Scientific Research Publishing

American Journal of Molecular Biology, 2024, 14, 245-260
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

Prevalence and Antibiotic Resistance of Urinary Tract Pathogens, with Molecular Identification of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Acinetobacter* spp., Using Multiplex Real-Time PCR

Hawa Tarnagda[1,2], Djénéba Ouermi[1,3], Tani Sagna[1,3,4], Wendyam Marie Christelle Nadembega[1,3], Abdoul Karim Ouattara[1,3,5], Lassina Traoré[1,3], Rogomenoma Alice Ouedraogo[1,3,6], Prosper Bado[1], Bapio Valérie Elvira Jean Télesphore Bazie[1,3,4], Nicole Bouda/Zongo[7], Luc Zongo[1,2,7], Albert Théophane Yonli[1], Théodora Mahoukèdè Zohoncon[1,2,3,7], Florencia Wendkuuni Djigma[1,3], Jacques Simpore[1,2,3,7]

[1]Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), Ouagadougou, Burkina Faso
[2]University Saint Thomas d'Aquin (USTA), Ouagadougou, Burkina Faso
[3]Laboratoire de Biologie Moléculaire et de Génétique (LABIOGENE), Université Joseph KI-ZERBO, Ouagadougou, Burkina Faso
[4]Institute for Research in Applied Sciences (IRSS/CNRST), Ouaga, Burkina Faso
[5]Université Norbert Zongo/Centre Universitaire de Manga, Avenue Maurice Yameogo, Koudougou, Burkina Faso
[6]Université Nazi BONI, Bobo-Dioulasso, Burkina Faso
[7]Hôpital Saint Camille de Ouagadougou (HOSCO), Ouagadougou, Burkina Faso
Email: simpore93@gmail.com

How to cite this paper: Tarnagda, H., Ouermi, D., Sagna, T., Nadembega, W.M.C., Ouattara, A.K., Traoré, L., Ouedraogo, R.A., Bado, P., Bazie, B.V.E.J.T., Bouda/Zongo, N., Zongo, L., Yonli, A.T., Zohoncon, T.M., Djigma, F.W. and Simpore, J. (2024) Prevalence and Antibiotic Resistance of Urinary Tract Pathogens, with Molecular Identification of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Acinetobacter* spp., Using Multiplex Real-Time PCR. *American Journal of Molecular Biology*, **14**, 245-260
https://doi.org/10.4236/ajmb.2024.144018

Received: September 17, 2024
Accepted: October 7, 2024
Published: October 10, 2024

Abstract

Urinary tract infections (UTIs) caused by uropathogens are a significant public health problem, and their treatment primarily relies on antibiotic therapy. However, the increasing global development of antibiotic resistance necessitates updating diagnostic techniques to ensure higher sensitivity and specificity, especially with advancements in science and medicine. This study aimed to evaluate the prevalence of UTIs and antibiotic resistance profiles through urine culture, as well as to identify *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Acinetobacter* spp. in urine samples using a molecular approach with multiplex real-time PCR. From May 3 to July 25, 2023, at the Pietro Annigoni Biomolecular Research Center (CERBA) and Saint Camille Hospital of Ouagadougou (HOSCO), 209 urine samples collected from patients with suspected UTIs were analyzed using both urine culture and multiplex real-time PCR. Among the 209 patients, 52.15% were male and 47.85% female, with an average age of 46.87 ± 21.33 years. Urine cultures revealed an overall UTI

prevalence of 23.44%, with a prevalence of 8.13% in men versus 15.31% in women (P = 0.023). The bacterial prevalence rates were as follows: *Escherichia coli* (12.92%), *Klebsiella* spp. (7.18%), *Enterobacter cloacae* (1.44%), *Staphylococcus aureus* (0.96%), and other bacteria. *Klebsiella* spp. demonstrated 100% resistance to Amoxicillin and Amoxicillin/Clavulanic Acid, while *Escherichia coli* showed 96.2% and 65.4% resistance to Amoxicillin and Amoxicillin/Clavulanic Acid, respectively. PCR analysis of the target bacteria revealed mono-infection prevalence rates of *Klebsiella pneumoniae* (10.39%), *Klebsiella oxytoca* (7.79%), and *Acinetobacter* spp. (7.79%), along with a co-infection prevalence rate of *Klebsiella pneumoniae*/*Acinetobacter* spp. (1.30%). This study demonstrated that PCR, with its high sensitivity and specificity, could effectively distinguish *Klebsiella pneumoniae* from *Klebsiella oxytoca* and detect *Acinetobacter* spp. in less than 24 hours—something urine culture alone could not achieve. The relative ease of automating urine PCR testing, combined with its diagnostic accuracy and rapid turnaround time, makes it a valuable addition to modern medical practice for the laboratory diagnosis of UTIs.

Keywords

Urinary Tract Infections, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Acinetobacter* spp., Urine Culture, Real-Time PCR

1. Introduction

Among the most common bacterial infections are Urinary Tract Infections (UTIs), which pose a major public health challenge, affecting individuals of all ages [1]. Uropathogens such as *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., *Staphylococcus* spp., and other pathogenic bacteria are frequent causes of nosocomial infections, including UTIs, which contribute significantly to mortality in clinical settings [2]-[6]. UTIs are the leading cause of morbidity in the general population and are the second most common infectious disease after respiratory infections [7]. Each year, approximately 150 million people worldwide are diagnosed with UTIs, resulting in a global economic burden exceeding US$6 billion [8]. Historically, UTIs have been recognized for millennia. As early as the 16th century BC, they were described in the Ebers Papyrus, one of the oldest surviving medical texts. The Egyptians attributed UTIs to heat in the bladder and treated them with herbal decoctions, bloodletting, and rest (Bent *et al*., 2002) [9]. Effective treatments for UTIs only emerged in the 1930s with the discovery of antibiotics.

Despite advances in diagnostics and the widespread use of antibiotic therapy, antimicrobial resistance (AMR) in uropathogens remains a significant concern [10]. UTIs, caused by the colonization of the urinary tract by infectious agents, lead to an inflammatory response in the urothelium. Among the pathogens involved, bacteria of the Klebsiella and Acinetobacter genera play a predominant role [11].

The diagnosis of UTIs is based on clinical symptoms, as well as the detection and identification of microorganisms in urine [9]. UTIs are prevalent in both community and hospital settings and affect people across all demographics [12]. However, women are four times more likely than men to develop UTIs due to anatomical differences [13].

Currently, particularly in low-income countries, the standard method for diagnosing UTIs remains the Cytobacteriological Examination of Urine (CEBU) through urine culture [14]. Urine culture provides valuable information regarding the presence or absence of a uropathogen, facilitates identification of the causative organism, and in some cases, determines antibiotic susceptibility or resistance. However, the main limitation of this method is the time required to obtain results, which typically takes 2 to 3 days [14]. To alleviate patient symptoms, empirical antibiotic therapy is often initiated while awaiting these results. This practice, however, can contribute to the overuse of antibiotics.

The development of a rapid diagnostic system based on molecular biology, such as automated urine analysis, could provide same-day results, detecting the presence or absence of significant bacteriuria, identifying the pathogen, and determining genetic resistance. Such a system could also reduce the need for urine culture in many negative cases, preventing unnecessary antibiotic prescriptions for patients who do not require them.

In this context, molecular characterization using the Polymerase Chain Reaction (PCR) has emerged as a vital innovation for the rapid and precise identification of pathogens in urinary tract infections. Multiplex real-time PCR allows for the specific detection and amplification of DNA fragments unique to targeted bacteria, offering a more sensitive and specific method compared to traditional urine culture. Urine culture often struggles to differentiate between *Klebsiella* species (*K. pneumoniae* and *K. oxytoca*) and is unable to detect *Acinetobacter* spp. [15].

Given the limitations of traditional urine culture, particularly its lengthy turnaround time and difficulty in distinguishing certain uropathogens like *Klebsiella* spp. and *Acinetobacter* spp., there is a pressing need for faster, more accurate diagnostic methods. The emergence of molecular techniques, such as multiplex real-time PCR, offers a solution by enabling rapid identification of pathogens and their genetic resistance profiles within the same day. This advancement can significantly reduce unnecessary antibiotic use and improve patient outcomes by offering precise diagnoses in a timely manner. The objectives of this study were: (i) to evaluate, using urine culture, the prevalence rate of urinary tract infections (UTIs) and the resistance rate, and (ii) to identify *Klebsiella* spp. and *Acinetobacter* spp. in urine through a molecular approach using multiplex real-time PCR.

2. Materials and Methods

2.1. Type and Period of Study

This cross-sectional study, with both descriptive and analytical objectives, was conducted from May 3 to July 25, 2023, at the Pietro Annigoni Biomolecular

Research Center (CERBA) and the medical biology laboratory of Saint Camille Hospital Ouagadougou (HOSCO).

2.2. Sampling

A total of 209 urine samples, each ranging from 20 to 30 mL and collected from the first morning void, were obtained from patients prior to any antibiotic intake. After perineal cleansing, midstream urine was collected in a sterile, dry container with a screw lid and promptly transported to the laboratory.

2.3. Inclusion and Non-Inclusion Criteria

The examination report should include the following details: the patient's identity (name, first name, age, sex), the nature of the sample, the clinical assessment, the consultation and/or hospitalization department, and the results of the cytobacteriological examination of the urine (CBEU). Samples collected in non-sterile equipment or suspected of external contamination were excluded from the study.

2.4. Culture, Identification and Antibiotic Resistance Testing

2.4.1. Isolation and Identification

The urine cultures were performed using the tight streaking technique with a 10 µL calibrated loop. The culture media used were chromogenic agars from "UriSelect™4 Medium" (Oxoid, United Kingdom), which enabled bacterial identification based on colony coloration, as each bacterium produces a distinctive color. The inoculated media were incubated at 37˚C for 24 hours.

2.4.2. Antibiotic Sensitivity Tests

Antibiotic susceptibility and resistance testing were performed using antibiograms in accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2023). Fifteen (15) antibiotics were used to assess antimicrobial susceptibility: Amikacin (AMK) (30 µg), Amoxicillin (AML) (25 µg), Amoxicillin/Clavulanic Acid (AMC) (30 µg), Ampicillin/Sulbactam (SAM) (20 µg), Cefepime (FEP) (30 µg), Cefixime (CFM) (5 µg), Cefoxitin (FOX) (30 µg), Ceftazidime (CAZ) (10 µg), Ceftriaxone (CRO) (30 µg), Ciprofloxacin (CIP) (5 µg), Fosfomycin (FOS) (200 µg), Gentamicin (GM) (10 µg), Imipenem (IPM) (10 µg), Levofloxacin (LEV) (5 µg), and Tobramycin (TOB) (10 µg).

2.5. Molecular Analysis of Urine

The pathogens tested were bacteria commonly associated with urinary tract infections (UTIs), such as *Klebsiella* spp. and *Acinetobacter* spp. For molecular analysis, multiplex real-time PCR was employed to screen for *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Acinetobacter* spp. A total of 77 out of 209 samples, including both *Klebsiella* spp. positive and negative cultures, were analyzed.

2.5.1. Extraction of Bacterial Genomic DNA

Bacterial genomic DNA was extracted from all strains following the manufacturer's instructions using the SaMag Bacterial DNA Extraction Kit (Sacace Biotechnologies, Como, Italy). The quantification and purity of the extracted DNA were assessed using a Nanodrop spectrophotometer. A 260/280 nm absorbance ratio between 1.8 and 2.0 was considered indicative of "pure" DNA.

2.5.2. Preparation of Tubes for PCR and Amplification Program

A reaction volume of 35 µL was prepared according to the manufacturer's instructions. For each clinical sample and control, one strip of PCR Reaction Mix-1 and one strip of PCR Reaction Mix-2 were used. PCR was performed using the SACACE Biotechnologies thermocycler with the following reaction mixture: 10 µL of Taq polymerase, 5 µL of isolated DNA samples, 5 µL of negative control (in the tube labeled C–, negative extraction control), and 5 µL of positive control (in the tube labeled C+, positive amplification control). All strips were sealed, briefly centrifuged (2 - 3 seconds), and then transferred to the thermocycler, which was pre-programmed with the Bac Multi-Screen Real-TM amplification protocol (Table 1).

Table 1. Amplification program (Sacace biotechnologies, 2022).

Steps	Temperature ˚C	Min	Seconds	Cycles
1: denaturation	80	0	30	1
	94	1	30	
2: hybridation	94	0	30	5
	64	0	15	
3: extension	94	0	10	45
	64	0	15	

2.5.3. Detection of Target Genes by Multiplex PCR Amplification and Interpretation of Results

According to the manufacturer's instructions, the targets were detected by fluorescent signals in the FAM, HEX, ROX, and Cy5 channels. The results were interpreted using the detection program of the Bac Multi-Screen Real-TM amplification protocol and were analyzed directly through the software provided with the real-time PCR instrument (SACACE thermocycler, Como, Italy). The data were presented as amplification curves. Results were validated based on the Ct (threshold cycle) values of each fluorochrome, which were compared to those of the positive controls. A Ct value lower than 26 was considered positive, while a Ct value higher than 26 was considered negative.

2.6. Data Analysis and Ethical Considerations

2.6.1. Data Analysis

The data was collected using Microsoft Excel 2016 software. Data analysis was performed using SPSS version 20.0, EpiInfo 7. Results were considered statistically significant if $P < 0.05$.

2.6.2. Ethical Considerations

The Institutional Ethics Committee of HOSCO/CERBA, through Deliberation No. 2023-02-15 dated April 18, 2023, provided a favorable opinion for this study. Confidentiality and anonymity regarding the information obtained from the various patient records were strictly maintained.

3. Results

3.1. Sociodemographic Characteristics

During this study, 209 cytobacteriological examination of urine (CBEU) samples were collected between May 2023 and July 2024. The samples came from both male and female patients, spanning various age groups. Table 2 provides the distribution of patients according to age and sex. Male patients accounted for 52.15% (109/209) of the total, while female patients represented 47.85% (100/209). The age group of 20 to 39 years, across both sexes, was the most represented (35.41%). The average age of all patients was 46.87 ± 21.33 years

3.2. Results of Clinical Examinations

Among the patients admitted for urine culture examinations at CERBA or HOSCO, some reported experiencing pain or burning during urination, while others complained of urethral discharge, pelvic pain, dark urine, dysuria, or polyuria.

3.3. Culture Results

3.3.1. Frequency of Urinary Tract Infections (UTI)

The frequency of Urinary Tract Infections (UTIs) by gender is presented in Table 3. The results of this study show an overall UTI prevalence rate of 23.44% (49/209). The prevalence rates for males and females were 8.13% and 15.31%, respectively, with a statistically significant difference (P = 0.023).

3.3.2. Prevalence of Urinary Tract Infections Found in the Urine Culture of this Study

The analysis of the pathogens involved, as shown in Table 4, revealed the following prevalence rates: *Escherichia coli* at 12.92% (27/209), *Klebsiella* spp. at 7.18% (15/209), *Enterobacter cloacae* at 1.44% (3/209), *Staphylococcus aureus* at 0.96% (2/209), *Staphylococcus* spp. at 0.48% (1/209), and *Pseudomonas* spp. at 0.48% (1/209).

3.3.3. Resistance of Isolated Pathogenic Microorganisms

Table 5 presents the resistance rates of the isolated pathogenic microorganisms. *Klebsiella* spp. exhibited 100% resistance to both Amoxicillin and Amoxicillin/Clavulanic Acid, while *Escherichia coli* showed 96.2% and 65.4% resistance to Amoxicillin and Amoxicillin/Clavulanic Acid, respectively. However, *Klebsiella* spp. remained 91.7% susceptible to Imipenem, 80.0% to Gentamicin, and 73.3% to Tobramycin.

3.4. PCR Results: Prevalence Rates of the Three Germs Tested for by Multiplex Real-Time PCR

The analysis of the three pathogens involved revealed the following prevalence rates for mono-infections: *Klebsiella pneumoniae* at 10.39% (8/77), *Klebsiella oxytoca* at 7.79% (6/77), and *Acinetobacter* spp. at 7.79% (6/77). Additionally, the prevalence of co-infection with *Klebsiella pneumoniae* and *Acinetobacter* spp. was 1.30% (1/209) as shown in Table 6. This molecular study, conducted using multiplex real-time PCR, also demonstrated an overall positivity rate of 23.38% (18/77). The prevalence rates by sex were 12.99% (10/77) for women and 10.39% (8/77) for men, with no statistically significant difference (P = 0.316).

Table 2. Sociodemographic characteristics of the 209 patients.

	Females		**Males**		Total		Mean age		
Age grouos, years	N1	%	N2	%	N	%	Mean	Standard deviation	P
X < 20	6	2.87	11	5.26	17	8.13	13.35	6.47	
20 à 39	47	22.49	27	12.92	74	35.41	29.32	5.06	
40 à 59	24	11.48	22	10.53	46	22.01	49.33	6.34	
60 à 79	17	8.13	41	19.62	58	27.75	68.00	5.85	
X > 79	6	2.87	8	3.83	14	6.70	85.71	4.30	
Total	100	47.85	109	52.15	209	100.00	46.87	21.33	
Sex									
Females*					100	47.8	42.55	19.75	
Males°					109	52.2	50.83	22.04	**0.005**
Total	**F --->M: P = 0.002**				209	100.0	46.87	21.33	

Table 3. Distribution of the study population by sex based on the positivity of germs isolated by the bacterial culture technique.

Sex	Negative	Positive	Total	P
Males	92/209 (44.02%)	17/209 (8.13%)	109/209 (52.15%)	
Females	68/209 (32.54%)	32/209 (15.31%)	100/209 (47.85%)	0.023
Total	160/209 (76.56%)	49/209 (23.44%)	209/209 (100.0%)	

Table 4. Distribution of microorganisms isolated from bacterial culture.

Isolated uropathogens	N = 209	Percentage
Mono-infection		
Escherichia coli	27	12.92
Klebsiella spp.	15	7.18
Enterobacter cloacae	3	1.44
Staphylococcus aureus	2	0.96
Staphylococcus sp.	1	0.48
Candida albicans	1	0.48
Pseudomonas sp.	1	0.48
Acinetobacter spp.	0	0.00
Klebsiella oxytoca	0	0.00
Co-infection		
E. coli/ *Klebsiella* spp.	1	0.48

Table 5. Resistance rate of pathogenic microorganisms isolated from bacterial culture.

Antibiotics	*Escherichia coli* (%)		*Klebsiella* spp. (%)		*Enterobacter cloacae* (%)	
	Resistance	Sensibility	Resistance	Sensibility	Resistance	Sensibility
AML	96.2	3.8	100.0	0.0	-	-
AMC	65.4	34.6	100.0	0.0	-	-
SAM	65.4	34.6	76.9	23.1	0.0	100.0
CIP	55.6	44.4	60.0	40.0	66.7	33.3
LEV	57.7	42.3	60.0	40.0	0.0	100.0
GM	18.5	81.5	20.0	80.0	33.3	66.7
AMK	48.1	51.9	40.0	60.0	-	-
TOB	37.0	63.0	26.7	73.3	-	-
FEP	57.7	42.3	60.0	40.0	-	-
CAZ	60.0	40.0	73.3	26.7	-	-
CRO	60.0	40.0	60.0	40.0	-	-
FOX	-	-	26.7	73.3	-	-
IPM	-	-	8.3	91.7	-	-
CFM	-	-	73.3	26.7	-	-
FOS	-	-	53.3	46.7	33.3	66.7

Amikacin (AMK), Amoxicillin (AML), Amoxicillin + Clavulanic Acid (AMC), Ampicillin/sulbactam (SAM), Cefepim (FEP), Cefixime (CFM), Cefoxitin (FOX), Ceftazidime (CAZ), Ceftriaxone (CRO), Ciprofloxacin (CIP), Fosfomycin (FOS), Gentamicin (GM), Imipenem (IPM), Levofloxacin (LEV), Tobramycin (TOB).

Table 6. Mono- and co-infection rates of bacteria isolated by multiplex real-time PCR and their prevalence by sex.

Isolated	Negative	Positive	Total	P
Mono-infection				
K. pneumoniae	69 (89.61%)	8 (10.39%)	77 (100.00%)	
K. oxytoca	71 (92.21%)	6 (7.79%)	77 (100.00%)	
Acinobacter spp.	71 (92.21%)	6 (7.79%)	77 (100.00%)	
Co-infections				
K. pneumoniae/K. oxytoca	77 (100.00%)	0 (0.00%)	77 (100.00%)	
K. pneumoniae/Acinobacter spp.	76 (98.70%)	1 (1.30%)	77 (100.00%)	
K. oxytoca/Acinobacter spp.	77 (100.00%)	0 (0.00%)	77 (100.00%)	
Sex				
Females	24/77 (31.17%)	10/77 (12.99%)	34/77 (44.16%)	0.616
Males	35/77 (45.45%)	8/77 (10.39%)	43/77 (55.84%)	
Total	59/77 (76.62%)	18/77 (23.38%)	77/77 (100.0%)	

4. Discussion

In this study, we evaluated the prevalence of uropathogens, their resistance to antimicrobials, and the potential for diagnosing pathogens that are difficult to identify using conventional bacterial culture, through multiplex real-time PCR.

Uropathogens Identified by Urine Cultures

Among the 209 patients who underwent urine culture, 23.44% (49/209) were diagnosed with a urinary tract infection (UTI). This rate is comparable to the findings in Burkina Faso (24.07%) [16], in Bamako (21.9%) [17], in Ghana (22.1%) [18], and

in Gabon (29.2 %) [8]. However, it is higher than the 18.5% reported in N'Djamena, Chad (18.5%) [19]; but lower than the 59.8% found in Cameroun [20]. The prevalence of uropathogenic bacteria varies across regions and continents [21], highlighting the need to understand local epidemiology and its changes over time to select effective first-line antibiotic therapies tailored to each area [8].

In this study, the most prevalent uropathogens, in decreasing order, were: *Escherichia coli* (12.92%), *Klebsiella pneumoniae* (4.31%), *Enterobacter cloacae* (1.44%), and *Staphylococcus aureus* (0.96%). The prevalence of *Escherichia coli* in this study is lower than that reported in Ouagadougou (32.76%) [22] and (35.5%) [23]. Similarly, the prevalence of *Klebsiella pneumoniae* is lower than that found in Ouagadougou (22%) [23] and (10.45%) [22] and in Mbarara, in Uganda (12.57%) [24]. Variations in prevalence rates could be attributed to differences in study periods, sampling locations, and identification techniques.

Given that UTIs typically spread through an ascending route and that *E. coli* is a natural component of the digestive flora, this likely explains its strong predominance in urins samples [25]. As several authors have indicated, among the enterobacteria isolated in UTIs, the genus *Escherichia* is most involved, followed by Klebsiella [26]-[28].

Moreover, this study revealed that UTIs were more prevalent in women (13.40%) compared to men (7.18%), which is consistent with findings from other studies [24] [26] [29]. Factors such as environmental pressure, seasonal variation [24], insufficient hygiene or the anatomical configuration of the female urinary tract could contribute to this difference. However, genetically, women are more resistant to bacterial infections than men due to the "Multiple Copies in T-cell Lymphoma-1" (MCTS1) gene, located at the Xq24 locus on the X chromosome. This gene regulates *Interferon gamma* (IFN-γ) and the "Janus Kinase 2" (JAK2) gene, which activate immune responses to infections. Since women have two X chromosomes (XX) and men have one (XY), a mutation in the MCTS1 gene on the X chromosome in men could make them more susceptible to infections [30]. This genetic difference may explain why certain infectious diseases, such as COVID-19 and AIDS, have higher mortality rates in men than in women [31] [32].

Antibiotic resistance

In this study, *Klebsiella pneumoniae* exhibited 100% resistance to Amoxicillin (AML) and Amoxicillin/Clavulanic Acid (AMC), while *Escherichia coli* showed 96.2% and 65.4% resistance to AML and AMC, respectively. However, *Klebsiella pneumoniae* remained 87.5% sensitive to Imipenem (IPM) and 77.8% sensitive to Gentamicin (GM). Numerous studies have confirmed the high resistance of uropathogens to antibiotics in Africa [33]-[35]. Furthermore, *Klebsiella* spp. resistance to Ceftriaxone (60.0%), Imipenem (26.7%) and Cefoxitin (26.7%) in this study is comparable to that found in Uganda (72.7%), 31.8% and 27.3% respectively [24].

However, the primary risk factor for antibiotic resistance is repeated prior exposure to the same antibiotic [8]. The overuse or misuse of an antibiotic, or a class of antibiotics, fosters the development of bacterial resistance, which can extend to

other antibiotic families through the cross-transmission of mobile genetic elements carrying antibiotic resistance genes [36] [37]. Since UTIs often arise from the ascending contamination of the perineal flora, which reflects the digestive flora, this selection pressure has significant clinical implications [8].

Although urine culture is currently considered the "gold standard" in bacteriology, it has several limitations. One major drawback is the time required cultures take at least two days to yield results, including sensitivity profiles. This delay in treatment increases the risk of complications, such as upper urinary tract infections (e.g., pyelonephritis), especially when early intervention with empirical antibiotics could have mitigated the symptoms [38]. Additionally, certain rare pathogenic species are difficult to identify using conventional bacterial culture techniques. In contrast, molecular diagnostic methods offer a solution to these identification challenges.

Identification of Uropathogens by Multiplex Real-Time PCR

Multiplex real-time PCR analysis of three uropathogens yielded an overall positivity prevalence rate of 23.38% (18/77), with prevalence rates of *Klebsiella pneumoniae* at 10.39% (8/77), *Klebsiella oxytoca* at 7.79% (6/77), and *Acinetobacter* spp. at 7.79% (6/77). Among the 18 positive cases, the majority were female, with a prevalence of 12.99%, compared to 10.39% in males (P = 0.462). Additionally, the multiplex real-time PCR identified one co-infection involving *Klebsiella pneumoniae* and *Acinetobacter* spp.

Comparison Between the Diagnosis of Uropathogens by Bacterial Culture and the Multiplex PCR Technique

According to Sacace Biotechnologies, multiplex real-time PCR offers 100% sensitivity [79.41 - 100] and 100% specificity [98.71 - 100] [39]. The results of this study demonstrated the effectiveness and reliability of multiplex real-time PCR by confirming the positivity of all fifteen (15) *Klebsiella* spp. samples identified by urine culture, including nine (09) samples of *Klebsiella pneumoniae* and six (06) of *Klebsiella oxytoca*, which were further identified by PCR.

Although urine culture is currently considered the gold standard for diagnosing bacterial infections, it faces challenges such as high vulnerability to contamination, with an average contamination rate of 15% in many laboratories [40].

Contamination, often occurring during the pre-analytical phase of urine culture, can be minimized with proper urine collection, storage, and transport methods [41]. False-positive results due to contamination can lead to inappropriate or unnecessary treatments, increasing bacterial resistance, compromising patient outcomes, and escalating healthcare costs [38]. Furthermore, the time-consuming nature of culture techniques, often requiring several days for results, has driven the search for faster diagnostic methods like multiplex real-time PCR [42]. Multiplex real-time PCR-based bacterial diagnostics represent a significant advancement in DNA-based laboratory technologies and have become widely available in clinical settings. The use of multiplex PCR, which employs multiple primers to simultaneously detect multiple targets, has reduced both the cost and time of

analysis, which explains its growing adoption in clinical medicine [43]. According to Xu *et al*. (2021), PCR not only shows higher detection rates of single pathogens compared to urine culture [44] [45], but it is also highly effective at detecting multiple pathogens in urine samples [46] such as *Klebsiella oxytoca* and *Acinetobacter* spp., which were identified in this study—pathogens that standard urine cultures consistently failed to detect.

5. Conclusion

Bacteriological culture, first pioneered in the 19th century by Louis Pasteur, revolutionized medical diagnostics. However, with ongoing advances in medical technology, newer diagnostic tools with greater sensitivity and specificity—such as multiplex real-time PCR, expanded quantitative urine culture (EQUC), and next-generation sequencing (NGS)—now offer more comprehensive pathogen analyses in significantly less time. This study, for instance, demonstrated that molecular characterization of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Acinetobacter* spp. Using multiplex real-time PCR was completed in less than 24 hours, whereas traditional urine culture required two to three days. The ability to automate urine PCR testing, combined with its accuracy and rapid results, makes multiplex real-time PCR a powerful addition to the diagnostic tools available for managing urinary tract infections in modern medicine.

Acknowledgements

The authors thank Saint Camille Hospital of Ouagadougou (HOSCO) and the Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA) for facilitating data collection.

Author Contributions

JS, HT, TMZ, DO, CN, TS, designed the study.

JS, TMZ, DO, TS, FWD, AKO, LT, RAO, wrote the manuscript.

HT, JS, DO, TS, CN, AKO, NB, ATY, PB collected the data.

JS, HA, AKO, computerized the data.

HT, DO, TS, AKO, LZ, JS, TMZ, EJTV performed statistical analysis.

JS, TMZ, DO, TS, DO, FWD, AKO, LT, LZ, RAO, revised and edited the manuscript.

Ethical Statement

The HOSCO/CERBA Institutional Ethics Committee approved this study in Deliberation No. 2023-02-05 of 15 April 18, 2023.The study was conducted according to the declaration of Helsinki.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Kiros, T., *et al.* (2023) Bacterial Etiology of Urinary Tract Infection and Antibiogram Profile in Children Attending Debre Tabor Comprehensive Specialized Hospital, Northwest Ethiopia. *Interdisciplinary Perspectives on Infectious Diseases*, **2023**, Article ID: 1035113.

[2] Xu, Y., Zhu, F., Zhou, Z., Ma, S., Zhang, P., Tan, C., *et al.* (2024) A Novel mRNA Multi-Epitope Vaccine of Acinetobacter Baumannii Based on Multi-Target Protein Design in Immunoinformatic Approach. *BMC Genomics*, **25**, Article No. 791. https://doi.org/10.1186/s12864-024-10691-7

[3] Dissinviel, S.K., Nathalie, G., Juste, I.B., Mohamed, B.O., Fernique, K., Abraham, A., *et al.* (2017) Prevalence and Resistance Profile of Extended-Spectrum -Lactamases-Producing Enterobacteriaceae in Ouagadougou, Burkina Faso. *African Journal of Microbiology Research*, **11**, 1120-1126. https://doi.org/10.5897/ajmr2017.8598

[4] Salah, F.D., Soubeiga, S.T., Ouattara, A.K., Sadji, A.Y., Metuor-Dabire, A., Obiri-Yeboah, D., *et al.* (2019) Distribution of Quinolone Resistance Gene (*qnr*) in ESBL-Producing *Escherichia coli* and *Klebsiella* spp. in Lomé, Togo. *Antimicrobial Resistance & Infection Control*, **8**, Article No. 104. https://doi.org/10.1186/s13756-019-0552-0

[5] Kpoda, D.S., Guessennd, N., Somda, N.S., Ajayi, A., Bonkoungou, J.I., Konan, F., *et al.* (2017) Antimicrobial Susceptibility of Extended-Spectrum β-Lactamase Producing *Enterobacteriaceae* Causing Urinary Tract Infections in Ouagadougou, Burkina Faso. *African Journal of Clinical and Experimental Microbiology*, **18**, 139-144. https://doi.org/10.4314/ajcem.v18i3.2

[6] Diagbouga, S., Salah, F.D., *et al.* (2016) Detection of High Prevalence of TEM/SHV/CTX-M Genes in ESBL Producing and Multidrug Resistant *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *JBR Journal of Clinical Diagnosis and Research*, **4**, Article 130. https://doi.org/10.4172/2376-0311.1000130

[7] Addis, T., Mekonnen, Y., Ayenew, Z., Fentaw, S. and Biazin, H. (2021) Bacterial Uropathogens and Burden of Antimicrobial Resistance Pattern in Urine Specimens Referred to Ethiopian Public Health Institute. *PLOS ONE*, **16**, e0259602. https://doi.org/10.1371/journal.pone.0259602

[8] Mouanga Ndzime, Y., Onanga, R., Kassa Kassa, R.F., Bignoumba, M., Mbehang Nguema, P.P., Gafou, A., *et al.* (2021) Epidemiology of Community Origin *Escherichia coli* and *Klebsiella pneumoniae* Uropathogenic Strains Resistant to Antibiotics in Franceville, Gabon. *Infection and Drug Resistance*, **14**, 585-594. https://doi.org/10.2147/idr.s296054

[9] Bent, S. (2002) Does This Woman Have an Acute Uncomplicated Urinary Tract Infection? *JAMA*, **287**, 2701-2710. https://doi.org/10.1001/jama.287.20.2701

[10] Tuon, F.F., Zequinao, T., da Silva, M.S. and Silva, K.O. (2024) eHealth and mHealth in Antimicrobial Stewardship to Reduce Mortality in Empirical Antimicrobial Therapy and a Systematic Review with a Meta-Analysis of Adequate Therapy. *Infectious Disease Reports*, **16**, 707-723. https://doi.org/10.3390/idr16040054

[11] Bunduki, G.K., Masoamphambe, E., Fox, T., Musaya, J., Musicha, P. and Feasey, N. (2024) Prevalence, Risk Factors, and Antimicrobial Resistance of Endemic Healthcare-Associated Infections in Africa: A Systematic Review and Meta-Analysis. *BMC Infectious Diseases*, **24**, Article No. 158. https://doi.org/10.1186/s12879-024-09038-0

[12] Mbuvi, C.M., Musila, B.N. and Nyamache, A.K. (2024) Urogenital Infections among Women Attending Mwingi Hospital, Kitui County, Kenya: Safeguarding Antibiotics Through Microbiological Diagnosis. *The East African Health Research Journal*, **8**, 99-105.

[13] Spurbeck, R.R., Dinh, P.C., Walk, S.T., Stapleton, A.E., Hooton, T.M., Nolan, L.K., *et al.* (2012) *Escherichia coli* Isolates That Carry *vat*, *fyuA*, *chuA*, and *yfcV* Efficiently Colonize the Urinary Tract. *Infection and Immunity*, **80**, 4115-4122. https://doi.org/10.1128/iai.00752-12

[14] Broeren, M.A.C., Bahçeci, S., Vader, H.L. and Arents, N.L.A. (2011) Screening for Urinary Tract Infection with the Sysmex Uf-1000i Urine Flow Cytometer. *Journal of Clinical Microbiology*, **49**, 1025-1029. https://doi.org/10.1128/jcm.01669-10

[15] van der Kolk, J.H., Endimiani, A., Graubner, C., Gerber, V. and Perreten, V. (2019) Acinetobacter in Veterinary Medicine, with an Emphasis on Acinetobacter Baumannii. *Journal of Global Antimicrobial Resistance*, **16**, 59-71. https://doi.org/10.1016/j.jgar.2018.08.011

[16] Sagna, T., Somda, W.D.N., Koné, A., Sagna, Y., Tialla, D., *et al.* (2019) Antibiotic Susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* Strains, Urinary Tract Infections Cases in Bobo-Dioulasso, Burkina Faso. *EC Microbiology*, **15**, 172-178. https://ecronicon.net/assets/ecmi/pdf/ECMI-15-00605.pdf

[17] Soula, G., Pichard, E., Soula, G.G. and Kodio, A. (1990) Étude bactériologique des infections Urinaires à Bamako: Orientation pratique. *Médecine d'Afrique Noire*, 37, 243-249. https://www.santetropicale.com/Resume/53703.pdf

[18] Gambrah, E., Owusu-Ofori, A., Biney, E., Oppong, C. and Coffin, S.E. (2021) Diagnosis and Treatment of Urinary Tract Infections in Hospitalized Adults in Ghana: The Role of the Clinical Microbiology Laboratory in Improving Antimicrobial Stewardship. *International Journal of Infectious Diseases*, **102**, 497-500. https://doi.org/10.1016/j.ijid.2020.10.068

[19] Yandai, F.H., Ndoutamia, G., Nadlaou, B. and Barro, N. (2019) Prevalence and Resistance Profile of *escherichia Coli* and *klebsiella Pneumoniae* Isolated from Urinary Tract Infections in N'djamena, Tchad. *International Journal of Biological and Chemical Sciences*, **13**, 2065-2073. https://doi.org/10.4314/ijbcs.v13i4.13

[20] Nzalie, R.N., Gonsu, H.K. and Koulla-Shiro, S. (2016) Bacterial Etiology and Antibiotic Resistance Profile of Community-Acquired Urinary Tract Infections in a Cameroonian City. *International Journal of Microbiology*, **2016**, Article ID: 3240268. https://doi.org/10.1155/2016/3240268

[21] El bouamri, M.C., Arsalane, L., Kamouni, Y., Yahyaoui, H., Bennouar, N., Berraha, M., *et al.* (2014) Profil actuel de résistance aux antibiotiques des souches d'*Escherichia coli* uropathogènes et conséquences thérapeutiques [Current Antibiotic Resistance Profile of Uropathogenic *Escherichia coli* Strains and Therapeutic Consequences]. *Progrès en Urologie*, **24**, 1058-1062. https://doi.org/10.1016/j.purol.2014.09.035

[22] Karou, S.D., Ilboudo, D.P., Nadembega, W.M.C., Ameyapoh, Y., Ouermi, D., Pignatelli, S., *et al.* (2009) Antibiotic Resistance in Urinary Tract Bacteria in Ouagadougou. *Pakistan Journal of Biological Sciences*, **12**, 712-716. https://doi.org/10.3923/pjbs.2009.712.716

[23] Savadogo, H., Dao, L., Tondé, I., Tamini/Toguyeni, L., Ouédraogo, A.I., Ouermi, A.S., *et al.* (2021) Infections du tractus urinaire en milieu pédiatrique: Écologie bactérienne et sensibilité aux antibiotiques au Centre hospitalier universitaire pédiatrique Charles-de-Gaulle de Ouagadougou (Burkina Faso). *Néphrologie & Thérapeutique*, **17**, 532-537. https://doi.org/10.1016/j.nephro.2021.04.003

[24] Turugurwa, J., Mwesigye, J., Kassaza, K., Byarugaba, F., Kabanda, T. and Musinguzi, B. (2019) Antimicrobial Resistance Patterns and Molecular Characterization of *Klebsiella pneumoniae* in Clinical Isolates at Mbarara Regional Referral Hospital. *Advances in Infectious Diseases*, **9**, 197-225. https://doi.org/10.4236/aid.2019.93015

[25] Niang, A., Ka, R., Sarr, H. and Diop, A. (2020) Phenotypic Characterization of Uropathogenic Bacteria Isolated at Fann CHNU. *Dakar Uro' Andro*, **2**, 56-60.

[26] Dia, M., Chabouny, H., Diagne, R., Kâ, R. and Ba-Diallo, A. (2014) Antibiotic Susceptibility Pattern of Uropathogenic Bacterial Isolates in a Dakar Senegalese Teaching. *Uro' andro*, **2**, 71-78.

[27] Adeleye, Q., Ndubuisi, E. and Isa, F. (2024) Etiologic and Anti-Microbial Susceptibility Profiles of Bacterial Urinary Tract Infection and Bacterial Enteritis among Children at a Private Multi-Specialty Healthcare Facility in Abuja, Nigeria: A 5-Year Separate and Comparative Review. *Nigerian Journal of Clinical Practice*, **27**, 35-46. https://doi.org/10.4103/njcp.njcp_299_23

[28] Kebbeh, A., Dsane-Aidoo, P., Sanyang, K., Darboe, S.M.K., Fofana, N., Ameme, D., *et al.* (2023) Antibiotics Susceptibility Patterns of Uropathogenic Bacteria: A Cross-Sectional Analytic Study at Kanifing General Hospital, the Gambia. *BMC Infectious Diseases*, **23**, Article No. 723. https://doi.org/10.1186/s12879-023-08373-y

[29] Leotard, S. and Negrin, N. (2010) Épidémiologie des entérobactéries sécrétrices de bêta-lactamases à spectre étendu (E-BLSE) au centre hospitalier de Grasse (2005-2008) [Epidemiology of Enterobacteriaceae Producing Extended-Spectrum β-Lactamase in Grasse Hospital (2005-2008)]. *Pathologie Biologie*, **58**, 35-38. https://doi.org/10.1016/j.patbio.2009.07.014

[30] Bohlen, J., Zhou, Q., Philippot, Q., Ogishi, M., Rinchai, D., Nieminen, T., *et al.* (2023) Human Mcts1-Dependent Translation of JAK2 Is Essential for Ifn-γ Immunity to Mycobacteria. *Cell*, **186**, 5114-5134.e27. https://doi.org/10.1016/j.cell.2023.09.024

[31] Beegle, K., Demombynes, G., de Walque, D., Gubbins, P. and Veillard, J. (2024) COVID-19 Increased Existing Gender Mortality Gaps in High Income More than Middle Income Countries. *International Journal of Infectious Diseases.* https://doi.org/10.1016/j.ijid.2024.107167

[32] Ahmadpour, E., Valilou, S., Ghanizadegan, M.A., Seyfi, R., Hosseini, S.A., Hatam-Nahavandi, K., *et al.* (2024) Global Prevalence, Mortality, and Main Characteristics of Hiv-Associated Pneumocystosis: A Systematic Review and Meta-Analysis. *PLOS ONE*, **19**, e0297619. https://doi.org/10.1371/journal.pone.0297619

[33] Asamoah, B., Labi, A., Gupte, H.A., Davtyan, H., Peprah, G.M., Adu-Gyan, F., *et al.* (2022) High Resistance to Antibiotics Recommended in Standard Treatment Guidelines in Ghana: A Cross-Sectional Study of Antimicrobial Resistance Patterns in Patients with Urinary Tract Infections between 2017-2021. *International Journal of Environmental Research and Public Health*, **19**, Article 16556. https://doi.org/10.3390/ijerph192416556

[34] Adekanmbi, A.O., Akinlabi, O.C., Usidamen, S., Olaposi, A.V. and Olaniyan, A.B. (2022) High Burden of ESBL-Producing *Klebsiella* Spp., Proteus Mirabilis, Enterobacter Cloacae and Pseudomonas Aeruginosa in Diagnosed Cases of Urinary Tract Infection in a Nigerian Teaching Hospital. *Acta Microbiologica et Immunologica Hungarica*, **69**, 127-134. https://doi.org/10.1556/030.2022.01747

[35] Campbell, J.S.O., van Henten, S., Koroma, Z., Kamara, I.F., Kamara, G.N., Shewade, H.D., *et al.* (2022) Culture Requests and Multi-Drug Resistance among Suspected Urinary Tract Infections in Two Tertiary Hospitals in Freetown, Sierra Leone (2017-21): A Cross-Sectional Study. *International Journal of Environmental Research and Public Health*, **19**, Article 4865. https://doi.org/10.3390/ijerph19084865

[36] Wang, Z., Chen, J., You, Z., Liu, X. and Zhang, J. (2024) [Advances in Mechanisms of Biofilm Formation and Drug Resistance of *Staphylococcus aureus*]. C*hinese Journal of Biotechnology*, **40**, 2038-2051.

[37] Brintz, B.J., Madaras-Kelly, K., Nevers, M., Echevarria, K.L., Goetz, M.B. and Samore, M.H. (2024) Predicting Antibiotic Resistance in Enterobacterales to Support Optimal Empiric Treatment of Urinary Tract Infections in Outpatient Veterans. *Antimicrobial Stewardship & Healthcare Epidemiology*, **4**, e118. https://doi.org/10.1017/ash.2024.377

[38] Xu, R., Deebel, N., Casals, R., Dutta, R. and Mirzazadeh, M. (2021) A New Gold Rush: A Review of Current and Developing Diagnostic Tools for Urinary Tract Infections. *Diagnostics*, **11**, Article 479. https://doi.org/10.3390/diagnostics11030479

[39] (2022) Real Time PCR Kit for Detection of DNA of Opportunistic Bacteria of the Classes Bacilli, β-Proteobacteria and γ-Proteobacteria Causing Nosocomial and Community-Acquired Infections. Sacace™ Bac Multi-Screen Real-TM Handbook.

[40] Bekeris, L.G., Jones, B.A., Walsh, M.K. and Wagar, E.A. (2008) Urine Culture Contamination: A College of American Pathologists Q-Probes Study of 127 Laboratories. *Archives of Pathology & Laboratory Medicine*, **132**, 913-917. https://doi.org/10.5858/2008-132-913-uccaco

[41] LaRocco, M.T., Franek, J., Leibach, E.K., Weissfeld, A.S., Kraft, C.S., Sautter, R.L., *et al.* (2016) Effectiveness of Preanalytic Practices on Contamination and Diagnostic Accuracy of Urine Cultures: A Laboratory Medicine Best Practices Systematic Review and Meta-Analysis. *Clinical Microbiology Reviews*, **29**, 105-147. https://doi.org/10.1128/cmr.00030-15

[42] Valentine-King, M.A. and Brown, M.B. (2017) Antibacterial Resistance in Ureaplasma Species and Mycoplasma Hominis Isolates from Urine Cultures in College-Aged Females. *Antimicrobial Agents and Chemotherapy*, **61**, e01104-17. https://doi.org/10.1128/aac.01104-17

[43] Elnifro, E.M., Ashshi, A.M., Cooper, R.J. and Klapper, P.E. (2000) Multiplex PCR: Optimization and Application in Diagnostic Virology. *Clinical Microbiology Reviews*, **13**, 559-570. https://doi.org/10.1128/cmr.13.4.559-570.2000

[44] Gaydos, C.A., Theodore, M., Dalesio, N., Wood, B.J. and Quinn, T.C. (2004) Comparison of Three Nucleic Acid Amplification Tests for Detection of *chlamydia Trachomatis* in Urine Specimens. *Journal of Clinical Microbiology*, **42**, 3041-3045. https://doi.org/10.1128/jcm.42.7.3041-3045.2004

[45] Pillay, A., Radebe, F., Fehler, G., Htun, Y. and Ballard, R.C. (2006) Comparison of a Taqman-Based Real-Time Polymerase Chain Reaction with Conventional Tests for the Detection of Trichomonas Vaginalis. *Sexually Transmitted Infections*, **83**, 126-129. https://doi.org/10.1136/sti.2006.022376

[46] Wojno, K.J., Baunoch, D., Luke, N., Opel, M., Korman, H., Kelly, C., *et al.* (2020) Multiplex PCR Based Urinary Tract Infection (UTI) Analysis Compared to Traditional Urine Culture in Identifying Significant Pathogens in Symptomatic Patients. *Urology*, **136**, 119-126. https://doi.org/10.1016/j.urology.2019.10.018

Abbreviations

AMC	Amoxicillin + Clavulanic acid
AMK	Amikacin
AML	Amoxicillin
AMR	Antimicrobial resistance
CAZ	Ceftazidime
CBEU	Cytobacteriological examination of urine
CERBA	Pietro Annigoni Biomolecular Research Center
CFM	Cefixime
CIP	Ciprofloxacin
CRO	Ceftriaxone
Ct	Threshold cycle
DNA	Deoxyribonucleic acid
FEP	Cefepim
FOS	Fosfomycin
FOX	Cefoxitin
GM	Gentamicin
HOSCO	Saint Camille Hospital of Ouagadougou
IPM	Imipenem
LEV	Levofloxacin
PCR	Polymerase chain reaction
SAM	Ampicillin/sulbactam
SPSS	Statistical Package for the Social Sciences
TOB	Tobramycin
UTI	Urinary tract infections