

# Epidemiological insights into seasonal, sex-specific and age-related distribution of bacterial pathogens in urinary tract infections

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**Abstract.** Urinary tract infections (UTIs) are prevalent and recurrent bacterial infections that affect individuals worldwide, posing a significant burden on healthcare systems. The present study aimed to explore the epidemiology of UTIs, investigating the seasonal, gender-specific and age-related bacterial pathogen distribution to guide clinical diagnosis. Data were retrospectively collected from electronic medical records and laboratory reports of 926 UTIs diagnosed in Fuding Hospital (Fujian University of Traditional Chinese Medicine, Fuding, China). Bacterial isolates were identified using standard microbiological techniques.  $\chi^2$  tests were performed to assess associations between pathogens and the seasons, sex and age groups. Significant associations were found between bacterial species and seasons. *Enterococcus faecium* exhibited a substantial prevalence in spring ( $\chi^2$ , 12.824;  $P=0.005$ ), while *Acinetobacter baumannii* demonstrated increased prevalence in autumn ( $\chi^2$ , 16.404;  $P=0.001$ ). Female patients showed a higher incidence of UTIs. Gram-positive bacteria were more prevalent in males, with *Staphylococcus aureus* showing significant male predominance ( $\chi^2$ , 14.607;  $P<0.001$ ). *E. faecium* displayed an age-related increase in prevalence ( $\chi^2$ , 17.775;  $P<0.001$ ), whereas *Escherichia coli* tended to be more prevalent in younger patients ( $\chi^2$ , 12.813;  $P=0.005$ ). These findings highlight the complex nature of UTIs and offer insights for tailored diagnostic and preventive strategies, potentially enhancing healthcare outcomes.

## Introduction

Urinary tract infections (UTIs) are an extensive and pervasive health concern, ranking among the most common and recurring bacterial infections, impacting a vast number of individuals worldwide each passing year (1-4). From 1990 to 2019, the total cases of UTIs surged by 60.40%, rising from 252.25 million to 404.61 million (2). These infections affect people of all ages, backgrounds, and geographical locations, representing a substantial burden on healthcare systems and impacting the overall well-being of affected populations (5,6).

Given the wide-ranging prevalence of UTIs, a comprehensive exploration of their epidemiology and underlying factors becomes of paramount necessity (2,7). Investigating the seasonal distribution of UTIs can shed light on potential environmental and climatic influences, helping identify periods of heightened risk and enabling more proactive public health interventions (8,9). Furthermore, understanding the sex-specific patterns of UTI occurrence can provide valuable insights into the complex interplay between anatomical differences, hormonal factors and behavioral aspects that may contribute to susceptibility (10,11).

Age-related distribution of bacterial pathogens in UTIs is another crucial area of investigation (12). Age has been associated with the incidence of UTIs and with treatment failure of UTIs in multiple observational studies, sometimes yielding conflicting results (13-15). Therefore, recognizing how UTI incidence varies across different age groups can aid in the development of targeted prevention strategies and tailor-made treatment approaches, considering the unique physiological and immunological characteristics of specific populations (16,17).

A heightened understanding of the factors contributing to the development and spread of UTIs can pave the way for the implementation of effective public health measures, including educational campaigns to raise awareness about preventive practices and hygiene habits (18). Community-wide efforts can thus foster a culture of proactive health management, ultimately reducing the incidence of UTIs and improving the overall quality of life for individuals worldwide (2,19).

However, the distribution of bacterial pathogens in UTIs is not well understood, especially regarding seasonal, sex-specific

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**Abbreviations:** UTI, urinary tract infection; CFU, colony-forming unit

**Key words:** UTI, Gram-positive bacteria, *Escherichia coli*, *Acinetobacter baumannii*, bacterial pathogen

and age-related patterns, creating a crucial knowledge gap. The present study aimed to address this gap by investigating the prevalence and distribution of UTI bacterial pathogens in hospitalized cases, and exploring their complex interaction with various demographic characteristics. The valuable insights gained from this research are expected to result in more effective diagnosis, treatment and prevention strategies. With this knowledge, the impact of UTIs can be proactively mitigated, ultimately promoting better health outcomes and well-being for individuals.

## Patients and methods

**Study design.** This study employed a retrospective observational design to investigate the seasonal, sex-specific and age-related distribution of bacterial pathogens in UTIs. A total of 926 consecutive patients diagnosed with UTIs in Fuding Hospital (Fujian University of Traditional Chinese Medicine, Fuding, China) were enrolled. The patients included 343 men and 583 women diagnosed with UTIs. The data collection period spanned from July 1, 2022, to June 30, 2023. Data from electronic medical records and laboratory reports were collected and analyzed to identify patterns of bacterial prevalence.

**Inclusion and exclusion criteria.** Inclusion criteria were as follows: Participants with three urine samples exhibiting  $>10^5$  colony-forming units (CFU)/ml were included. Clean catch midstream urine samples were obtained following proper instructions. Exclusion criteria included participants with inflammatory conditions unrelated to UTIs, those with cancer and pregnant patients.

**Data collection and outcome measures.** Data were obtained from 926 patients diagnosed with UTIs in Fuding Hospital between July 1, 2022, and June 30, 2023. Demographic information, including age and sex, was extracted from electronic health records. Additionally, information on the date of UTI diagnosis was recorded to categorize cases according to seasonal variations.

Urine samples were obtained through either bladder catheterization or the midstream clean catch method. The urine specimens for routine urinalysis were tested using a Fully Automated Urine Chemistry Analyzer UC-3500 and a Partical Analyzer UF-5000 (Sysmex Corporation). Pyuria was defined as the presence of  $>5$  white blood cells per high-power field in the urine sediment, and significant bacteriuria was identified as the growth of a single pathogenic microorganism  $>100,000$  CFU per ml of urine (20). Patients exhibiting pyuria in the urine analysis underwent a urine culture to confirm the presence of a UTI and detect the causative organism. Cultures were performed using cysteine, lactose, and electrolyte-deficient agar. The culture was considered positive when it detected the presence of at  $\geq 50,000$  CFUs per ml of a single urinary pathogen (21). Bacterial isolates derived from urine cultures underwent identification processes that integrated conventional microbiological techniques with the Microbial Analyzing System of VITEK<sup>®</sup> 2 COMPACT (BioMérieux, Inc.) (22). This comprehensive approach involved methodologies such as Gram staining and biochemical tests. For Gram

staining, a standard procedure was followed at room temperature. Bacterial smears were first fixed to a slide by passing through a flame. The slide was then stained with crystal violet for 1 min, followed by the application of iodine solution for 1 min. After a brief rinse with water, the slide was decolorized with alcohol for 20 sec and then counterstained with safranin for 30 sec. The slides were observed under a light microscope at  $\times 1,000$  magnification. The classification of bacterial species into either Gram-positive or Gram-negative groups was established based on their unique responses to Gram staining.

The reference strains (*Escherichia coli*, ATCC 25922; *Staphylococcus aureus*, ATCC 25923; *Pseudomonas aeruginosa*, ATCC 27853; and *Enterococcus faecalis*, ATCC 29212) were utilized as positive controls and were all obtained from Fujian Provincial Inspection Center of China.

**Classification based on season, sex and age.** To examine the seasonal distribution of bacterial pathogens, UTI cases were categorized into four seasons: Spring, summer, autumn and winter, based on the date of UTI diagnosis and corresponding calendar months.

Patients were classified into two sex groups: Male and female, based on their recorded sex in the electronic health records.

Age-related distribution of bacterial pathogens was explored by categorizing patients into four groups: Age group 1, 0-49 years; age group 2, 50-69 years; age group 3, 70-79 years; and age group 4,  $\geq 80$  years.

**Ethics.** The present study was conducted in accordance with ethical guidelines and received approval from the Medical Ethics Committee of Fuding Hospital, Fujian University of Traditional Chinese Medicine (approval no. Fuding Hospital 2022325). The requirement for written informed consent was waived by the Medical Ethics Committee of Fuding Hospital, Fujian University of Traditional Chinese Medicine, due to the retrospective nature of the study.

**Statistical analysis.** Statistical analysis was conducted using SPSS version 22.0 software (IBM Corp.) and GraphPad Prism 8.0 (Dotmatics). Count data were expressed as  $n$  (%). Data normality was assessed using the Kolmogorov-Smirnov test. For non-normally distributed measurements, medians and interquartile ranges were reported. Non-parametric tests were analyzed with the Kruskal-Wallis H test for multiple groups.  $\chi^2$  tests or Fisher's exact tests, as appropriate, were used to determine the significance of differences in bacterial prevalence across different seasons, sexes and age groups.  $P < 0.05$  was considered to indicate a statistically significant difference.

To address the issue of multiple comparisons within the four groups, the Bonferroni correction was applied as a post-hoc analysis after initial tests (Kruskal-Wallis H test and  $\chi^2$  tests) to indicate significant differences. With six possible pairwise comparisons (4 groups choose 2), the adjusted significance level ( $\alpha_{\text{adjusted}}$ ) was calculated as  $\alpha/6 \approx 0.0083$  (23).

## Results

The age distribution of the subjects in the four seasonal groups was tested using the Kolmogorov-Smirnov test, yielding

Table I. Clinical parameters of the four seasonal groups in the study.

Parameter	Spring	Summer	Autumn	Winter	Kruskal-Wallis/ $\chi^2$ test	P-value
Median age (IQR), years	70.0 (57.0-78.0)	68.0 (58.3-76.8)	69.0 (57.0-75.0)	69.0 (57.0-79.0)	2.526	0.471
Sex, n	246	212	273	195	1.751	0.626
Male, n (%) (n=343)	88 (35.8)	79 (37.3)	109 (39.9)	67 (34.4)		
Female, n (%) (n=583)	158 (64.2)	133 (62.7)	164 (60.1)	128 (65.6)		

Kolmogorov-Smirnov test was performed on the age data for the four seasonal groups with the following results:  $Z=0.118-0.140$ , all  $P<0.001$ . IQR, interquartile range.

results of  $Z=0.118-0.140$ , all  $P<0.001$ . These results indicated a non-normal distribution of age data, prompting the use of the non-parametric Kruskal-Wallis H test for statistical analysis. Additionally, a  $\chi^2$  test was performed to compare sex distribution among the four groups. The results of both the sex and age tests are documented in Table I. The statistical analysis revealed that there was no statistically significant difference in age or sex distribution among the four seasonal groups ( $P>0.05$ ).

A total of 926 bacterial strains were isolated from the studied patients, with 280 (30.2%) belonging to Gram-positive bacteria and 646 (69.8%) to Gram-negative bacteria. Among these, the top nine pathogenic bacteria were identified as follows: *E. coli* (50.0%), *Enterococcus faecium* (15.6%), *E. faecalis* (9.6%), *Klebsiella pneumoniae* (6.8%), *P. aeruginosa* (3.5%), *A. baumannii* (2.7%), *Streptococcus agalactiae* (2.5%), *S. aureus* (2.1%) and *Proteus mirabilis* (1.2%). The remaining bacterial species accounted for 6.2% of the total isolates (Fig. 1).

Further analysis of specific bacterial species revealed associations with the different seasons: *E. faecium* demonstrated a substantial prevalence during the spring season, with 22.0% of isolates ( $P=0.005$ ); *A. baumannii* displayed a notable association with the autumn season, accounting for 5.9% of isolates ( $P=0.001$ ) (Table II).

A total of 926 hospitalized subjects were enrolled in the study, including 343 (37.0%) men and 583 (63.0%) women. Gram-positive bacteria were found to be more prevalent in men compared with women (35.9 vs. 26.9%;  $P=0.004$ ). Particularly, *E. faecalis* and *S. aureus* showed significant sex differences, being more common in men (12.5%,  $P=0.021$ ; and 4.4%,  $P<0.001$ , respectively). Furthermore, among Gram-negative bacteria, *P. aeruginosa* and *A. baumannii* demonstrated notable sex disparities, with a higher prevalence in men compared with women (7.4%,  $P=0.001$ ; and 5.0%,  $P=0.001$ , respectively). Conversely, *E. coli* exhibited a higher prevalence in women compared with men (60.4%,  $P<0.001$ ) (Table III).

The data in Table IV revealed associations between age groups and the prevalence of bacterial pathogens in UTIs. Gram-positive bacteria demonstrated a significant increase in prevalence in the older age groups (age groups 3 and 4) ( $\geq 70$  years old) ( $P=0.005$ ), indicating that these

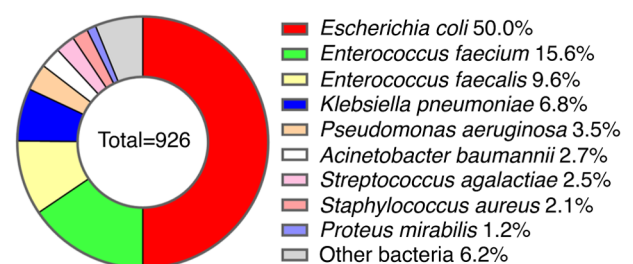


Figure 1. Distribution of the top 9 frequently isolated strains among 926 pathogens.

bacterial species are more commonly found in elderly patients. Additionally, *E. faecium* exhibited notable age-related associations ( $P<0.001$ ), being more prevalent in age groups 3 and 4 ( $\geq 70$  years old) (18.4 and 23.8%) compared with that in the other age groups.

Among the Gram-negative bacteria, *E. coli* showed a significant age association ( $P=0.005$ ). Notably, a higher prevalence was observed in younger age groups, with the highest proportion in the 50-69 years age group (57.3%) and  $\leq 50$  years age group (49.2%) (Table IV).

The findings in Table IV suggested that the prevalence of certain bacterial pathogens in UTIs was influenced by the age of the patients. Gram-positive bacteria, *E. faecium* tended to be more prevalent in older patients, while *E. coli* was more common in younger patients.

## Discussion

The present study sheds light on the distribution of bacterial pathogens in UTIs concerning seasonality, sex, and age factors. The predominant pathogenic bacteria identified were *E. coli* (50.0%), *E. faecium* (15.6%), *E. faecalis* (9.6%), *K. pneumoniae* (6.8%), *P. aeruginosa* (3.5%), *A. baumannii* (2.7%), *S. agalactiae* (2.5%), *S. aureus* (2.1%) and *P. mirabilis* (1.2%). These findings align with those of previous studies (24-26). Another similar study by Faine *et al* (27) showed that among the patients with positive urine cultures, 84.7% had cultures that grew Enterobacterales, with *E. coli* (63.2%) being the most common pathogen isolated. Pathogenic *E. coli* was

Table II. Association between season and distribution of bacteria (n=926) in urinary tract infections.

Bacteria	Spring (n=246)	Summer (n=212)	Autumn (n=273)	Winter (n=195)	$\chi^2$ test	P-value
Gram-positive, n (%)	87 (35.4)	70 (33.0)	68 (24.9)	50 (25.6)	9.442	0.024
<i>Enterococcus faecium</i> , n (%)	54 (22.0)	32 (15.1)	29 (10.6)	29 (14.9)	12.824	0.005 <sup>b</sup>
<i>Enterococcus faecalis</i> , n (%)	20 (8.1)	25 (11.8)	29 (10.6)	15 (7.7)	2.930	0.403
<i>Streptococcus agalactiae</i> , n (%)	4 (1.6)	10 (4.7)	6 (2.2)	3 (1.5)	4.993	0.162 <sup>a</sup>
<i>Staphylococcus aureus</i> , n (%)	9 (3.7)	3 (1.4)	4 (1.5)	3 (1.5)	3.593	0.302 <sup>a</sup>
Gram-negative, n (%)	159 (64.4)	142 (67.0)	205 (75.1)	145 (74.4)	9.442	0.024
<i>Escherichia coli</i> , n (%)	130 (52.8)	99 (46.7)	127 (46.5)	107 (54.9)	4.895	0.180
<i>Klebsiella pneumoniae</i> , n (%)	17 (6.9)	8 (3.8)	24 (8.8)	14 (7.2)	4.819	0.187
<i>Pseudomonas aeruginosa</i> , n (%)	7 (2.8)	5 (2.4)	14 (5.1)	6 (3.1)	3.412	0.342
<i>Acinetobacter baumannii</i> , n (%)	1 (0.4)	5 (2.4)	16 (5.9)	3 (1.5)	16.404	0.001 <sup>b</sup>
Other bacteria n (%)	4 (1.6)	25 (11.8)	24 (8.8)	15 (7.7)	4.926	0.260

<sup>a</sup>Fisher's exact test used for statistical analysis. <sup>b</sup>P<0.05. Pairwise tests were performed using Bonferroni correction afterward: i) *Enterococcus faecium*: Spring vs. autumn,  $\chi^2$ , 12.361 and P<0.001. Results of other pairwise tests:  $\chi^2$ , 0.004-3.568 and P>0.0083, showing no significance. ii) *Acinetobacter baumannii*: Spring vs. autumn,  $\chi^2$ , 12.150 and P<0.001. Results of other pairwise tests:  $\chi^2$ , 0.354-5.456, P>0.0083, demonstrating no significance.

regarded as the most common pathogen of UTIs (27-30). The present analysis of seasonal distribution revealed a significant association between specific bacterial species and particular seasons. Notably, *E. faecium* showed higher prevalence during the spring and *A. baumannii* during the autumn. These findings aligned with a previous study by Alrashid *et al* (31), which also demonstrated a seasonal pattern, with the highest number of confirmed UTIs in January and the lowest in April. These results emphasized the seasonal variations in UTIs and suggested that the prevalence of bacterial pathogens was influenced by seasonal factors (9). This complexity adds to our understanding of UTIs and highlights the need to consider seasonal variations while devising diagnostic and preventive strategies, as corroborated by the previous study by Simmering *et al* (8).

The study enrolled a total of 926 hospitalized subjects, with 343 (37.0%) men and 583 (63.0%) women, indicating a higher incidence of UTIs in women, which is consistent with previous studies (5,32,33). UTIs were much more common in women than in men due to certain anatomical and physiological characteristics, such as a shorter urethra, the proximity of the urethral opening to the anus, changes during menopause, pregnancy and other factors, with this difference increasing with age (34,35). The analysis of sex distribution revealed noteworthy differences in the prevalence of bacterial pathogens between male and female groups. Men with UTIs exhibited a greater susceptibility to Gram-positive bacterial infections compared with their female counterparts (35.9 vs. 26.9%; P=0.004). *E. faecalis*, *S. aureus*, *P. aeruginosa* and *A. baumannii* showed a significant sex difference, being more common in men. Silva *et al* (36) observed a higher prevalence of UTIs caused by *E. faecalis* and *P. aeruginosa* in men compared with women (8.8% for *E. faecalis* and 8.1% for *P. aeruginosa* in men, compared with 1.8 and 1.6% in females, respectively). A previous study conducted by

Magliano *et al* (37) demonstrated that *P. aeruginosa* was found more frequently in men, whereas the prevalence of *E. faecalis* aligned with the findings of the current study. In the present study, the occurrence of *E. faecalis* in men and women was 12.5 vs. 7.9% (P<0.05), compared with 9.5 vs. 5.4% (P<0.05) as reported by Magliano *et al* (37). *E. coli* demonstrated notable sex disparities, with a higher prevalence in women compared with men (P<0.001). Amna *et al* (38) determined that non-*E. coli* bacteria were more prone to infect men, citing the increased complexity of UTIs in men, often attributed to the frequent use of catheters (39,40). Specifically, *Enterococcus* and *Pseudomonas* have been linked to infections associated with urinary tract catheters (41,42). These findings shed light on the intricate interplay between biological, anatomical and behavioral factors in UTI prevalence.

The higher occurrence of Gram-positive bacteria in men suggests the possibility of sex-related disparities in immunity or physiological factors that may render men more susceptible to UTIs (11,43). The finding of the present study, showing a rise in *S. aureus* among men, aligns with the research conducted by Stokes *et al* (44), which demonstrated a higher prevalence of *S. aureus* in men, particularly in older age groups with comorbidities. Understanding the sex-specific variations in bacterial prevalence holds promise for targeted interventions and tailored awareness campaigns for each sex group (45). By addressing the unique factors influencing UTIs in men and women, progress can be made towards reducing UTI rates and enhancing overall health outcomes (46).

The present investigation into the age distribution of bacterial pathogens revealed significant associations between certain species and different age groups. The study highlighted that Gram-positive bacteria, especially *E. faecium*, exhibited a marked increase in prevalence among elderly individuals with UTIs. These findings were consistent with those of previous studies (47,48). The notable age-related associations observed for *E. coli* in the



Table III. Association between sex and bacterial distribution in urinary tract infections.

Microorganisms	Infection Status	Male group, n (%) (n=343)	Female group, n (%) (n=583)	$\chi^2$ test	P-value <sup>a</sup>
Gram-positive bacteria	Yes	123 (35.9)	157 (26.9)	8.164	0.004 <sup>b</sup>
	No	220 (64.1)	426 (73.1)		
<i>Enterococcus faecium</i>	Yes	56 (16.3)	88 (15.1)	0.250	0.617
	No	287 (83.7)	495 (84.9)		
<i>Enterococcus faecalis</i>	Yes	43 (12.5)	46 (7.9)	5.366	0.021 <sup>b</sup>
	No	300 (87.5)	537 (92.1)		
<i>Staphylococcus aureus</i>	Yes	15 (4.4)	4 (0.7)	14.607	<0.001 <sup>b</sup>
	No	328 (95.6)	579 (99.3)		
<i>Streptococcus agalactiae</i>	Yes	7 (2.0)	16 (2.7)	0.441	0.506
	No	336 (98.0)	567 (97.3)		
<i>Enterococcus pseudoavium</i>	Yes	2 (0.6)	3 (0.5)	0.000	1.000 <sup>c</sup>
	No	341 (99.4)	580 (99.5)		
Gram-negative bacteria	Yes	220 (64.1)	426 (73.1)	8.164	0.004 <sup>b</sup>
	No	123 (35.9)	157 (26.9)		
<i>Escherichia coli</i>	Yes	111 (32.4)	352 (60.4)	67.798	<0.001 <sup>b</sup>
	No	232 (67.6)	231 (39.6)		
<i>Klebsiella pneumoniae</i>	Yes	30 (8.7)	33 (5.7)	3.243	0.072
	No	313 (91.3)	550 (94.3)		
<i>Pseudomonas aeruginosa</i>	Yes	18 (5.2)	14 (2.4)	5.244	0.022 <sup>b</sup>
	No	325 (94.8)	569 (97.6)		
<i>Acinetobacter baumannii</i>	Yes	17 (5.0)	8 (1.4)	10.560	<0.001 <sup>b</sup>
	No	326 (95.0)	575 (98.6)		
<i>Proteus mirabilis</i>	Yes	6 (1.7)	5 (0.9)	1.463	0.227
	No	337 (98.3)	578 (99.1)		
Other bacteria	Yes	38 (11.1)	14 (2.4)	30.678	<0.001 <sup>b</sup>
	No	305 (88.9)	569 (97.6)		

<sup>a</sup>Calculated by  $\chi^2$  tests. <sup>b</sup>P<0.05. <sup>c</sup>Continuity correction. Other bacteria in the male group: *Enterobacter cloacae* complex (6 strains); *Morganella morganii* (n=6); *Pseudomonas putida* (n=4); *Stenotrophomonas maltophilia* (n=4); *Serratia plymuthica* (n=4); *Enterobacter aerogenes* (n=3); *Citrobacter freundii* (n=3); *Providencia rettgeri* (n=1); *Klebsiella Oxytoca* (n=1); *Citrobacter diversus* (n=1); *Aeromonas caviae* (n=1); *Burkholderia cepacia* complex (n=1). Other bacteria in the female group: *Enterobacter aerogenes* (n=4); *Morganella morganii* (n=2); *Serratia plymuthica* (n=2); *Citrobacter freundii* (n=1); *Citrobacter diversus* (n=1); *Elizabethkingia meningoseptica* (n=1); *Proteus vulgaris* (n=1); *Stenotrophomonas maltophilia* (n=1); *Enterobacter cloacae* complex (n=1).

present study demonstrated higher prevalence in younger individuals with UTIs. These age-related patterns of bacterial pathogens add another layer of complexity to our understanding of UTIs. The substantial changes in bacteria prevalence of UTIs with age emphasize the importance of age-specific management strategies leading to better healthcare outcomes (49,50).

The retrospective design, the single tertiary hospital setting, and the lack of detailed information on UTI types are limitations of the present study. Future research with prospective designs and multicenter collaborations through a focused analysis of UTI types is a proactive way to address these limitations and enhance the overall understanding of UTI epidemiology.

In conclusion, the present study provided a comprehensive exploration of UTIs, revealing intricate patterns in bacterial pathogen distribution concerning seasonality, sex, and

age. *E. coli* was the predominant pathogen among UTIs. Significant associations between bacterial species and seasons were observed, with *E. faecium* prevalent in the spring and *A. baumannii* in the autumn. Notable sex-specific differences were found in bacterial prevalence, with *S. aureus* and *E. faecalis* found more frequently in men. Additionally, age-related associations revealed significant dynamics, with *E. faecium* exhibiting a marked increase in prevalence with advancing age, while *E. coli* had a higher prevalence in younger individuals with UTIs. Future research with prospective designs and multicenter collaborations aims to enhance our understanding of UTI epidemiology.

#### Acknowledgements

Not applicable.

Table IV. Association between age and distribution of bacteria (n=926) in urinary tract infections.

Bacteria	Age group 1 (≤50 years; n=128)	Age group 2 (50-69 years; n=351)	Age group 3 (70-80 years; n=283)	Age group 4 (>80 years; n=164)	$\chi^2$ test	P-value <sup>a</sup>
Gram-positive (n=275), n (%)	43 (33.6)	80 (22.8)	95 (33.6)	57 (34.8)	12.989	0.005 <sup>b</sup>
<i>Enterococcus faecium</i> , n (%)	18 (14.1)	36 (10.3)	52 (18.4)	39 (23.8)	17.775	<0.001 <sup>b</sup>
<i>Enterococcus faecalis</i> , n (%)	13 (10.2)	33 (9.4)	27 (9.5)	15 (9.1)	0.092	0.993
<i>Streptococcus agalactiae</i> , n (%)	5 (3.9)	8 (2.3)	8 (2.8)	2 (1.2)	2.350	0.503
Gram-negative (n=651), n (%)	85 (66.4)	271 (77.2)	188 (66.4)	107 (65.2)	17.775	<0.001 <sup>b</sup>
<i>Escherichia coli</i> , n (%)	63(49.2)	201 (57.3)	126 (44.5)	73 (44.5)	12.813	0.005 <sup>b</sup>
<i>Klebsiella pneumoniae</i> , n (%)	7 (5.5)	25 (7.1)	16 (5.7)	15 (9.1)	2.426	0.489
<i>Pseudomonas aeruginosa</i> , n (%)	5 (3.9)	11 (3.1)	10 (3.5)	6 (3.7)	0.212	0.976
<i>Acinetobacter baumannii</i> , n (%)	5 (3.9)	10 (2.8)	10 (3.5)	0 (0.0)	6.038	0.110
<i>Proteus mirabilis</i> , n (%)	1 (0.8)	6 (1.7)	4 (1.4)	0 (0.0)	3.088	0.378
Other bacteria, n	11 (8.6)	21 (6.0)	30 (10.6)	14 (8.5)	4.506	0.210

<sup>a</sup>Calculated by  $\chi^2$  tests. <sup>b</sup>P<0.05. Significant differences in Gram-positive bacterial prevalence were found between age groups 2 and 3 ( $\chi^2$ , 9.106; P=0.003) and between age groups 2 and 4 ( $\chi^2$ , 8.194; P=0.004). For *Enterococcus faecium*, significant differences were observed between age groups 2 and 3 ( $\chi^2$ , 8.638; P=0.004) and between age groups 2 and 4 ( $\chi^2$ , 16.431; P<0.001). Similarly, *Escherichia coli* exhibited significant differences between age groups 2 and 3 ( $\chi^2$ , 10.185; P=0.002) and between age groups 2 and 4 ( $\chi^2$ , 7.301; P=0.007). No statistically significant differences were found across other pairwise tests ( $\chi^2$ , 0.001-5.734; P>0.0083).

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## Availability of data and materials

The data generated in the present study may be requested from the corresponding author upon reasonable request.

## Authors' contributions

ZSZhan and JS contributed equally to this work, and were involved in the conception and design of the study, data collection, analysis and interpretation of data, and drafting the manuscript. ZSZheng contributed to the design of the study, data analysis and critically revised the manuscript for important intellectual content. XXZ contributed reagents, materials, analysis tools and data, and analyzed and interpreted the data. JC and XYZ played significant roles in data interpretation, and provided substantial contributions to the manuscript revision. SYZ was responsible for the study concept, analyzed and interpreted the data, wrote and analyzed the methods, and reviewed and edited the manuscript. XXZ and XYZ confirm the authenticity of all the raw data. All the authors read and approved the final manuscript.

## Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Fuding Hospital, Fujian University of Traditional Chinese Medicine (Fuding, China; approval no. Fuding Hospital

2022325). All methods were conducted according to relevant guidelines and regulations. The requirement for informed consent from participants was waived by the Medical Ethics Committee of Fuding Hospital, Fujian University of Traditional Chinese Medicine, due to the retrospective nature of the study.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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