Original article Casp. J. Vet. Sci., 2025; 2(1): 37-41

Amol University of Special Modern Technologies

Caspian Journal of Veterinary Science
doi: 10.22034/cjvs.2025.491105.1035
Journal homepage: https://Cjvs.ausmt.ac.ir/

Antibiotic resistance in isolated bacteria from dogs with urinary tract infection in Babol city

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Article Info

Article history:

Received: 27 November 2024

Accepted: 12 February 2025

Keywords:

Antibiotic resistance

Babol

Dog

Urinary tract infection

Abstract

Urinary tract is the most common site of infection after the respiratory tract. Various factors play a role in causing infection in the urinary tract, and in 80% of cases, *Escherichia coli* bacteria is the cause of infection. In the meantime, increasing antibiotic resistance of bacteria leads to treatment failure and disease recurrence. Due to the different pattern of antibiotic resistance of bacteria in each region, it is necessary to study and check the antibiotic resistance of bacteria. The aim of this study was to determine the antibiotic resistance of bacteria in urine samples of dogs in Babol city. To do so, 100 samples of dogs with urinary tract infection referred to private clinics of Babol city were examined. One hour after collecting the samples, 0.01 ml of urine was cultured directly on the blood agar medium using a standard loop and incubated at 35-37°C for 24-48 hours. After that, the antibiogram was performed and finally the area of non-growth around the antibiotic discs was measured. Our findings showed that gram-negative bacteria were the most abundant in this study, indicating the most resistant to clindamycin (92.59%), penicillin (87.04%) and amoxicillin (79.63%). The least resistant to amikacin and gentamicin (12.96%), and trimethoprim (28.52%) was observed. According to the results, it is necessary to perform a precise antibiogram test using reliable discs before prescribing the drug, as well as rationally prescribing antibiotics and not arbitrarily taking them.

Introduction

A urinary tract infection (UTI) occurs when the host's defense mechanisms are compromised, allowing a pathogenic microbe to adhere to, multiply in, and persist within part of the urinary tract. Host defenses include natural flushing, anatomical structures, the mucosal barrier, urine, and the immune system's strength. While most UTIs are caused by bacteria, fungi and viruses can also infect the urinary tract. UTIs may affect more than one anatomical site and should be classified as either upper (kidneys and ureters) or lower urinary tract infections (bladder, urethra, and vagina). Most bacterial UTIs result from the ascending migration of pathogens from the genital and urethral regions to the bladder, ureters, and one or both kidneys. Rectal, perineal, and genital bacteria serve as the main reservoirs of infection (Olin and Bartges, 2022).

Asymptomatic bacterial UTIs are common in dogs, estimated to occur in approximately 14% of the dog population at some point in their lives. Often, the infectious agents involved are pathogenic bacteria from the intestinal flora that ascend into the urinary system through the genitourinary tract. However, the incidence of UTI in cats is less common than in dogs. Nonetheless, this trend is reportedly increasing in some European countries. Due to its high prevalence, UTIs are reported as the second or third most common reason for antibiotic use in dogs, accounting for 12% of all antibiotic prescriptions. The rise in multidrug-resistant bacteria is a potential cause. Antibiotic failure in animals and the presence of potential bacterial reservoirs shared between humans and animals present a serious threat to global public health (Hernando *et al.,* 2021).

Although the prevalence of UTIs depends on the bacterial diversity in the region, the duration of antibiotic treatment for UTIs in dogs is generally longer than the recommended treatment duration for human UTI patients. For dogs with uncomplicated UTIs, the recommended antibiotic treatment duration is less than 7 days (Grabe *et al.,* 2015). For complicated infections, the recommended treatment duration is typically 7 to 14 days, but it may be extended to 21 days depending on underlying conditions. In comparison, the recommended duration for antibiotic treatment in dogs with complicated UTIs can be up to 4 weeks (Jessen *et al.,* 2015).

Various bacteria can cause UTIs, but one of the most important UTI-causing bacteria is *Escherichia coli*. *E. coli* can lead to multiple infections, including UTIs, pneumonia, septicemia, meningitis, and wound infections. This bacterium is a part of the normal intestinal flora of humans and animals, and many of its strains are opportunistic pathogens, meaning they cause disease when they enter tissues that are not part of their usual flora (Blount, 2015).

Materials and Methods

Study design

In this study, 100 suspected dogs referred to the clinic with symptoms of UTI, who had not received antibiotics, were included. Urine samples were collected, and after culturing and identifying positive cases, cultures were further tested to determine bacterial resistance to selected antibiotics based on antibiogram testing. Additionally, this study, with the ethical code (IR.IAU.BABOL.REC.1399.101), was approved by the Ethics Committee of Islamic Azad University, Babol branch.

Sampling

Mid-stream urine samples were collected in specialized urine culture containers. Within one hour of collection, 0.01 ml of each sample was directly cultured on blood agar using a standard loop and incubated at 35–37°C for 24 to 48 hours. After incubation, colony morphology was examined. Negative cases with no growth were identified, while positive cases were further cultured and Gram staining was performed. Gram-positive and Gram-negative bacteria were differentiated. Finally, an antibiogram test was conducted to assess bacterial resistance to antibiotics, and results were categorized as "sensitive," "resistant," or "intermediate" based on the susceptibility profile.

Culture and sensitivity measurement

In this study, blood agar, a non-selective and nutrient-rich medium, was used. The blood agar medium was prepared with 10% blood, along with casein, papaya, sodium chloride, peptone, and distilled water. Additionally, Mueller-Hinton agar was used to determine the sensitivity of bacterial samples.

Gram stain

After preparing and fixing the bacterial smear on the slide, it was placed in crystal violet solution for one minute. Then, it was rinsed with water and placed in iodine solution for one minute. Next, after rinsing with water, the slide was decolorized with a decolorizing solution for 20 seconds, and finally, after another rinse, it was placed in fuchsin solution for 45 seconds.

Data analysis

Quantitative data were analyzed using one-way ANOVA and Duncan's post hoc test. A P value of less than 0.05 was considered statistically significant (Salehi *et al.,* 2022).

Results

Overall, our findings showed that regarding the Gram-positive bacteria, 53.85% were sensitive to gentamicin and sulfamethoxazole + trimethoprim, while 23.08% were sensitive to ceftriaxone. Resistance varied, with 38.46% resistant to tetracycline, clindamycin, and amikacin. Moreover, several bacteria showed intermediate resistance, such as 38.46% for cefazolin and tetracycline. On the other hand, higher sensitivity was noted for antibiotics like amikacin (75.93%) and gentamicin (72.22%) in Gram-negative bacteria. Resistance was observed in up to 35.19% for tetracycline and 33.33% for enrofloxacin. Significant intermediate resistance was found for penicillin (87.04%) and amoxicillin (79.63%).

More specifically, in this study conducted on 100 suspected samples, it was observed that 33% of the total samples were negative (Table 1), while the remaining 67% were positive and infected with UTIs. Among the positive cases, there were 54 cases with Gram-negative bacteria and 13 cases with Gram-positive bacteria (Fig. 1).

The antibiogram results for these isolates from UTI patients showed that resistance of Gram-negative bacteria was as follows: clindamycin (92.59%), penicillin (87.04%), amoxicillin (79.63%), tetracycline (48.15%), enrofloxacin (35.19%), ceftriaxone (33.33%), cefazolin (25.93%), trimethoprim (18.52%), gentamicin (12.56%), and amikacin (12.96%) (Table 2). For Gram-positive bacteria, resistance was observed in the following order: clindamycin, tetracycline, amikacin, ceftriaxone (38.46%), cefazolin, penicillin, enrofloxacin, trimethoprim (23.08%), gentamicin, and amoxicillin (15.38%) (Fig. 2).

Table1. Infection rate of Gram-positive and Gram-negative bacteria.

Samples	Percentage
Negative	**33**
Positive gram-positive	**54**
Positive gram-negative	**13**
Total	**100**

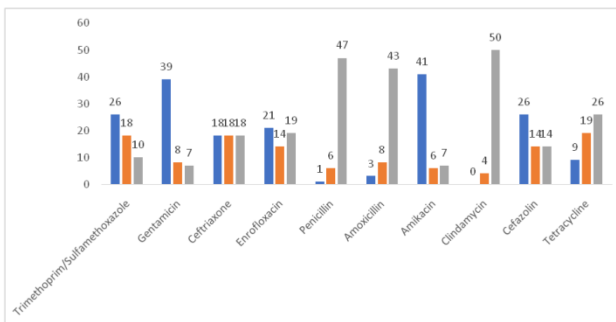

Fig. 1. Antibiotic sensitivities of Gram-negative bacteria in different treatments.

Table 2. Percentages of bacterial resistance to antibiotic; S = Sensitive, R = Resistant, I = Intermediate. Results are expressed as number (percentage).

		Sulfamethoxazole + Trimethoprim	**Gentamicin**	**Ceftriaxone**	**Enrofloxacin**	**Penicillin**	**Amoxicillin**	**Amikacin**	**Clindamycin**	**Cefazolin**	**Tetracycline**
Gram-positive bacteria n=13	S	7(53.85)	7(53.85)	3(23.08)	3(46.15)	6(46.15)	6(46.15)	4(30.77)	4(30.77)	5(38.46)	3(23.08)
	R	3(23.08)	4(30.77)	5(38.46)	4(30.77)	4(30.77)	5(38.46)	4(30.77)	4(30.77)	5(38.46)	5(38.46)
	I	3(23.08)	2(15.38)	٥(38.46)	3(23.08)	3(23.08)	2(15.38)	5(38.46)	(38.46)	3(23.08)	5(38.46)
Gram-negative bacteria n=54	S	26(48.15)	39(72.22)	18(33.33)	21(38.89)	1(1.85)	3(5.56)	41(75.93)	0(0)	26(48.15)	9(16.67)
	R	18(33.33)	8(14.81)	18(33.33)	14(25.93)	6(11.11)	8(14.81)	6(11.11)	4(7.41)	14(25.93)	19(35.19)
	I	10(18.52)	7(12.96)	18(33.33)	19(35.19)	47(87.04)	43(79.63)	7(12.96)	50(92.59)	14(25.93)	26(48.15)

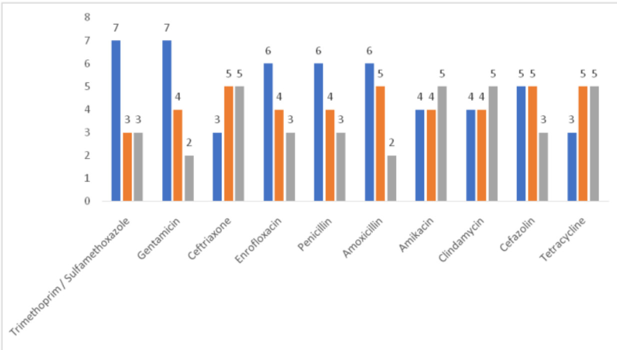

Fig. 2. Antibiotic sensitivities of Gram-positive bacteria in different treatments.

Discussion

UTIs are a common global issue across human societies, and without appropriate or timely treatment, they can lead to severe complications. UTIs affect all age groups, but antibiotic resistance among pathogens frequently results in treatment failure. Therefore, it is recommended to base UTI treatments on antibiotic susceptibility tests; however, the 48-hour delay in lab results often leads to empirical treatment. Since empirical antibiotic treatment should consider the local resistance patterns of uropathogens, identifying and monitoring antibiotic resistance patterns is essential goal of this research was to identify antibiotics that were initially effective against bacteria but have now become less effective due to the emergence and spread of resistant strains, causing hard-to-treat UTIs (Mirmahdavi and Ahmadian, 2001). This study highlights stance patterns vary significantly by region and depend on local antibiotic use. Several factors contribute to these differences, including environmental health, income levels, variations in animal populations, climate, and levels of antibiotic usage.

The findings indicated that the most effective antibiotics for Gram-negative bacteria isolated from UTI-infected dogs in Babol were amikacin and gentamicin, with a resistance rate of 12.96%. In contrast, the highest resistance rates were observed for clindamycin (92.59%) and penicillin (87.04%). Resistance rates for other antibiotics were as follows: trimethoprim (18.52%), cefazolin (25.93%), ceftriaxone (33.33%), enrofloxacin (35.19%), tetracycline (48.15%), and amoxicillin (79.63%). These antibiotics should be used cautiously. For example, the lowest resistance was against gentamicin and amoxicillin (15.38%), while the highest resistance was noted for clindamycin, tetracycline, ceftriaxone, and amikacin (38.46%). These results align with previous studies in Amol and Shahrekord cities, which also demonstrated varied resistance levels in different regions (Bahadoran *et al.,* 2008; Khoshbakht *et al.,* 2019).

Given the World Health Organization's warning (WHO's warnings) that antibiotic resistance may become one of the leading causes of mortality in coming decades, continuous bacterial and antibiotic resistance pattern surveillance is essential. Responsible and judicious use of antibiotics, alongside regular antibiograms in laboratories, is crucial to prevent further resistance development. Reducing unnecessary antibiotic use, especially in prophylactic contexts such as surgeries or catheterization, is also important. Additionally, the use of antibiotics in livestock contributes to resistance. Since many bacteria are shared between humans and animals, indiscriminate use in animal husbandry can lead to resistant strains that can spread to humans through food or the environment, leading to severe health issues and economic burdens.

Despite straightforward lab diagnosis, treatment of UTIs is increasingly challenged by resistant pathogens. Therefore, careful antibiotic use in UTI treatment based on antibiogram results is crucial. Studies in different regions have also shown geographic variations in the prevalence of pathogens and their resistance patterns (Weaver and Pillinger, 1977; Ling *et al.,* 1998; Seguin *et al.,* 2003; Ball *et al.,* 2007; Black *et al.,* 2009; Pour Mirbolok Jalali *et al.,* 2010; Vahhabi *et al.,* 2010; Farhadi *et al.,* 2012; Hall *et al.,* 2013; Namroodi *et al.,* 2017).

In conclusion, findings from this study and worldwide indicated that antibiotic resistance patterns are specific to each region. It is essential to heed WHO's warnings and curb indiscriminate antibiotic use, especially without antibiogram testing. We recommend routinely performing susceptibility testing in UTI treatment, as resistance patterns can vary over time and between regions. Similar studies in other climates and further examination of other antibiotics used in Babol for UTI treatment would also be valuable.

Acknowledgment

This article is derived from Ms. Fatemeh Hadinejad's thesis for obtaining a Doctor of Veterinary Medicine (DVM) degree from the Islamic Azad

University, Babol branch. We extend our heartfelt gratitude to the Islamic Azad University of Babol for their support in conducting this research. The authors declare no conflict of interest in this study.

Conflict of Interest

The authors declare no conflict of interest in this study.

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