| ORIGINAL SCIENTIFIC ARTICLE |

Identification of Bacterial Isolates from Canine Urine Samples by MALDI-TOF MS and Determination of Their Antimicrobial Susceptibility Profiles

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Abstract

Urinary tract infections are common bacterial infections in dogs and frequently require antimicrobial therapy in small-animal practice. Accurate identification of bacterial agents and determination of antimicrobial susceptibility profiles are essential for rational therapeutic decision-making. This study aimed to identify bacteria isolated from urine samples of dogs evaluated for suspected urinary tract infection using MALDI-TOF MS and to determine their antimicrobial susceptibility profiles. Urine samples from 30 dogs were examined bacteriologically. Samples were first enriched in brain heart infusion broth and then inoculated onto 5% sheep blood agar, MacConkey agar, and eosin methylene blue agar. Pure colonies were identified by MALDI-TOF MS. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method with amoxicillin-clavulanic acid, amikacin, ampicillin, ceftiofur, cefpodoxime, doxycycline, enrofloxacin, nitrofurantoin, and sulfamethoxazole-trimethoprim discs. Bacterial growth was detected in 17 of 30 urine samples (56.6%), yielding 21 bacterial isolates, of which four positive samples each yielded two bacterial isolates. MALDI-TOF MS identified eight bacterial species: *Enterococcus faecalis* (n=4, 19.0%), *Staphylococcus intermedius* (n=4, 19.0%), *Staphylococcus haemolyticus* (n=3, 14.3%), *Micrococcus luteus* (n=3, 14.3%), *Escherichia coli* (n=2, 9.5%), *Staphylococcus pseudintermedius* (n=2, 9.5%), *Streptococcus canis* (n=2, 9.5%), and *Enterococcus faecium* (n=1, 4.8%). The highest resistance rates were observed against sulfamethoxazole-trimethoprim (76.2%), ceftiofur (57.1%), and enrofloxacin (47.6%). Multidrug resistance was detected in 12 isolates (57.1%). These findings indicate that bacterial isolates recovered from canine

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urine samples may exhibit considerable variation in antimicrobial susceptibility. MALDI-TOF MS identification of cultured isolates combined with antimicrobial susceptibility testing may support microbiological evaluation and rational antimicrobial use in dogs with suspected urinary tract infection.

Keywords: *antimicrobial resistance; canine urine; dog; MALDI-TOF MS; multidrug resistance; urinary tract infection.*

Introduction

The urinary system plays an essential role in the elimination of metabolic waste products and in the maintenance of fluid, electrolyte, and acid-base balance. Disorders affecting this system are therefore clinically important in veterinary medicine and may have substantial effects on animal health (Cavrenne and Mai, 2009). Urinary tract infection is a common clinical problem in dogs and a frequent reason for antimicrobial use in small-animal practice. Approximately 14% of dogs experience at least one episode of urinary tract infection during their lifetime (Ling, 1984). *Escherichia coli* is frequently reported as the most common bacterial agent associated with canine urinary tract infections. Other bacteria, including *Staphylococcus* spp., *Enterococcus* spp., *Proteus* spp., *Streptococcus* spp., *Klebsiella* spp., and *Pasteurella multocida*, have also been described among urinary tract pathogens in dogs. Most infections are considered to develop via an ascending route, whereas haematogenous and lymphatic spread are less common (Temmerman et al., 2024). In a study of canine urinary tract disease, *E. coli* was the most frequently isolated bacterium from urine samples, followed by *Staphylococcus* species (Windahl et al., 2014). Increasing antimicrobial resistance among bacterial urinary tract pathogens in dogs is a significant challenge for treatment. Empirical antimicrobial treatment without consideration of susceptibility testing may contribute to therapeutic failure and selection of resistant strains. Furthermore, close contact between companion animals and humans may facilitate the circulation of antimicrobial-resistant bacteria in shared environments, highlighting the relevance of antimicrobial resistance in canine urinary bacteria within a One Health framework (Guardabassi et al., 2004). Therefore, identification of bacteria isolated from canine urine samples and determination of their antimicrobial susceptibility profiles are important for both clinical treatment decisions and antimicrobial resistance monitoring. Bacterial culture and antimicrobial susceptibility testing remain fundamental approaches in the microbiological evaluation of suspected urinary tract infections in dogs. In recent years, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has become increasingly used in microbiology laboratories for the rapid identification of bacteria isolated from culture (Croxatto et al., 2012; Moreno et al., 2018; Svetličić et al., 2022). MALDI-TOF MS is considered an alternative to conventional or automated bacterial identification systems; however, antimicrobial susceptibility testing is still required to support appropriate therapeutic decisions.

This study aimed to identify bacterial isolates recovered from urine samples of dogs with suspected urinary tract infection using MALDI-TOF MS and to determine their antimicrobial susceptibility profiles.

Materials and Methods

Study material and sample collection

This laboratory-based study was conducted using urine samples submitted to the microbiology laboratory from 30 client-owned dogs evaluated for suspected urinary tract infections at various veterinary clinics and animal hospitals in the provinces of İzmir and Aydın, Türkiye. Dogs were considered to have a suspected urinary tract infection based on the clinical evaluation of the attending veterinarians. Clinical suspicion was based on signs compatible with lower urinary tract disease, such as dysuria, pollakiuria, haematuria, stranguria, or abnormal urine appearance, when recorded in the clinical history. Urine samples were collected aseptically by attending veterinarians using routine urine collection methods, including cystocentesis, catheterisation, or free-catch collection. The ethical protocol of the study was evaluated by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK), and no ethical objection was reported (Decision no. 64583101/2015/103, 14 August 2015). After collection, the samples were transported under cold-chain conditions to the Routine Diagnosis Laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, and processed for bacteriological analysis. Because the samples were obtained as routine diagnostic submissions from multiple clin-

ical settings, urine collection reflected real-world clinical practice rather than a standardised prospective sampling protocol. Accordingly, the findings were interpreted as culture-based bacterial isolates recovered from submitted urine samples of dogs with suspected urinary tract infection. Detailed urinalysis findings, previous antimicrobial treatment history, recurrent infection status, and comorbidities were not consistently available in the submitted clinical records.

Bacterial isolation and culture conditions

Urine samples submitted to the laboratory underwent pre-enrichment for bacterial isolation. For this purpose, 0.1 mL of each urine sample was inoculated into brain heart infusion broth and incubated at 37°C for 12 h. Pre-enrichment was used to improve bacterial recovery before culture-based identification, particularly because the samples were received as routine diagnostic submissions from various clinical settings. However, because enrichment may increase the recovery of low-abundance bacteria and may influence the interpretation of bacterial growth in urine samples, the results were evaluated at the isolate level rather than as quantitative diagnostic urine culture results. After enrichment, samples were streaked onto 5% sheep blood agar, MacConkey agar, and eosin methylene blue agar. The plates were incubated aerobically at 37°C for 24–48 h. After incubation, colonies were evaluated for morphological characteristics and subcultured onto tryptic soy agar plates to obtain pure cultures. Purified colonies were examined by Gram staining and classified as Gram-positive or Gram-negative. Isolates obtained from pure cultures were then subjected to MALDI-TOF MS identification. Quantitative urine culture was not performed, and colony-forming unit per millilitre values or diagnostic bacterial count thresholds were not used. Accordingly, bacterial growth results were interpreted as culture-based recovery of bacterial isolates from submitted urine samples of dogs with suspected urinary tract infection, rather than as microbiological confirmation of definitive urinary tract infection.

MALDI-TOF MS identification

Species-level identification of bacterial isolates obtained from pure cultures was performed by MALDI-TOF MS using the VITEK MS system (bioMérieux, France), with software version 4.1 and Knowledge Base version v4.2. Colonies purified on tryptic soy agar were sampled using a sterile 1-µL calibrated inoculating loop and spread as a thin layer onto VITEK MS target slides. For quality control and calibration, *Escherichia coli* ATCC 8739, with a known mass spectrum profile, was applied to the calibration well located in the center of each group of 16 wells on the prepared slide. Subsequently, 1 µL CHCA (α-cyano-4-hydroxycinnamic acid) matrix solution was added to each well, and the slides were allowed to dry at room temperature. After approximately 2 min, the slides were placed into the instrument. Mass spectra were obtained for each isolate, compared with the VITEK MS Knowledge Base, and the identification results were recorded.

Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles of the 21 identified isolates were determined using the Kirby-Bauer disc diffusion method. Colonies obtained from pure cultures were homogenised in brain heart infusion broth and adjusted to a 0.5 McFarland turbidity standard. A 100 µL aliquot of each bacterial suspension was evenly spread onto the surface of Mueller-Hinton agar no. 2 (HiMedia, M1084). Antibiotic discs (Oxoid, UK) were placed on the inoculated agar surface according to Clinical and Laboratory Standards Institute (CLSI) recommendations, and the plates were incubated aerobically at 37°C for 24 h. The following antibiotic discs were used for antimicrobial susceptibility testing: amoxicillin-clavulanic acid (30 µg), amikacin (30 µg), ampicillin (10 µg), ceftiofur (30 µg), cefpodoxime (10 µg), doxycycline (30 µg), enrofloxacin (5 µg), nitrofurantoin (300 µg), and sulfamethoxazole-trimethoprim (25 µg). After incubation, inhibition zone diameters were measured, and the results were interpreted as susceptible, intermediate, or resistant according to the veterinary guidelines (CLSI, 2023) and European Committee on Antimicrobial Susceptibility Testing breakpoint tables (EUCAST, 2024). Interpretive criteria were applied according to the antimicrobial agent and bacterial group whenever available in the referenced standards. For antimicrobial-bacterial group combinations without directly applicable veterinary-specific interpretive criteria, results were interpreted cautiously using the most relevant available CLSI or EUCAST criteria, and this limitation was considered during data interpretation. The breakpoint sources and interpretive approach used for the tested antimicrobial agents and bacterial groups are summarised in Supplementary Table S1.

Data evaluation

Bacterial isolation results, MALDI-TOF MS identification data, and antimicrobial susceptibility testing findings were evaluated at the isolate level. The culture positivity rate, distribution of isolated bacterial species, and antimicrobial susceptibility results were expressed as numbers and percentages. Antimicrobial susceptibility testing

Figure 1. Study workflow. The figure summarizes urine sampling; BHIB enrichment; culture-based isolation on blood, MacConkey, and EMB agars; MALDI-TOF MS identification; antimicrobial susceptibility testing; and MDR assessment. MDR: multidrug resistance; UTI: urinary tract infection; BHIB: Brain Heart Infusion Broth; EMB: Eosin Methylene Blue agar.

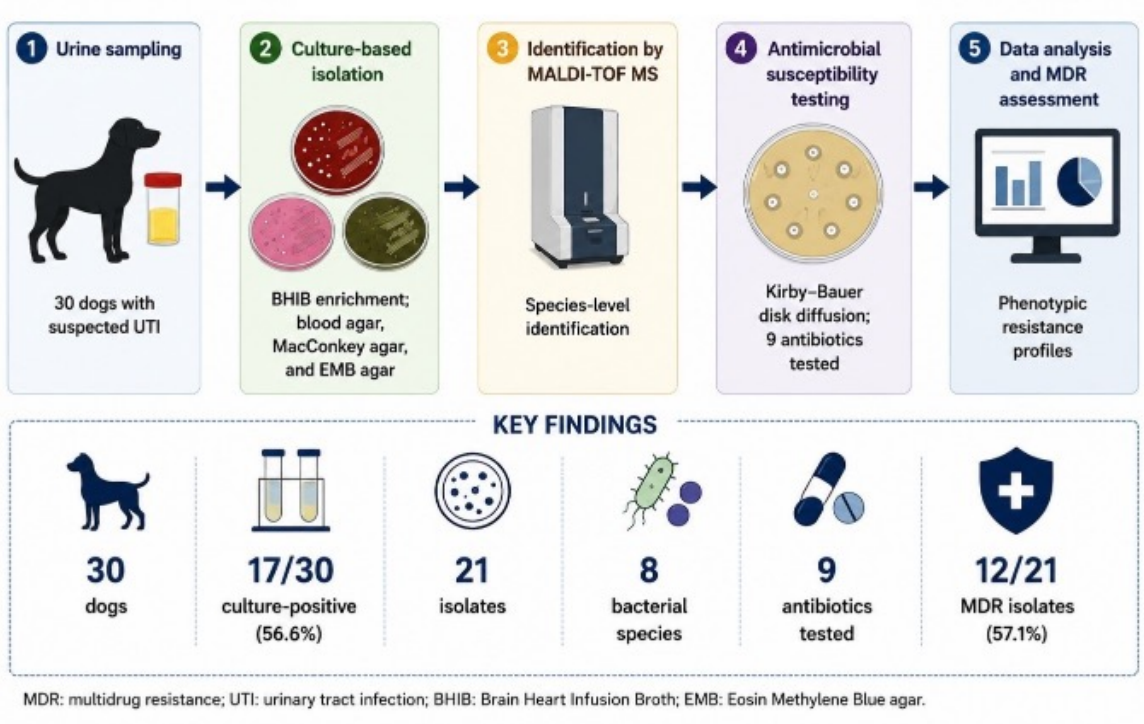
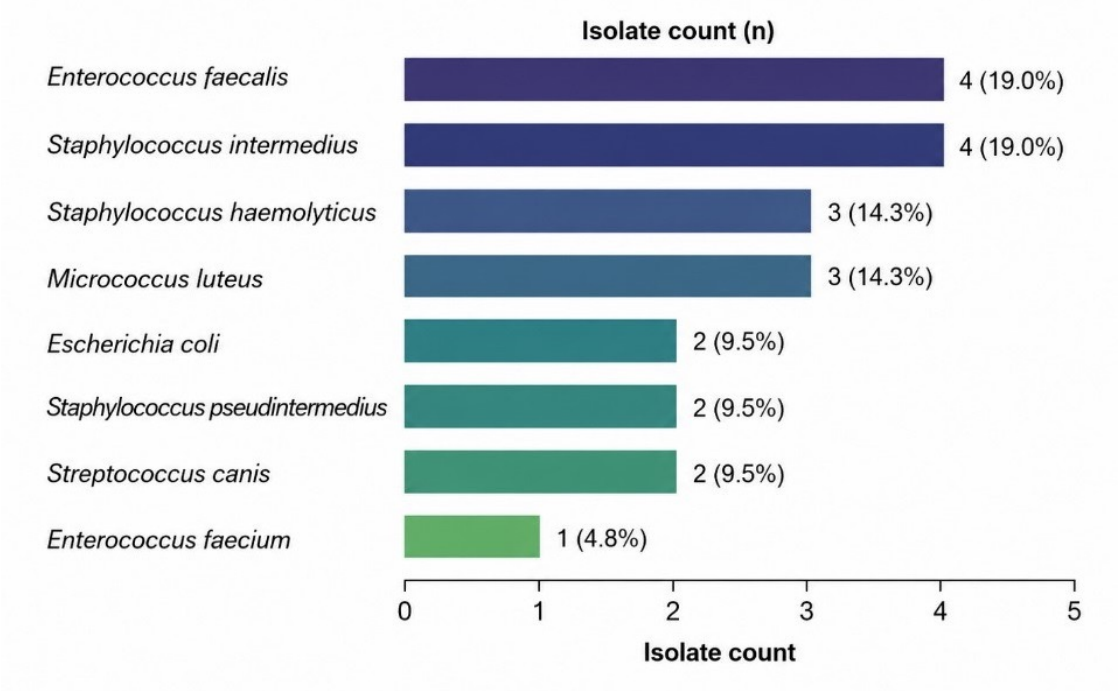


Table 1. Summary of study material and main microbiological findings.

Parameter	Value
Dogs included in the study	30
Culture-positive urine samples	17/30
Culture positivity rate	56.6%
Culture-negative urine samples	13/30
Total bacterial isolates obtained	21
Number of bacterial species identified by MALDI-TOF MS	8
Number of antibiotics tested	9
Multidrug-resistant isolates	12/21
Multidrug resistance rate	57.1%

results were classified as susceptible, intermediate, or resistant according to the interpretive criteria summarised in Supplementary Table S1. For MDR evaluation, the tested antimicrobials were grouped into the following classes/categories: penicillins/beta-lactam combinations, cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones, nitrofurans, and folate pathway inhibitors. Multidrug resistance (MDR) was defined as resistance to at least one antimicrobial agent in three or more antimicrobial classes/categories (Yamanaka et al., 2019). Intermediate results were retained as a separate interpretive category and were not counted as resistant in the MDR calculation. MDR was evaluated based on isolate-level phenotypic resistance profiles. Due to the limited sample size and small number of isolates within individual bacterial species, the analysis was restricted to descriptive statistics, and no inferential comparisons between bacterial species were performed. The general microbiological workflow of the study is summarised in Figure 1.

Figure 2. Distribution of bacterial isolates identified by MALDI-TOF MS. The figure shows the species-level distribution of 21 bacterial isolates obtained from culture-positive urine samples. Percentages were calculated based on the total number of isolates.



Results

Bacterial isolation and MALDI-TOF MS identification findings

Urine samples from 30 dogs with suspected urinary tract infection were examined microbiologically. Bacterial growth was detected in 17 of 30 urine samples (56.6%), yielding 21 bacterial isolates. Four culture-positive samples yielded two distinct bacterial isolates each. No bacterial growth was observed in 13 samples (43.3%). The study material and main microbiological findings are summarized in Table 1.

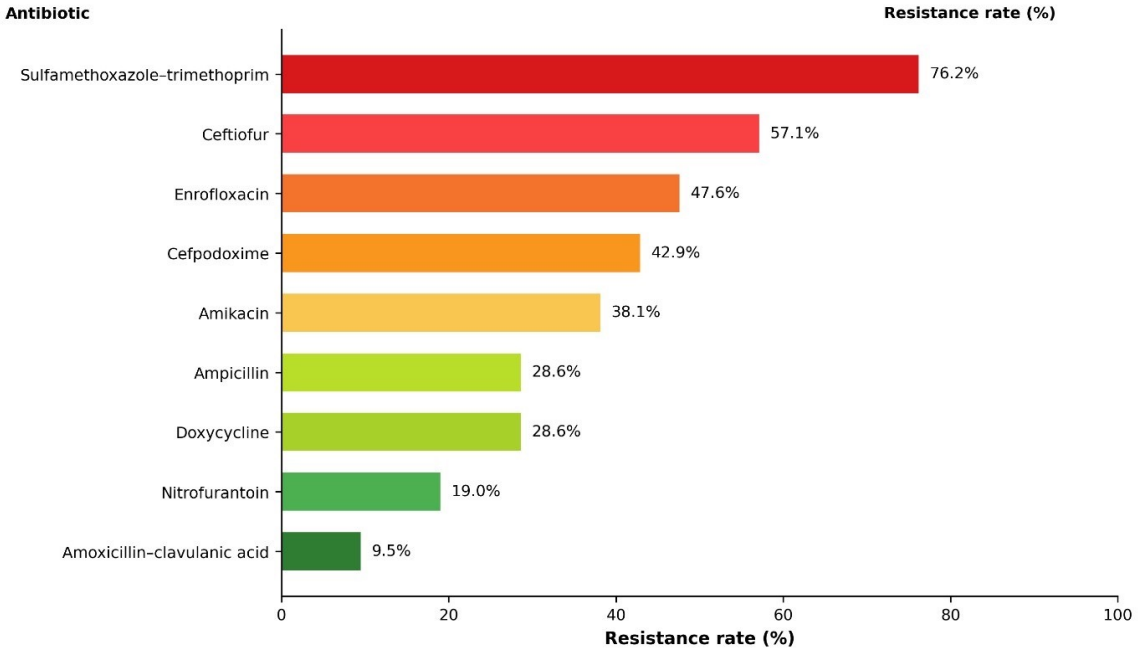
MALDI-TOF MS identification revealed that the isolates represented eight different bacterial species. The most frequently detected species were *Enterococcus faecalis* (n=4, 19.0%) and *Staphylococcus intermedius* (n=4, 19.0%), followed by *Staphylococcus haemolyticus* (n=3, 14.3%), *Micrococcus luteus* (n=3, 14.3%), *Escherichia coli* (n=2, 9.5%), *Staphylococcus pseudintermedius* (n=2, 9.5%), *Streptococcus canis* (n=2, 9.5%), and *Enterococcus faecium* (n=1, 4.8%). Overall, Gram-positive bacteria predominated among the recovered isolates, accounting for 19 of 21 (90.5%), whereas the two Gram-negative isolates (9.5%) were both *Escherichia coli*. At the genus level, *Staphylococcus* species constituted the largest group, with nine isolates. The species-level distribution of bacterial isolates identified by MALDI-TOF MS is shown in Figure 2.

Antimicrobial susceptibility findings

When all 21 bacterial isolates were considered together, the highest pooled resistance rates were observed for sulfamethoxazole-trimethoprim (76.2%), ceftiofur (57.1%), and enrofloxacin (47.6%). Other antimicrobial resistance rates were 42.9% for cefpodoxime, 38.1% for amikacin, 28.6% for both ampicillin and doxycycline, 19.0% for nitrofurantoin, and 9.5% for amoxicillin-clavulanic acid. Since these pooled resistance rates were calculated from a taxonomically heterogeneous collection of bacterial isolates, they should be interpreted descriptively and not treated as species-specific resistance estimates. The pooled resistance rates according to the tested antimicrobials are shown in Figure 3.

When nitrofurantoin susceptibility was evaluated, all *Staphylococcus* spp. (n=9), *Enterococcus faecalis* (n=4), *Streptococcus canis* (n=2), and *Escherichia coli* (n=2) isolates were susceptible. In contrast, nitrofurantoin resistance was detected in all three *Micrococcus luteus* isolates and in the single *Enterococcus faecium* isolate. Resistance to amoxicillin-clavulanic acid was detected in only two isolates: one *Staphylococcus haemolyticus* isolate and the single *E. faecium* isolate. All *Enterococcus* isolates were resistant to amikacin, whereas all *E. faecalis* isolates were susceptible to amoxicillin-clavulanic acid, ampicillin, and nitrofurantoin. Both *E. coli* isolates were resistant to ceftiofur and sulfamethoxazole-trimethoprim; neither isolate was classified as resistant to any of the remain-

Figure 3. Resistance rates were calculated from 21 bacterial isolates tested using the Kirby-Bauer disc diffusion method. The highest resistance rates were observed against sulfamethoxazole-trimethoprim, ceftiofur, and enrofloxacin.



ing seven tested antimicrobials. Intermediate results were recorded for amoxicillin-clavulanic acid (2/21 isolates), ampicillin (1/21), cefpodoxime (2/21), ceftiofur (1/21), doxycycline (2/21), and enrofloxacin (3/21). No intermediate results were observed for amikacin, nitrofurantoin, or sulfamethoxazole-trimethoprim.

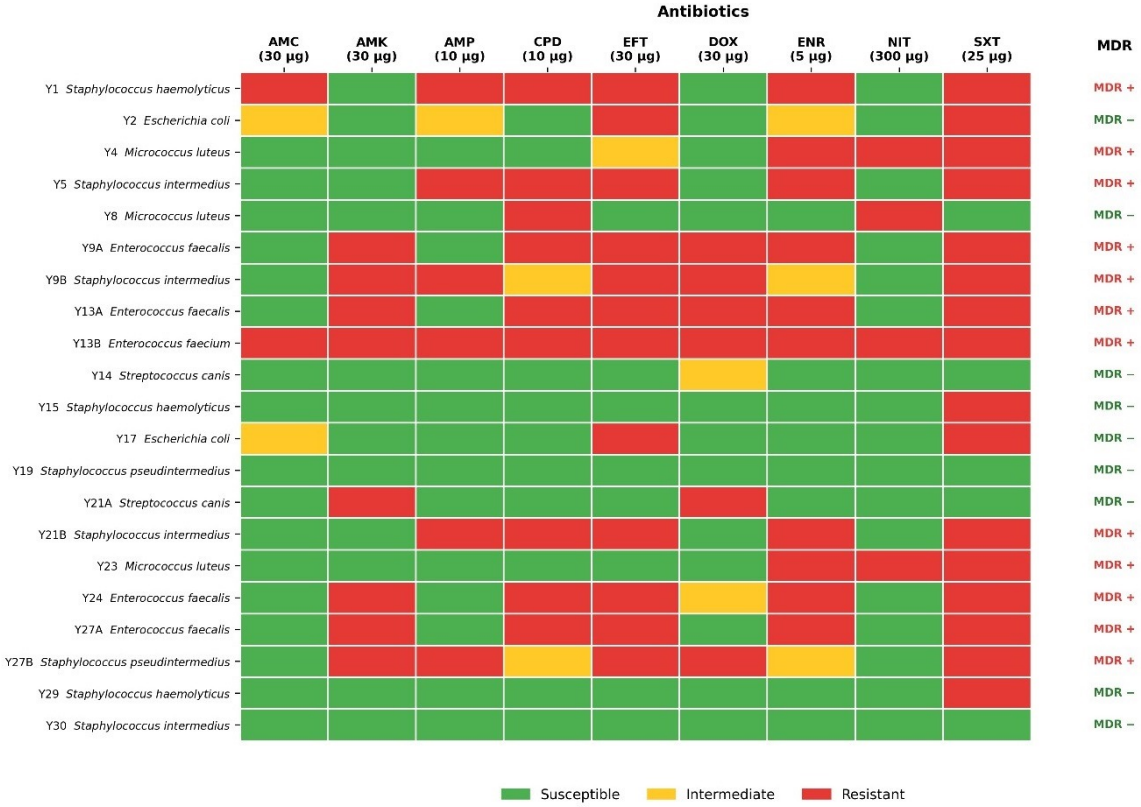
Multidrug resistance findings

Based on isolate-level phenotypic resistance profiles, multidrug resistance was detected in 12 of 21 isolates (57.1%). At the species level, MDR was detected in *E. faecalis* (4/4 isolates), *E. faecium* (1/1), *Staphylococcus intermedius* (3/4), *M. luteus* (2/3), *Staphylococcus pseudintermedius* (1/2), and *S. haemolyticus* (1/3). MDR was not detected in *E. coli* (0/2) or *S. canis* (0/2). The single *E. faecium* isolate was resistant to all nine tested antimicrobials. In addition, one *S. pseudintermedius* isolate and one *S. intermedius* isolate, obtained from two different dogs, were susceptible to all nine tested antimicrobials. Isolate-level antimicrobial susceptibility and resistance profiles, together with MDR status, are shown as a heatmap in Figure 4.

Discussion

This study identified bacterial isolates recovered from urine samples of dogs evaluated for suspected urinary tract infection by culture using MALDI-TOF MS, and determined their antimicrobial susceptibility profiles. Because quantitative urine culture and diagnostic bacterial count thresholds were not used, the findings were interpreted as culture-based bacterial recovery from submitted urine samples rather than as microbiological confirmation of definitive urinary tract infection. Bacterial growth was detected in 17 of 30 urine samples examined (56.6%). This rate was close to the bacterial growth rate of 60.27% reported by Yamanaka et al. (2019) in dogs with cystitis, supporting the relevance of bacterial culture in the microbiological evaluation of dogs with suspected urinary tract infection. The bacterial species recovered in this study were broadly consistent with those reported in canine urinary samples. Commonly reported bacteria in canine urinary tract infections include *Escherichia coli*, *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Proteus* spp., and *Klebsiella* spp. (Temmerman et al., 2024). Windahl et al. (2014) and Hall et al. (2013) reported *E. coli* as the most commonly isolated bacterium from canine urine samples. In contrast, Yamanaka et al. (2019) reported *Staphylococcus* species as the most frequently isolated group, which is closer to the genus-level distribution observed here. In the current dataset, *Enterococcus faecalis* and *Staphylococcus intermedius* were the most frequently detected species, while *Staphylococcus* species constituted the largest genus, with nine isolates. *Escherichia coli* was recovered at a lower frequency, with two isolates (9.5%). The recovery of *Micrococcus luteus* from three urine samples (14.3% of isolates) should be interpreted cau-

Figure 4. Isolate-level antimicrobial susceptibility profiles of the 21 bacterial isolates. Rows represent individual bacterial isolates and columns represent the nine tested antimicrobials. Susceptible, intermediate, and resistant classifications are shown for each isolate, together with multidrug resistance (MDR) status (positive or negative).



tiously, as this skin-associated commensal may represent contamination rather than a clinically relevant urinary tract infection, particularly in samples obtained by non-cystocentesis methods. The use of different routine urine collection methods may therefore have influenced the recovery of this organism. These differences may reflect variation in study populations, sample sources, sampling conditions, geographical regions, and laboratory workflows. Previous studies from Türkiye have reported a range of bacteria from canine urine samples, including *Klebsiella pneumoniae*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus, Streptococcus* spp., *Enterococcus* spp., *Escherichia coli, Proteus vulgaris*, *Bacillus* spp., *Pasteurella* spp., *Neisseria* spp., and *Flavobacterium* spp. (Gülen and Kırkan, 2012; Kırkan et al., 2019). Seyhan and Öztürk (2023) reported *E. coli* and coagulase-negative *Staphylococcus* species as the most frequent isolates in dogs with urinary tract infection. The recovery of *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus canis*, and *E. coli* in this study is generally consistent with the bacterial diversity reported in previous studies. However, the predominance of *E. faecalis* and *S. intermedius* suggests that regional and laboratory-based surveillance may be useful for understanding the distribution of local isolates.

The antimicrobial susceptibility findings showed marked variation among the tested agents. Because the pooled resistance rates were calculated across a taxonomically heterogeneous collection of bacterial isolates, these values should be interpreted descriptively. They should not be regarded as species-specific resistance estimates. Species-level observations should also be interpreted cautiously because of the small number of isolates representing each species. The 76.2% resistance rate to sulfamethoxazole-trimethoprim was notable. Previous studies reported resistance to sulfamethoxazole-trimethoprim as 33.6% in Italy, 56.75% in South Korea, and 18.8% in Portugal (Tagliasacchi et al., 2025; Lee et al., 2024; Garcês et al., 2022). The higher rate observed here suggests that the empirical use of this antimicrobial combination should be considered with caution and interpreted in the light of regional isolate profiles and susceptibility testing results. Resistance to amoxicillin-clavulanic acid was relatively low, at 9.5%. Previous studies reported amoxicillin-clavulanic acid resistance rates of 48.64% among *Klebsiella* isolates from canine urinary tract infections in South Korea, 24.2% among bacteria isolated from canine and feline urinary tract infections in Portugal, and 27.08% among *Staphylococcus pseudintermedius* isolates in the United States

(Lee et al., 2024; Garcês et al., 2022; Yudhanto et al., 2022). In the current dataset, resistance to amoxicillin-clavulanic acid was not detected in the two *S. pseudintermedius* isolates. However, since the number of species-specific isolates was limited, this observation should be interpreted cautiously and confirmed in studies with larger sample sizes. Nitrofurantoin susceptibility varied by species. All *Staphylococcus* species, *E. coli*, *E. faecalis*, and *Streptococcus canis* isolates were susceptible to nitrofurantoin, whereas resistance was detected in three *Micrococcus luteus* isolates and one *E. faecium* isolate. This pattern indicates that nitrofurantoin susceptibility should not be generalised across all urinary isolates, and instead be interpreted at the species or isolate level. The amikacin resistance rate of 38.1% was also notable. Previous studies reported amikacin resistance rates of 1.1% in Portugal, 5.4% in South Korea, and 0% among *E. coli* and *Proteus mirabilis* isolates in the United States (Garcês et al., 2022; Lee et al., 2024; Yudhanto et al., 2022). Although amikacin resistance was not detected in *E. coli* isolates in this study, the higher overall rate may be related to the composition of the isolate panel. In particular, resistance among *Enterococcus* isolates may have contributed to the overall rate of amikacin resistance. Further molecular studies are required to determine whether the observed phenotypic resistance patterns are associated with specific resistance genes or mechanisms. Resistance to enrofloxacin, a fluoroquinolone, was 47.6%. Previous studies reported enrofloxacin resistance rates of 36.6% in Italy, 19.6% in Portugal, and 29.17% among *S. pseudintermedius* isolates in the United States (Tagliasacchi et al., 2025; Garcês et al., 2022; Yudhanto et al., 2022). The rate observed here was higher than that reported in some of the compared studies. Because fluoroquinolones are frequently used in veterinary medicine for their broad-spectrum activity, resistance in this group should be monitored. Similarly, the 57.1% resistance rate observed for ceftiofur highlights the importance of considering cephalosporin susceptibility data when interpreting antimicrobial treatment options.

Multidrug resistance was one of the clinically relevant findings. MDR was detected in 12 of 21 bacterial isolates (57.1%). Considering that an MDR rate of 65.9% has been reported among isolates obtained from dogs with cystitis, the rate observed here appears comparable with previous data (Yamanaka et al., 2019). MDR was detected in all *Enterococcus* isolates, and one *Enterococcus faecium* isolate was resistant to all nine tested antimicrobials, though this finding should be interpreted with caution, given that it is based on a single isolate. Nevertheless, the broad phenotypic resistance observed in this isolate indicates limited *in vitro* activity of the antimicrobial agents tested and warrants confirmation across larger collections of isolates. In contrast, MDR was not detected in *Escherichia coli* or *Streptococcus canis* isolates, and one *Staphylococcus pseudintermedius* isolate and one *Staphylococcus intermedius* isolate were susceptible to all tested antimicrobials. These findings suggest that resistance profiles may differ markedly among species and isolates. However, given the limited number of isolates tested, these results should be confirmed in larger studies. The presence of antimicrobial-resistant bacteria in companion animals is relevant within a One Health framework (Guardabassi et al., 2004). The resistance profiles detected in this study do not demonstrate zoonotic transmission, rather they underscore the importance of antimicrobial resistance surveillance in companion animals as part of integrated One Health monitoring. Continued surveillance of bacteria recovered from canine clinical samples may contribute to veterinary antimicrobial stewardship and provide useful regional data on resistance trends. MALDI-TOF MS was used to identify pure bacterial isolates obtained after culture. This method supports rapid species-level identification of cultured isolates in microbiology laboratories (Croxatto et al., 2012; Moreno et al., 2018; Svetličić et al., 2022). Compared with conventional biochemical identification workflows, MALDI-TOF MS can shorten the time required for post-culture identification and reduce the number of manual biochemical tests needed for species-level identification. These practical advantages may reduce laboratory workload, particularly when multiple bacterial isolates require identification. The identification of eight bacterial species by MALDI-TOF MS supports its usefulness for post-culture identification in the present laboratory workflow. However, MALDI-TOF MS identification alone does not determine antimicrobial susceptibility profiles; therefore, antimicrobial susceptibility testing remains necessary to support appropriate therapeutic decisions.

This study has several limitations. First, the sample size was limited and the findings should be interpreted with respect to the study population. Second, because the samples were obtained as routine diagnostic submissions from veterinary clinics and animal hospitals in Aydın and İzmir provinces, larger multicentre studies are needed before the findings can be generalised to broader canine populations. Third, urine collection reflected real-world clinical practice rather than a standardised prospective sampling protocol. Furthermore, the use of different routine urine collection methods may have influenced bacterial recovery and introduced a risk of contamination, particularly for skin-associated organisms. Detailed clinical metadata, including complete urinalysis findings, previous antimicrobial treatment history, recurrent infection status, and comorbidities, were not consistently available in the submitted clinical records. In addition, quantitative urine culture was not performed, and colony-forming unit per millilitre thresholds were not used. The use of

pre-enrichment may have improved bacterial recovery but may also influence the interpretation of bacterial growth in urine samples. Together, these methodological factors limit the ability to distinguish clinically relevant bacteriuria from incidental or contaminating bacterial recovery. Finally, the study was based on culture-based identification and phenotypic antimicrobial susceptibility testing; molecular investigation of resistance genes was beyond its scope. Overall, these preliminary culture-based findings indicate that the combined use of bacterial culture, MALDI-TOF MS identification, and antimicrobial susceptibility testing may contribute to the microbiological evaluation of urine samples from dogs with suspected urinary tract infection. The bacterial diversity and MDR rates observed in this study underscore the importance of rational antimicrobial use and regional monitoring of resistance in canine urinary isolates.

Conclusions

In this preliminary culture-based study, bacterial isolates recovered from urine samples of dogs with suspected urinary tract infection were identified by MALDI-TOF MS, and their antimicrobial susceptibility profiles were determined. The findings showed that different bacterial species were recovered and that multidrug resistance was detected in 12 of 21 isolates (57.1%). Pooled resistance was most frequently observed for sulfamethoxazole-trimethoprim, ceftiofur, and enrofloxacin; however, these findings were derived from a taxonomically heterogeneous collection of isolates with small species-specific sample sizes and should therefore be interpreted cautiously. MALDI-TOF MS identification of bacterial isolates recovered by culture, together with antimicrobial susceptibility testing, may assist clinicians in selecting appropriate antimicrobial therapy. Since urine collection methods were not standardised, differences in sampling procedures may have influenced bacterial recovery. Further studies should include larger sample sizes, standardised prospective sampling protocols, quantitative urine cultures, broader geographical coverage, and participation of more veterinary clinics and hospitals to improve the representativeness of the findings.

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> Identifikacija bakterijskih izolata iz uzoraka mokraće pasa MALDI-TOF MS tehnikom i određivanje njihove osjetljivosti na antimikrobne lijekove

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Bakterijske infekcije mokraćnog sustava česte su u pasa te redovito zahtijevaju primjenu antimikrobnog liječenja. Točna identifikacija bakterijskih uzročnika i određivanje njihove osjetljivosti na antimikrobne lijekove ključni su za racionalno donošenje odluka o liječenju. Cilj ovog istraživanja bio je identificirati bakterije izolirane iz uzoraka urina pasa pregledanih zbog sumnje na infekciju mokraćnog sustava primjenom MALDI-TOF MS tehnike te odrediti njihovu osjetljivost prema antimikrobnim lijekovima. Bakteriološki je pretraženo 30 uzoraka urina pasa. Uzorci su najprije obogaćeni u Brain Heart Infusion bujonu, a zatim precijepljeni na krvni agar s 5 % ovčje krvi, MacConkey-ev agar i Eosin Methylene Blue agar. Čiste bakterijske kulture identificirane su MALDI-TOF MS tehnikom. Određivanje osjetljivosti na antimikrobne lijekove provedeno je Kirby-Bauerovom disk-difuzijskom metodom primjenom diskova s amoksicilin-klavulanskom kiselinom, amikacinom, ampicilinom, ceftiofurom, cefpodoksimom, doksiciklinom, enrofloksacinom, nitrofurantoinom i sulfametoksazol-trimetoprimom. Rast bakterija utvrđen je u 17 od 30 uzoraka mokraće (56,6 %), pri čemu je dobiven 21 bakterijski izolat. Iz četiri uzorka s pozitivna uzorka izolirane su po dvije različite bakterijske vrste. MALDI-TOF MS tehnikom identificirano je osam bakterijskih vrsta: *Enterococcus faecalis* (n = 4; 19,0 %), *Staphylococcus intermedius* (n = 4; 19,0 %), *Staphylococcus haemolyticus* (n = 3; 14,3 %), *Micrococcus luteus* (n = 3; 14,3 %), *Escherichia coli* (n = 2; 9,5 %), *Staphylococcus pseudintermedius* (n = 2; 9,5 %), *Streptococcus canis* (n = 2; 9,5 %) i *Enterococcus faecium* (n = 1; 4,8 %). Najviši stupanj rezistencije utvrđen je prema sulfametoksazolu s trimetoprimom (76,2 %), ceftiofuru (57,1 %) i enrofloksacinu (47,6 %). Višestruka rezistencija na antimikrobne lijekove utvrđena je u 12 izolata (57,1 %). Dobiveni rezultati pokazuju da bakterijski izolati izdvojeni iz uzoraka urina pasa mogu imati značajne razlike u osjetljivosti na antimikrobne lijekove. Identifikacija uzgojenih bakterijskih izolata MALDI-TOF MS tehnikom u kombinaciji s određivanjem njihove osjetljivosti na antimikrobne lijekove može pridonijeti mikrobiološkoj dijagnostici i racionalnoj primjeni antimikrobnih lijekova u liječenju pasa sa sumnjom na infekciju mokraćnog sustava.

Ključne riječi: *antimikrobna rezistencija; urin pasa; pas; MALDI-TOF MS; višestruka rezistencija; infekcija mokraćnog sustava*.