped using Bakta (v1.8.2, DB: v5.0 - Light) and Prokka (version 1.14.6). Additionally, antimicrobial resistance genes were identified using the Resistance Gene Identifier tool integrated into the Proksee platform (https://proksee.ca/), allowing for cross-validation of resistance gene profiles. CRISPR arrays were also detected using the CRISPR identification tools available within the Proksee platform.

The presence of antimicrobial resistance genes, sequence types, and plasmid incompatibility groups was analyzed using the ResFinder, MLST, and PlasmidFinder tools available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). FastQ files from all genome

sequences were submitted to (www.ncbi. nlm.nih.gov) databases (Submission ID: SUB15682498). SRA records were accessible with the following: https://www.ncbi.nlm.nih.gov/sra/PRJNA1337466.

3. Results

3.1. Description of infected cats and collected isolates

The affected cats were young adult males aged 2–5 years, predominantly of domestic shorthair and longhair breeds, with two pedigrees and one Siamese. Most urine samples were obtained by free catch, with one sample collected via natural voiding and one case in which the collection method was not recorded. The most frequently observed urinary tract signs were pollakiuria and hematuria, followed by lower urinary tract symptoms, urinary incontinence, malodorous urine, and other general urinary abnormalities (Table 1, figure 2).

Among the eleven diseased cats diagnosed with UTI, a total of seven bacterial isolates were recovered in six cats, as one urine sample yielded two distinct isolates. The species identified included *Mammaliicoccus lentus* (formerly *Staphylococcus lentus*, n = 2), *Staphylococcus schleiferi* subsp. *coagulans* (n = 1), *Mammaliicoccus sciuri* (formerly *Staphylococcus sciuri*, n = 1), *Enterococcus faecalis* (n = 1), *Enterococcus casseliflavus* (n = 1), and *Klebsiella aerogenes* (n = 1) (Table 2).

3.2. Antimicrobial resistance profiles

Antimicrobial susceptibility testing of bacterial isolates from cats revealed diverse resistance patterns across species. *Mammaliicoccus. lentus* isolates (S62 and S104) display resistance to penicillin, ampicillin, oxacillin, clindamycin , and norfloxacin . *S. schleiferi* (S46) exhibited resistance to penicillinP, ampicillin, oxacillin, norfloxacin and trimethoprim. In contrast, *E. faecalis* strains (E67) exhibited high-level resistance to multiple agents, including ampicillin, linezolid, erythromycin, tetracycline, and high-level streptomycin resistance . *E. casseliflavus* (E58) was resistant to linzolid

and tetracycline, while *S. sciuri* (S86) showed resistance to erythromycin , cefamandole (fosfomycin , cefoxitin , and tetracycline.

K. aerogenes (V68) demonstrated broad-spectrum resistance to amoxicillin-clavulanate , cefotaxime, ceftazidime , cefoxitin , meropenem , fosfomycin, tetracycline and nalidixic acid (Table 2, Figure 3). Antimicrobial susceptibility testing of bacterial isolates from cats revealed diverse resistance patterns across species. *M. lentus* isolates (S62 and S104) exhibited resistance to penicillin, ampicillin, oxacillin, clindamycin, and norfloxacin. *M. sciuri* (S86) was resistant to erythromycin, cefamandole, fosfomycin, cefoxitin, and tetracycline. *S. schleiferi* (S46) was resistant to penicillin, ampicillin, oxacillin, norfloxacin, and trimethoprim. *E. faecalis* (E67) displayed high-level resistance to multiple agents, including ampicillin, linezolid, erythromycin, tetracycline, and streptomycin. *E. casseliflavus* (E58) showed resistance to linezolid and tetracycline. Finally, *Klebsiella aerogenes* (V68) demonstrated broad-spectrum resistance, including amoxicillin-clavulanate, cefotaxime, ceftazidime, cefoxitin, meropenem, fosfomycin, tetracycline, and nalidixic acid (Table 2, Figure 3).

3.3 WGS Analysis

3.3.1 Genome sizes

The genome sizes of the analyzed strains ranged from approximately 1.96 to 4.94 Mbp, reflecting notable interspecies variation. The smaller genomes (~2.0–2.8 Mbp) correspond to Gram-positive cocci. The intermediate-sized genomes (~3.0 Mbp) was observed in the *E. casseliflavus* E58 strain. In contrast, the largest genome (~4.9 Mbp) correspond to the Gram-negative strain *Klebsiella aerogenes* V68 (Table 2).

3.3.2 Resistomes

Several important antimicrobial resistance patterns were observed. For *E. faecalis* E67 the following genes were observed: *lsaA* (clindamycin and quinupristin-dalfopristin resistance), *dfrE/dfrG* (trimethoprim resistanceà, *vanT* (belonging to van cluster, vancomycin resistance), *efrA* (intrinsic

183 gene encoding multidrug efflux pump responsible to resistance towards: macrolides,
184 fluoroquinolones, rifamycin), *tet(M)* (tetracycline resistance), *tet(45)* (tetracycline resistance), *emeA*
185 (multidrug efflux pump), *aad(6)* (also known as *aac(6))* (aminoglycosides resistance), *aph(3')-IIIa*
186 (aminoglycosides resistance), *sat-4* (streptothrin resistance), *ermB* (macrolide-lincosamide-
187 streptogramin B resistance). In contrast, for *E. casseliflavus* E58 only genes encompassed in the
188 vanC cluster were observed including the *vanT, vanXY, vanC* genes in addition to, *vanY* gene of the
189 *vanB* cluster; interestingly, no acquired resistance genes were observed despite phenotypic resistance
190 towards tetracycline and to linezolid.

191 For *K. aerogenes* V68, its resistome was rich and concordant to its phenotypic resistance pattern. It
192 harboured several resistance-associated genes, including $bla_{\text{CMY-132}}$ (an AmpC-type β-lactamase that
193 hydrolyses cephalosporins), *oqxA* (the outer membrane component of the OqxAB efflux pump,
194 conferring resistance to fluoroquinolones and other agents), *fosA5* (a glutathione-S-transferase that
195 inactivates fosfomycin), *erm*(43) (macrolide-lincosamide-streptogramin B resistance), *eptB* (a
196 phosphoethanolamine transferase that modifies lipid A and lowers susceptibility to polymyxins),
197 *leuO* (a global transcriptional regulator that can affect stress responses and efflux pump expression),
198 CRP (cAMP receptor protein, a global regulator which can modulate expression of multiple
199 resistance/virulence pathways), *rsmA* (an RNA-binding post-transcriptional regulator that influences
200 expression of efflux systems), *PmrF* (also known as *arnT*, an L-Ara4N transferase that modifies
201 lipid A and contributes to polymyxin resistance), *emrR* (a repressor of the EmrAB efflux pump
202 system, whose loss or mutation leads to over-expression of the pump), *marA* (a transcriptional
203 activator of the Mar regulon, increasing expression of multiple efflux pumps and reducing porin
204 production), *KpnE/F/G* (components of the KpnEFG efflux pump system in *Klebsiella*, conferring
205 multidrug efflux capacity), and *vanG* (a vancomycin-resistance operon normally described in
206 Gram-positives, suggesting either horizontal gene acquisition or a cryptic/vestigial element).

M. lentus S62 isolate harbored multiple resistance- and regulation-associated genes. These included *efrA* (encodes EfrAB efflux pump contributing to fluoroquinolone and macrolide resistance), *fosI* (encodes fosfomycin-inactivating enzyme). *vanY/vanT/vanR/vanXY* (Vancomycin resistance operon components), *sepA* and *sdrM* (small multidrug resistance and MFS pumps), *emrR* and *AcrS* (regulators of EmrAB and AcrAB-TolC efflux systems), *rsmA*, (RNA-binding post-transcriptional regulator influencing efflux and virulence gene expression). Finally, the strain harbored also regulatory and stress-response genes *gadX* and *leuO* (that may indirectly modulate resistance), *salB* gene (associated to biofilm formation).

In the other hand the *M. lentus* strain S104, harbored a diverse repertoire of antimicrobial resistance and virulence-associated genes. Indeed, it contained *mecC*-type (methicillin-resistance) and *mecI* and *blaZ* genes (β-lactamase production), conferring broad resistance to β-lactam antibiotics. It harbored also the *qacG* gene that encodes a small multidrug resistance (SMR) efflux pump providing tolerance to quaternary ammonium compounds and other biocides. Additionally, it contained *sdrM* and *sepA* genes that encode multidrug efflux transporters implicated in reduced susceptibility to fluoroquinolones and other antimicrobial agents. Despite the absence of complete *van* operon, the strain harbored *vanY* and *vanT* genes. As biofilm is also considered as enhancer of antimicrobial resistance, Resfinder identified the *salB* gene, associated with biofilm formation and adherence, highlights the strain's potential to persist in host and environmental niches (Table 2).

Similarly the the *M. sciuri* S86 carried a broad set of resistance determinants conferring multidrug resistance. The presence of *vanY*, *vanT*, *vanW*, and *vanR* indicates a vancomycin resistance operon, while *tet*(45) and *tetB*(P) mediate tetracycline resistance through ribosomal protection and efflux mechanisms, respectively. The genes *aph(6)-Id*, *BcI*, and *BcII* encode aminoglycoside- and β-lactam-modifying enzymes, respectively. Efflux-related genes *sdrM*, *sepA*, and *adeF* contribute to decreased susceptibility to multiple drug classes, and *fosB* confers resistance to fosfomycin.

231 Finally, the *S. schleiferi* S46 harbored the *sepA* gene and a *fusE* mutation. The *sepA* gene encodes a
232 small multidrug efflux transporter belonging to the major facilitator superfamily (MFS), contributing
233 to decreased susceptibility to various antimicrobial compounds, including fluoroquinolones and dyes.
234 The *fusE* mutation, affecting elongation factor G (EF-G), is known to confer resistance to fusidic
235 acid.

236 **3.3.3** Phylogeny of strains according to MLST and/or Ribosomal Multilocus Sequence Typing
237 (rMLST)

238 The *E. faecalis* E67 strain was assigned to sequence type (ST) 19, corresponding to ribosomal
239 sequence type (rST) 18465 (Table 2). The lineage is internationally distributed lineage, although it is
240 not always described as a "pandemic clone" in the strictest sense. As MLST is not yet established for
241 the *E. casseliflavus* species, the *E. casseliflavus* E58 strain was assigned to the rST-261312 lineage.
242 The *K. aerogenes* V68 was assigned to ST242 and to rST-209866, an ST rarely reported globally.
243 The *M. lentis* S104 strain belonged to rST-170586 **;** however, the lineage of *M. lentis* S62 was not
244 determined. The *M. sciuri* S86 strain belonged to ST121, a lineage documented in animal-associated
245 studies of methicilllin-resistant *M. sciuri* populations. Finally, the lineage of *S. schleiferi* S46 was
246 not determined (Table 2).

247 **3.3.5 Plasmid content, transposons and CRISPR-Cas systems**

248 Plasmids were not detected in the seven isolates; however, transposon-associated sequences were
249 detected only in *E. casseliflavus* E58 (type Tn*916/1545*). CRISPR-Cas systems were variably
250 distributed among isolates. Several isolates, including *E. faecalis* E67 and *M. lentus* S104, carried
251 type I and type II Cas clusters, whereas other isolates such as *S. schleiferi* S46 and *K. aerogenes* V68
252 contained only type I signatures. *M. sciuri* showed numerous CRISPR arrays, which suggest
253 improved resistance against mobile genetic elements (Table 2).

254 **4. Discussion**

In this study, UTIs were observed primarily in young adult male cats aged 2–5 years, reflecting the known predisposition of males to feline lower urinary tract disease, however, female sex and age are considered as risk factors of FLUTD (Dorsch et al., 2019). According to several studies, feline UTIs are more common in cats older than ten years, who have undergone perineal urethrostomy or urinary catheterization or possess comorbidities (e.g., chronic renal failure or diabetes mellitus) (Dorsch et al., 2019; Fonseca et al., 2021; Yudhanto et al. 2025). Clinically, pollakiuria and hematuria were the most frequently reported signs, followed by lower urinary tract symptoms, urinary incontinence, malodorous urine, and other general urinary abnormalities, indicating a spectrum of LUTI manifestations in affected animals as reported previously (Dorsch et al., 2019 ; Sakauchi et al., 2025; Van Bohemen et al., 2025). Microbiologically, seven isolates were recovered from six diseased domestic cats, including two isolates from a single urine sample, demonstrating that feline UTIs can be polymicrobial. The bacterial species identified encompassed both Gram-positive and Gram-negative taxa. In a recent study in USA, from urine samples (n =2062) of cats with urinary tract infections in Illinois, 2020–2022, the most common Gram-positive bacterial species were *E. faecalis* (6.07%), *S. felis* (2.47%), *S. pseudintermedius* (1.38%), and *E. faecium* (1.38%). However, the most common Gram-negative bacteria were *E. coli* (15.89%), *Proteus mirabilis* (0.89%), and *Pasteurella multocida* (0.59%) (Yudhanto et al. 2025). Overall, the diversity highlights the multifactorial etiology of feline UTIs and underscores the importance of comprehensive bacterial identification to guide antimicrobial therapy (Hansen-Jones et al., 2018; Jousserand et al., 2025).

The antimicrobial susceptibility profiles of the feline urinary isolates reveal a concerning pattern of multidrug resistance across both Gram-positive and Gram-negative species. *M. lentus* (S62 and S104) and *S. schleiferi* S46 exhibited resistance to multiple β-lactams and other commonly used antibiotics, including clindamycin, norfloxacin, and trimethoprim, which may compromise the efficacy of first-line treatments for feline UTIs. Similarly, *M. sciuri* S86 carried resistance to erythromycin,

279 clindamycin, tetracycline, fosfomycine. Previous studies have also highlighted the occurrence of such
280 resistance phenotypes in these *Staphylococcus* spp. strains as well as those previously classified in
281 the staphylococci species (Darwich et al. 2021; Yudhanto et al. 2025). For enterococci,
282 cephalosporins, aminoglycosides, clindamycin, and trimethoprim-sulfamethoxazole may be active in
283 vitro but are not clinically effective. Therefore, antibiotics belonging to these classes are not
284 recommended for enterococcal UTI treatment (Tumpa et al 2022; Yudhanto et al. 2025). An increase
285 in resistance to antimicrobials recommended to treat UTIs in cats, including amoxicillin, might
286 hinder therapeutic strategies for enterococcal infections of the urinary tract and may lead to the
287 selection of resistant bacterial strains (Weese et al., 2019). Moreover, UTIs caused by MDR
288 enterococci are difficult to treat with currently available antimicrobials, and these strains are capable
289 of acquiring new resistance mechanisms, which might further limit treatment success (Dorsch et al.,
290 2019). The high-level resistance observed in *E. faecalis* E67 to ampicillin, linezolid, tetracycline,
291 erythromycin, and streptomycin is particularly concerning, as it encompasses drugs considered
292 critically important in human medicine. Similarly, *E. casseliflavus* E58 was resistant to linezolid and
293 tetracycline, in addition to its intrinsic resistance to glycopeptides (vancomycin and teicoplanin).
294 Despite linezolid is not used in veterinary medicine, the emergence of such linezolid-resistant
295 enterococci in companion animals is alarming since such strains can be transmitted between humans
296 and animals (Abdullahi et al., 2023). The unique Gram-negative strain, *K. aerogenes* V68,
297 demonstrated broad-spectrum resistance, including to β-lactams, carbapenems, fosfomycin,
298 tetracycline, and fluoroquinolones. Previously classified as *Enterobacter aerogenes*, *K. aerogenes* is
299 actually more closely related to *Klebsiella pneumoniae* than *Enterobacter* species, according to
300 genome-based analyses (Feng et al., 2024). In humans, *K. aerogenes* is a multidrug resistance
301 opportunistic pathogen that causes a variety of infections such as bloodstream infections, pneumonia,
302 and urinary tract infections, the majority of which are healthcare associated (Feng et al., 2024; Sun et

al. 2024). Cimilarly to our strain, several studies have reported MDR isolates from animal sources such as swine (Sun et al. 2024) and companion animals (dogs and cats) (Lee et al. 2021).

These findings underscore the clinical challenge of treating feline UTIs caused by multidrug-resistant strains and highlight the potential for companion animals to act as reservoirs of clinically relevant AMR genes. From a One Health perspective, the co-occurrence of resistance to critically important human antimicrobials in these isolates raises the risk of interspecies transmission, either directly to humans or indirectly through environmental dissemination. These results emphasize the need for prudent antimicrobial use in veterinary medicine and the integration of companion animal surveillance into broader One Health antimicrobial stewardship programs.

The genomic analysis of the two *Enterococcus* isolates revealed distinct antimicrobial resistance profiles reflecting their clinical significance. *E. faecalis* E67 harbored a wide array of resistance determinants, including *lsaA*, *dfrE/dfrG*, *vanT*, *tet(M)*, *tet(45)*, *ermB*, *aad(6) aph(3')*-IIIa, *sat*-4, and multiple multidrug efflux pumps (*efrA, emeA*). This extensive resistome indicates a high-level multidrug-resistant phenotype, posing challenges for therapeutic management of feline UTIs and highlighting its potential as a reservoir of clinically important resistance genes. In contrast, *E. casseliflavus* E58 carried only intrinsic vancomycin-associated genes (*vanC cluster*: *vanT*, *vanXY*, vanC, and *vanY* from *vanB* cluster), with no acquired resistance genes detected despite phenotypic resistance to tetracycline and linezolid. This discrepancy suggests the presence of non-characterized or mutation mechanisms in chromosomal genes. For instance, in the absence of the acquired resistance genes *optrA* and *poxtA*, linezolid resistance has been attributed to nucleotide substitutions in ribosomal protein–coding or regulatory genes such as *rplC*, *rplD*, and *rplV*, resulting in amino acid changes in ribosomal proteins L3, L4, and/or L22 (Bender et al., 2018).

The resistome of *K. aerogenes* V68 was extensive and fully concordant with its observed multidrug-resistant phenotype. The strain carried $bla_{\text{CMY-132}}$, an AmpC β-lactamase conferring resistance to

cephalosporins, and multiple efflux pump determinants, including *oqxA*, *KpnE/F/G*, *emrR*, and *marA*, which collectively contribute to reduced susceptibility to fluoroquinolones, β-lactams, and other antimicrobial classes. It is worth noting, that the *bla*$_{\text{CMY-132}}$ is rarely reported in Enterobacterales in contrast to the commonly reported *bla*$_{\text{CMY-2}}$ (Foudraine et al., 2021). Resistance to fosfomycin was mediated by *fosA5*, while *erm*(43) accounted for macrolide-lincosamide-streptogramin B resistance. Lipid A-modifying enzymes, *eptB* and *PmrF*, suggest potential tolerance to polymyxins, a last-resort therapeutic class. Regulatory genes such as *leuO*, *CRP*, and *rsmA* indicate that global stress response and efflux regulation may further enhance multidrug resistance. Interestingly, the detection of *vanG*, typically associated with Gram-positive bacteria (mainly enterococci), may reflect horizontal gene transfer or a vestigial operon, highlighting the genetic plasticity of *K. aerogenes*. These findings align with previous reports indicating that *Klebsiella aerogenes* oringinated from various sources including animal origin, can serve as a reservoir for genes encoding AmpC β-lactamases, aminoglycoside-modifying enzymes, efflux-mediated resistance determinants, regulatory elements, and a variety of other resistance markers, including *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{OXA-181}}$, *bla*$_{\text{OXA-10}}$, *aph(3')*-Ia, *aph(4)*-Ia, *aac(3)*-IV, *aadA1*, *aadA2*, *strA*/B, *qnrS1*, *cmlA1*, *sul3*, *dfrA14*, *arr*-2, *tetA*, *fosA*, and *floR*, that potentiate multidrug resistance (Merhi et al. 2023; Sun et al. 2024).

The genomic characterization of *M. lentus* (S62 and S104) and *M. sciuri* S86 strains highlights their potential as reservoirs of multidrug resistance determinants. *M. lentus* S62 harbored a broad set of resistance- and regulation-associated genes. Similarly, *M. lentus* S104 exhibited methicillin resistance (*mecC*-type, *mecI*, *blaZ*), biocide tolerance (*qacG*), multidrug efflux (*sepA*, *sdrM*), vancomycin-associated genes (*vanY*, *vanT*), and biofilm potential (*salB*), demonstrating a combination of intrinsic, acquired, and adaptive resistance mechanisms. To the best of our knowledge this the first Tunisian's report of *mecC* in *M. lentus*. Interestingly, in a neighboring country, Algeria, Belhout *et al.* (2023) reported the co-occurrence of *mecA* and *mecC* genes in five methicillin-resistant *M. lentus* strains isolated from the feces of healthy dromedary camels. In

Tunisia, Dhaouadi et al. (2022) have previously reported *mecC* in methicilllin-resistant/MDR *M. sciuri* strains collected from horses with skin infections. In our study, the *M. sciuri* S86 strain carried components of the vancomycin resistance operon (*vanY, vanT, vanW, vanR*), tetracycline resistance determinants (*tet(45)* (efflux pump gene), *tetB(P)* (ribosomal protective gene)) , aminoglycoside- and β-lactam-modifying enzymes (*aph*(6)-Id**,** *BcI***,** *BcII*), multidrug efflux pumps (*sdrM*, sepA, *adeF*), and *fosB*, conferring fosfomycin resistance. Collectively, these findings indicate that *M. lentus* and *M. sciuri* strains from feline UTIs harbor multifactorial resistance determinants, including genes encoding resistance to critically important antibiotics, efflux systems, biofilm formation, and stress adaptation. Clinically, such isolates may compromise therapeutic options for companion animals, while from a One Health perspective they represent potential reservoirs for horizontal gene transfer to other bacteria, including zoonotic pathogens.

The detection of the efflux pump gene *sepA* (staphylococcal efflux pump gene) alongside a *fusE* mutation in *S. schleiferi* S46 underscores the multifactorial basis of antimicrobial resistance in this companion-animal pathogen. The *sepA* gene, part of the small multidrug resistance (SMR) family, has been shown to mediate extrusion of dyes (e.g., ethidium bromide) and antiseptics and is implicated in decreased susceptibility not only to disinfectants but also to fluoroquinolones and other antimicrobial classes (Narui et al., 2003; Bay et al. 2008). While most studies of *S. schleiferi* have focused on methicillin resistance (e.g., *mecA*, *mecC*) and β-lactamase production in canine and human isolates, reports of efflux-mediated resistance remain rare in this species. The presence of a *fusE* mutation, which affects elongation factor G (EF-G), aligns with mechanisms documented for fusidic acid resistance in staphylococci (González-López and Selmer, 2025).

Taken together, the resistomes of the seven strains reveal a remarkable diversity of antimicrobial resistance mechanisms, including efflux pumps, β-lactamases, vancomycin resistance operons, aminoglycoside- and tetracycline-modifying enzymes, and regulatory genes that enhance survival

under antimicrobial or environmental stress. This genetic diversity is clinically important because it limits therapeutic options for treating infections in companion animals and may promote persistence and recurrence.

The MLST and rMLST analyses revealed that the feline urinary strains belong to diverse and clinically relevant lineages. *E. faecalis* E67 was assigned to ST19 and rST-18465. The ST19 is a globally distributed lineage associated with multidrug resistance in both human and animal infections, underscoring its potential zoonotic relevance (Larsen et al., 2011). *E. casseliflavus* E58 was assigned to rST-261312**,** reflecting the limited availability of MLST data for this species but highlighting its distinct genetic background.

Klebsiella aerogenes V68 was assigned to ST242 (rST-209866)**.** A comprehensive search of the literature and genome databases revealed that this clone has been reported only once, by Loncaric ***et al.*** (2024)**.** In that study, the strain *K. aerogenes* 22b ctx, isolated from the cloaca of a nestling osprey (*Pandion haliaetus*) at Mono Lake, California (USA), exhibited both AmpC and ESBL phenotypes and carried the bla_{CMY} and *fosA5* resistance genes. Globally, the most frequently described *K. aerogenes* lineages include ST4, ST53, ST93, ST143, ST209, and ST210 (Malek *et al.*, 2019; Merhi *et al.*, 2023).This suggests that companion animals may serve as reservoirs for under-characterized or emerging lineages.

M. lentus S104 was assigned to rST-170586**,** whereas the lineage of *M. lentus* S62 remained undetermined, highlighting gaps in reference databases for this species. *M. sciuri* S86 was classified as ST121**,** a lineage previously reported in methicillin-resistant animal isolates (Boonchuay et al., 2023), supporting its role as a potential reservoir for clinically relevant resistance genes. Finally, the lineage of *S. schleiferi* S46 could not be determined, reflecting limited MLST data for this species. Collectively, these findings demonstrate that feline UTI pathogens comprise genetically diverse lineages, some of which are associated with multidrug resistance and zoonotic potential.

Genomic analysis revealed that plasmids were absent in all seven isolates, suggesting that horizontal gene transfer *via* plasmids may not be the primary mechanism driving resistance in these feline urinary pathogens. However, transposon-associated sequences were detected in *E. casseliflavus* E58 (type Tn916/1545), a well-characterized conjugative element known to mediate the dissemination of tetracycline and macrolide resistance genes, indicating that mobile genetic elements still play a role in the spread of resistance in certain isolates (Lengliz et al., 2022). The variable distribution of CRISPR-Cas systems among isolates further underscores the heterogeneity of genomic defense and adaptation mechanisms within feline uropathogens. CRISPR-Cas loci, which provide adaptive immunity against foreign genetic elements such as phages and plasmids, are thought to influence horizontal gene transfer (HGT) dynamics and, consequently, the dissemination of antimicrobial resistance determinants (Garneau et al. 2010). The uneven presence of these systems suggests lineage-specific evolutionary pressures, where certain strains may prioritize acquisition of mobile genetic elements (MGEs) over defense, thereby enhancing genetic plasticity. In contrast, isolates with active CRISPR-Cas systems may exhibit restricted gene flow but improved genomic stability. Collectively, these observations indicate that, although plasmid-mediated resistance appears limited in these isolates, transposons, integrative elements, and other MGEs remain central drivers of gene flux and adaptive evolution. The interplay between these elements and CRISPR-Cas–mediated immunity likely defines the balance between genomic innovation and defense, shaping the resistome architecture of these bacteria. This dual role, facilitating resistance gene acquisition while modulating its spread, highlights a complex evolutionary equilibrium that contributes to the persistence and emergence of multidrug-resistant lineages. Understanding these dynamics is essential not only for elucidating resistance evolution in companion animals but also for assessing their potential role as reservoirs bridging veterinary and human antimicrobial resistance networks.

5. Conclusion

This study represents the first genomic investigation in Tunisia to employ whole-genome sequencing for the characterization of feline urinary pathogens. The findings revealed multidrug-resistant *Enterococcus*, *Staphylococcus*, *Mammaliicoccus* and *Klebsiella aerogenes* exhibiting clear zoonotic potential. The detection of last-resort resistance determinants underscores the urgent need to incorporate companion animals into One Health–based antimicrobial resistance surveillance frameworks.

To advance our understanding of antimicrobial resistance evolution and transmission, future studies should focus on plasmid epidemiology, cross-species gene flow, and comparative genomics with human clinical isolates. Such integrative approaches will be critical to elucidate the molecular mechanisms driving resistance dissemination and to better assess the public health implications of antimicrobial resistance at the human–animal interface.

Figures

Figure 1: One Health framework of resistant feline uropathogen transmission between domestic cats, environment, and human owners.

Figure 2: Summary of signalment, underlying disease conditions, urine collection methods and initial lower urinary tract signs.

Figure 3. Integrated Heatmap of AMR resistance Profiles observed in pathogens bacteria isolated from cats with urinary tract infection.

Tables

Table 1: Clinical characteristics of six cats with Urinary Tract Infection with identification of bacterial species

Table 2. Genomic features, antimicrobial resistance profiles, and CRISPR-Cas profiles of bacterial isolates from cats with urinary tract infections.

Data Availability Statement

All relevant data supporting the findings of this study are available in public repositories. Genome sequences have been deposited in the NCBI BioSample database under accession numbers, (Submission ID: SUB15682498). SRA records were accessible with the following: https://www.ncbi.nlm.nih.gov/sra/PRJNA1337466.

Ethics statement

All procedures involving the restraint and sampling of cats were performed in accordance with European legislation on animal ethics and welfare. Urine samples were collected by veterinarians from cats presenting with urinary tract infections, using gentle restraint to minimize discomfort and prevent unnecessary suffering. Verbal informed consent was obtained from the cat owners prior to inclusion in the study.

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Conflicts of Interest

The authors declare that they have no potential conflicts of interest with respect to the research, authorship and publication of this article.

Institutional Review Board Statement

The study was approved by the Ethics Committee CEEA of the National School of Veterinary Medecine of Sidi Thabet (89-CEEA-ENMV/25, 17 April 2025).

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Author Contributions*:*

Hajer Kilani, and Zaineb Hamzaoui contributed to the conceptualization of the study. Methodology was developed by Hajer Kilani, Sana Ferjani, Sana Lengliz and Lamia Kanzari. Formal analysis and data interpretation were performed by Hajer Kilani and Zaineb Hamzaoui. The original draft was prepared by Hajer Kilani, and the manuscript was reviewed and edited by Mohamed Salah Abbassi, Ghassan Tayh and Faten BenChedhida. Supervision was providedby Ilhem Boutiba-Ben Boubaker. All authors read and approved theFinal manuscript.

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Figure 1: One Health framework of resistant feline uropathogen transmission between domestic cats, environment, and human owners.

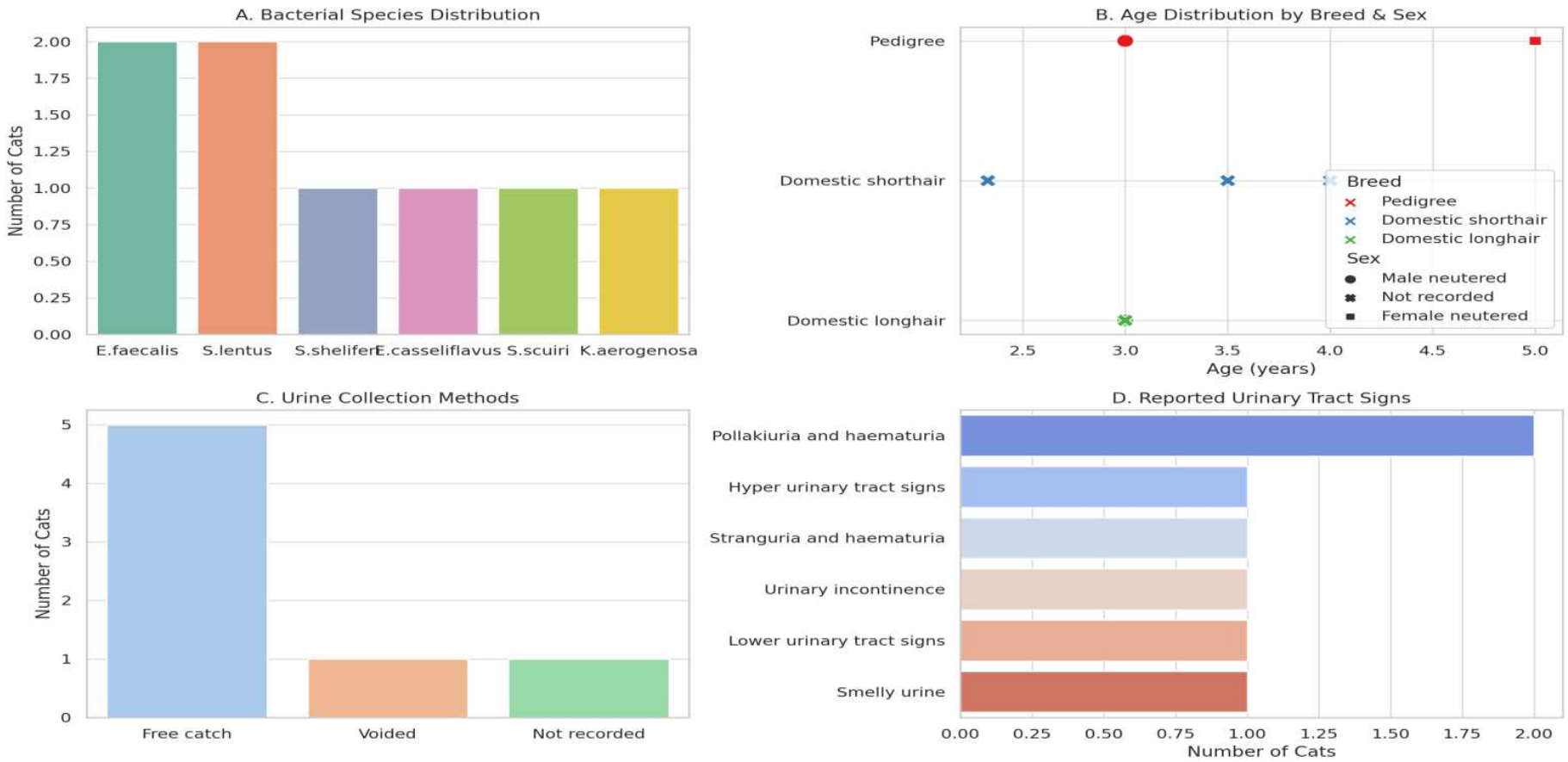


Figure 2. Summary of signalment, underlying disease conditions, urine collection method and initial presenting lower urinary tract signs identified. A. Bacterial Species Distribution → bar chart showing the frequency of isolates per species B. Age Distribution by Breed & Sex → scatter plot of ages, grouped by breed (all cats were male neutered) C. Urine Collection Methods → bar chart of sampling strategies (Free catch, Voided) D. Reported Urinary Tract Signs → bar chart showing the distribution of clinical symptoms.

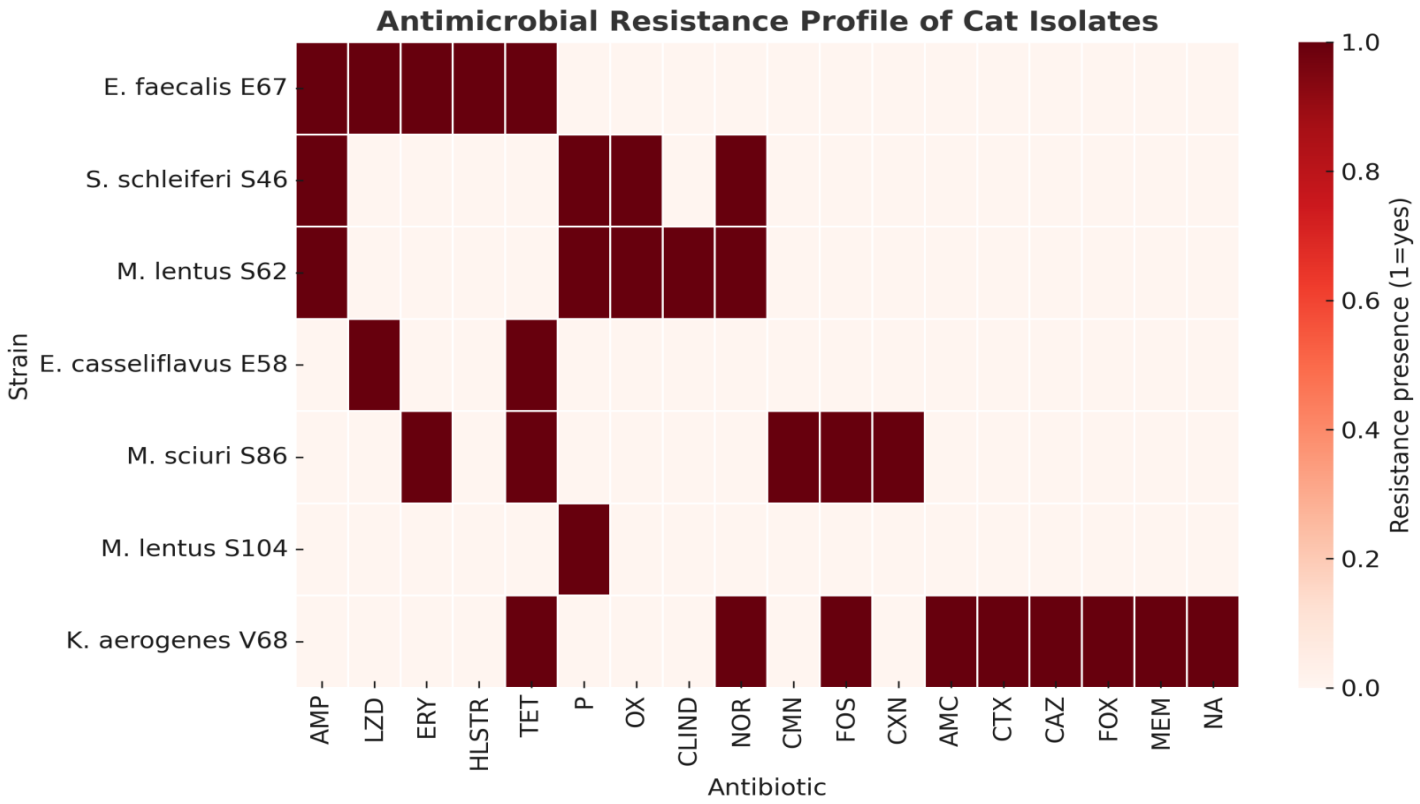


Figure 3. Integrated Heatmap of AMR resistance Profiles observed in pathogens bacteria isolated from cats with urinary tract infection: Rows = strains, columns = AMR presence ,brown = presence resistance to antibiotic , white = absent. Abbreviations: AMP: ampicillin, LZD: linezolid, ERY: erythromycin, HLSTR: high-level streptomycin, TET: tetracycline, P: penicillin, OX: oxacillin, CLIND: clindamycin, NOR: norfloxacin, CMN: gentamicin, FOS: fosfomycin,CXN: cefoxitin, AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FOX: cefoxitin, MEM: meropenem, NA: nalidixic acid.

Table 1. Clinical characteristics of 6 cats with Urinary Tract Infection with identification of bacteria species

Cat / Clinical caracteristics	**Race**	**Sex**	**Age**	**Breed**	**urinary tract signs**	**Method of urine collection**	**Species**
Cat 1	cross	Male neutered	3 years	Pedigree	Hyer urinary tract signs	Free catch	*E. faecalis*
Cat 2	cross	Male neutered	3 years and 4 months	Domestic shorthair	Stranguria and haematuria	Free catch	*S. sheliferi*
Cat3	common	Male neutered	3 years	Domestic longhair	Pollakiuria and haematuria	Voided	*S. lentus* *E. casseliflavus*
Cat 4	cross	Male neutered	4 years	Domestic shorthair	Urinary incontinence	Free catch	*S. scuiri*
Cat 5	cross	Male neutered	3 years and 6 months	Domestic shorthair	Pollakiuria and haematuria	Free catch	*S. lentus*
Cat 6	siamese	Male neutered	3 years	Domestic longhair	"Smelly urine" reported	Free catch	*K. aerogenosa*

Table 2. Antimicrobial resistance, genomic features and CRISPR-Cas profiles of isolates from six cats with urinary tract infection

Cat	Strains/BioSample accessions	Resistance profile	AMR genes	Genome size/Assembly length (bp)	ST/(rST)	Cas cluster
1	*E. faecalis E67* SAMN52299041	AMP, LZD, ERY, STR, TET	*lsaA, dfrE/dfrG, vanT, efrA, tet(M), tet(45), emeA, aad(6), SAT-4, aph(3')-IIIa, ermB*	~2.8 Mbp (2862000)	ST19 *(r ST-18465)*	*Cas1* (Type II), *Cas3* (Type I), *Cas9* (Type II)
2	*S. schleiferi S46* *SAMN52299036*	P, AMP, OX, NOR, TMN	*sepA, fusE mutation*	~2.0 Mbp (1958906)	ND	*Cas4* + *Cas9* (Type II), *1 array*
3	*M. lentus* (*S. lentus*) S62 SAMN52299040	P, AMP, OX, CLIND, NOR	*efrA, fosI, vanY/vanT/vanR/vanXY, sepA, sdrM, gadX, salB, erm(43), emrR, leuO, acrS, rsmA*	~2.7 Mbp (2771135)	ND	cas3 Type I
	E. casseliflavus E58 SAMN52299038	LZD , TET	*vanT-, vanXY-, vanT-, vanC* genes in *vanC* cluster, *vanY gene in vanB cluster*	~3.0 Mbp (3093305)	r ST-261312	Cas Type I/II
4	*M. sciuri* (*S. sciuri)* S86 SAMN52299039	ERY , CMN, FOS, CXN , TET	*vanY/vanT/vanW/vanR, tet(45), tetB(P), aph(6)-Id, BcII, BcI, sdrM, sepA, adeF, fosB*	2.8 Mbp (2812910)	ST121	*Cas3 Type I* (2 arrays)

5	*M. lentus* (*S. lentus*) S104 SAMN52299037	P	*mecC-type, blaZ, mecI, salB, qacG, vanY/vanT, sepA, sdrM*	~2.8 Mbp (2856337)	r ST-170586	*Cas* type IA and IIIB (2 arrays)
6	*K. aerogenes* V68 SAMN52299042	AMC, CTX, CAZ, FOX, MEM, FOS, TET, NA	*bla*$_{CMY-132}$*, eptB, leuO, CRP, rsmA, fosA5, PmrF, oqxA, emrR, marA, KpnE/F/G, vanG*	~4.9 Mbp (4941207)	ST242 (rST-209866)	*Cas3* Type I

Abbreviations : AMP: ampicillin, LZD: linezolid, ERY: erythromycin, STR: streptomycin, TET: tetracycline, P: penicillin, OX: oxacillin, CLIND: clindamycin, NOR: norfloxacin, TMN: tobramycin, CMN: gentamicin, FOS: fosfomycin, CXN: cefoxitin, AMC: amoxicillin–clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FOX: cefoxitin, MEM: meropenem and NA: nalidixic acid. ND: No determined.