Open Journal of Medical Microbiology, 2025, 15(1), 36-54
https://www.scirp.org/journal/ojmm
ISSN Online: 2165-3380
ISSN Print: 2165-3372

Characterization of Bacterial Pathogens of the Urinary Tract in Type 2 Diabetes Patients at Mzuzu Central Hospital, Northern Malawi

Thomas Stuart Mughogho[1*], Shiphrah Kuriah[2], Patrick Owili[3], Master Chisale[4], Pizga Kumwenda[4], Zhang Ning[5], Shakira Chimberenga[1]

[1]Ministry of Health, Laboratory Department, Mzuzu Central Hospital, Mzuzu, Malawi
[2]School of Community Health, Amref International University, Nairobi, Kenya
[3]African Population Health Research Center, Nairobi, Kenya
[4]Department of Biological Sciences, Faculty of Science, Technology and Innovations, Mzuzu University, Mzuzu, Malawi
[5]Department of Clinical Laboratory, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China
Email: *thommughogho@gmail.com

How to cite this paper: Mughogho, T.S., Kuriah, S., Owili, P., Chisale, M., Kumwenda, P., Ning, Z. and Chimberenga, S. (2025) Characterization of Bacterial Pathogens of the Urinary Tract in Type 2 Diabetes Patients at Mzuzu Central Hospital, Northern Malawi. *Open Journal of Medical Microbiology*, **15**, 36-54.
https://doi.org/10.4236/ojmm.2025.151003

Received: November 2, 2024
Accepted: March 10, 2025
Published: March 13, 2025

Abstract

Background: People with type 2 diabetes are more vulnerable to infections due to a weakened immune system caused by diabetes-related factors. Urinary tract infections caused by bacteria are associated with antibiotic resistance and severe recurrent disease. **Methods:** This cross-sectional study characterized uropathogenic bacteria and assessed beta-lactamases linked to antibiotic resistance with a sample size of 294. In this research, bacteria from urine culture were identified by mass spectrometry-MALDI-TOF-MS and standard biochemical assays. A phenotypic confirmatory disc diffusion test (PCDDT) was utilized to assess Extended Spectrum Beta Lactamase (ESBL) generating isolates. **Results:** The prevalence of Urinary Tract Infection was 11% (n = 294). Prevalence was significantly higher in those with a mean Random Blood Sugar (RBS) of >190 g/dl (13.1%) than in those with a mean RBS of ≤190 g/dl (X^2: $p = 0.029$). The prevalence of UTIs was also significantly higher among traders (29%, X^2: $p = 0.019$) than among employed (16.6%) and unemployed (7.2%) participants. UTI prevalence was significantly higher among those with type 2 diabetes between 5 and 10 years since medical diagnosis (17%, X^2: $p = 0.029$) than among those diagnosed < 5 years. The most isolated bacteria were *E. coli* (43.8%), *Klebsiella* spp. (34.4%), and *Enterobacter* spp. (12.5%). *E. coli* exhibited the highest level of antibiotic resistance, including resistance to 3rd generation Cephalosporin. Phenotypic ESBL confirmatory test showed 62.5% of the *E. coli* being positive for ESBL production. **Conclusion:** UTIs were common in type 2 diabetics, especially in those with >190 g/dl mean RBS. There was a concerning 62.5% ESBL-producing *E. coli* that were resistant to antibiotics, which is a serious threat to public health. Thus, regular UTI screening and AST should be utilized before prescription to facilitate Antibiotic resistance surveillance and promote early diagnosis, treatment, and infection control.

Keywords

Diabetes, Immunity, Urinary Tract Infections, Antibiotic Resistance, Phenotypic Confirmatory Disc Diffusion Test (PCDDT), Beta-Lactamase, Extended Spectrum Beta-Lactams (ESBL)

1. Introduction

Diabetes is a severe, chronic metabolic disease characterized by high blood glucose levels [1]. Diabetes is one of the fastest-growing global health crises of the 21st century [1] [2]. It is estimated that the number of people with diabetes in Africa will rise by 143% (47 million) by 2045 from 19.4 million among adults [2]. This is the highest predicted increase among all regions of the world [2]. About 7% (366,200) of deaths in 2019 were associated with diabetes [2]. In Malawi, the overall prevalence among adults was reported to be 5.6% by 2014 [3]. Age-sex-standardized prevalence of diabetes was 6.5%, and 4.7% in males and females respectively [3].

Infectious diseases are a major comorbidity in people living with type 2 diabetes mellitus because diabetes impacts negatively on the immune system [4]. This is due to the sustained hyperglycemia that impairs the general immune system in people with diabetes through various pathways that lead to an immune-compromised state [5]. The exponential increase in diabetes mellitus populations significantly contributes to increased hospitalizations [6] [7]. Despite the recent advances in medicine to keep the glucose levels of people with diabetes under control; it is still a major concern that infections continue to cause substantial morbidity and mortality in type 2 diabetes mellitus [7]. Bacteria are among the common etiological agents. Studies have reported a high prevalence of *E. coli*, and *Klebsiella* spp among urinary tract infections in type 2 diabetic patients [8] [9]. Although there have been substantial studies carried out around the world concerning type 2 diabetics, studies that have utilized advanced methods in Africa are few and these have mostly been conducted outside Sub-Saharan Africa such as in Egypt [10]. The studies done in Sub-Saharan Africa hsave shown a range of 20% to 39% percent prevalence of urogenital infections in diabetes and the incidence is more than double compared to people without diabetes [11]-[14]. These studies have led to various improved interventions in the fight to prevent and control infections in people with diabetes. However, it is evident that as the cases of diabetes increase, the infection rate that is associated with drug-resistant pathogens also increases [5] [15]. Resistance to a range of antibiotics which include beta-lactams and non-beta-lactams has been reported [5] [11]. Resistance to antimicrobials would eventually diabetic populations susceptible to an increased risk of persistent and recurrent infections resulting in higher medical costs, poor quality of life as well as increased mortality and morbidity.

Urinary tract infections are more frequent in people living with diabetes mellitus than in those without diabetes [15]. Presently, there is evidence that the prevalence of type 2 diabetes mellitus is substantially growing each year [2] [16] and most of the reported infections associated with these populations are developing multidrug resistance [9]. Bacterial uropathogens are significant contributors to disease and disability in type 2 diabetes mellitus patients [17].

In Sub-Saharan Africa, some studies have reported varying prevalences of uropathogens in people with type 2 diabetes with increased resistance to commonly used antibiotics being reported [11] [14]. However, studies on the same are short in Malawi. The increased prevalence of uropathogens as well as their increased resistance to medication has overall translated into over-prescription of drugs, resulting in ineffective treatment and more drug resistance [10]. This consequently translates to a poor quality of life for people living with diabetes i.e. poor disease prognosis, increased length and frequency of hospitalization, and increased disease-related costs. Therefore, there was a need for more studies to be conducted to generate relevant information on the spectrum of etiologies of infections, and phenotypic and genomic characteristics of bacterial uropathogens associated with disease in people with type 2 diabetes in Sub-Saharan Africa and indeed in Malawi.

2. Materials and Methods

Study design, site, and population

This was a cross-sectional study conducted at Mzuzu Central Hospital a referral Hospital in Mzuzu City, Malawi's northern region in South-East Africa. Demographic data on persons living with type 2 diabetes were collected and associations between the independent variables and dependent variables were determined. A total of 294 consenting participants above 18 with type 2 diabetes and without any other immunocompromising condition were recruited for this study.

Data and sample collection

A structured questionnaire was used to collect socio-demographic data such as gender, age, ethnicity, marital status, and occupation. It was also used to record information on Ethnicity and medical history such as duration of diabetes and medication. Each type 2 diabetic participant was instructed on how to collect a "clean-catch" mid-stream urine into sterile leak-proof containers.

Laboratory analysis

Urine samples from type 2 diabetic participants underwent a chain of laboratory tests to grow, isolate, and identify Gram-negative bacteria that cause

Urinary Tract Infections. The tests included bacterial culture, gram staining, bacterial subculture, biochemical tests (Indole, TSI, Citrate, Urease), Kirby Bauer disc diffusion antimicrobial susceptibility test, and ESBL testing following the standard procedures as described by clinical laboratory standard Institute (CLSI) [18].

Urine culture for bacteria

The urine samples were cultured on a standard 60 × 15 mm cysteine lactose electrolyte deficient (CLED) agar (Oxoid, UK) plate prepared by the manufacturing company's instructions, leaving a single streak across the center, and then spread the inoculum evenly distributed in a cross-zigzag pattern to the primary streak. The culture plates were incubated at 37˚C for 18 - 24 hours [18]. After which they were observed for growth. Bacterial growth of $\geq 10^5$ CFU/ml (colony-forming unit per milliliter) was indicative of significant growth [18].

Identification of bacteria

Bacteria identification from pure culture plates was based on colonial morphology, staining and biochemical tests. A representative colony on each plate was picked, Gram-stained, and further tested using sugar fermentation TSI, Indole, Citrate, Oxidase, and Urease biochemical tests [18]. Furthermore, the identification of gram-negative species was performed by Matrix-assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry (Vitek, biomerieux).

Antibiotic Susceptibility Testing (AST)

Antibiogram was conducted by the Kirby-Bauer disc diffusion method from pure bacterial colonies emulsified in saline and then compared with 0.5 McFarland standard [18]. The suspension was inoculated on a dried surface of the Mueller-Hinton agar plate (Oxoid) and the following commercially available antibiotics were applied on inoculated Mueller-Hinton agar plates: ampicillin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (30 µg), gentamicin (30 µg) (Oxoid, UK). After, 18 - 24 hours of incubation at 37˚C, zones of growth of inhibition were measured using a caliper and interpreted as per the Clinical Laboratory Standard Institute guidelines (CLSI) [18].

Phenotypic confirmatory disc diffusion test (PCDDT)

All *E. coli* isolates with reduced susceptibility to 3rd generation cephalosporin (cefotaxime, ceftazidime, or ceftriaxone) were selected for a phenotypic Extended Spectrum Beta-Lactamase (ESBL) confirmatory test following the CLSI 2019 guidelines [18]. Mueller-Hinton agar was inoculated with test organisms and after inoculation, a combination of cefotaxime (30 µg), cefotaxime plus clavulanic acid (30 µg/10µg) and ceftadizime (30 µg), ceftadizime plus clavulanic acid (30 µg /10µg) (Oxoid, UK) were placed 20 mm apart from center disc. Inoculated plates were incubated aerobically at 37˚C for 18 - 24 hours. According to CLSI (2019) [18] criteria, the formation of ESBL was indicated by an inhibition zone of ≥5 mm between cefotaxime plus clavulanic acid and cefotaxime, or between ceftadizime and ceftadizime plus clavulanic acid and ceftadizime [18]. *Klebsiella pneumoniae*

ATCC 700603, and *E. coli* ATCC 25922 were used as positive and negative controls respectively.

Statistical analysis and presentation

Data were analyzed using Stata SE 15.1. For socio-demographic categorical data (e.g. gender, age group, ethnicity), summary tables of counts and percentages were presented and for continuous variables, mean and standard deviations were presented after testing for normality. Bivariate analysis using Pearson's Chi-square test was used to determine factors associated with Urinary Tract Infections. A P-value of less than 0.05 (p- < 0.05) denoted statistical significance.

3. Results

Demographics of participants

The study recruited 294 participants. The majority of participants were females (54%) compared to Males (46%). The mean age was 55 years (age range; of 19 - 93 years). The highest level of education was between primary and secondary for the majority of participants with 45% and 39% respectively with the least being tertiary education level (9%). With regards to the duration of type 2 diabetes since diagnosis, the majority of participants were those diagnosed with type 2 diabetes between 5 - 10 years (41%) followed by those diagnosed with type 2 diabetes for less than 5 years since diagnosis (37%), and the least representation from those with diabetes for more than 10 years (22%). Most participants were in the category of having a mean random blood sugar (RBS) level of >190 g/dl (63%) than those with a mean RBS ≤ 190 g/dl (37%). In terms of occupation, most participants were unemployed (70%), followed by those employed (14%), with traders representing 10% of the participants and Artisans, and Students representing 6% of participants. Furthermore, in terms of marital status most participants were married (69.4%) followed by widows/widowers (22.4%), and the least were those who were single/divorced/separated (8.2%). In terms of ethnicity, most participants were Tumbukas (68%) compared to Chewas and other ethnic groups (Tonga/Ngonde/Yao) with 18% and 14% respectively as shown in Table 1.

Prevalence of Urinary Tract Infections in type 2 diabetes

An overall Urinary Tract Infection prevalence of 11% (n = 294) was established. Prevalence was higher in female participants (13.2%) than in male participants (8.1%). With regards to the period of type 2 diabetes since diagnosis; those with 5 - 10 years had the highest prevalence of Urinary Tract Infections (16.7%) followed by those with less than 5 years since diagnosis (7%) and the least Urinary Tract Infection prevalence was observed in those who had been diagnosed over 10 years with 6.2%. The prevalence was higher in those with a mean random blood sugar (RBS) level of >190 g/dl (13.1%) than those with a mean RBS ≤ 190 g/dl. The prevalence of Urinary Tract Infections was also higher in traders (28.6%) compared to the employed (16.6%), unemployed (7.2%), Artisans, and Students (11%). Equally, there was a higher prevalence of Urinary Tract Infections (12.1%) in those aged over 65 years, followed by those between 36 - 50 years of age with a

Table 1. Demographics.

Socio-demographic Characteristics	Number of participants (%)	n = 294
Gender		
Male	135 (46)	
Female	159 (54)	
mean RBS		
≤190 g/dl	109 (37)	
>190 g/dl	185 (63)	
Period with Diabetes		
<5 years	109 (37)	
5 - 10 years	120 (41)	
>10 years	65 (22)	
Occupation		
Unemployed	206 (70)	
Employed	42 (14)	
Trader	28 (10)	
Other (Artisan/Student)	18 (6)	
Age Groups (years)		
19 - 35	30 (10)	
36 - 50	85 (29)	
51 - 65	113 (38.5)	
>65	66 (22.5)	
Marital Status		
Married	204 (69.4)	
Widow/Widower	66 (22.4)	
Single/Divorced/Separated	24 (8.2)	
Level of education		
No formal/Primary	153 (52)	
Secondary	116 (39)	
Tertiary	25 (9)	
Ethnicity		
Tumbuka	200 (68)	
Chewa	53 (18)	
Other (Tonga/Ngonde/Yao)	42 (14)	

Urinary Tract Infections prevalence of 11.8% and the least prevalence Urinary Tract Infections of 9.7% in the 51 - 65 year age group. In terms of marital status, the highest prevalence was observed in widows/widowers (12.1%) followed by the married (11.8%), and least in those who were single/divorced/separated (4.2%). Those with no formal/only attained primary education (11.8%) had the highest prevalence compared to those who had attained secondary (10%) and tertiary (8%) education. In terms of ethnicity, the prevalence of Urinary Tract Infections, was also higher in Tumbukas (12.5%) compared to Chewas (3.8%) and other Ethnic groups (Tonga/Ngonde/Yao) 11.9%, as shown in Table 2.

Table 2. Prevalence of urinary tract infections in various demographics.

Demographic characteristics	Number of participants		UTI prevalence%
	With UTIs	Without UTIs	
Overall	**32**	**262**	**11**
Gender			
Male	11	124	8.1
Female	21	138	13.2
Average RBS			
≤190 g/dl	4	105	3.7
>190 g/dl	28	157	15.1
Period with Diabetes			
<5 years	8	101	7.3
5 - 10 years	20	100	16.7
>10 years	4	61	6.2
Occupation			
Unemployed	15	191	7.3
Employed	7	35	16.7
Trader	8	20	28.6
Other (Artisan/Student)	2	16	11.1
Age Groups (years)			
19 - 35	3	27	10.0
36 - 50	10	75	11.8
51 - 65	11	102	9.7
>65	8	58	12.1
Marital Status			
Married	23	181	11.3
Widow/Widower	8	58	12.1
Single/Divorced/Separated	1	23	4.2

Continued

Level of education			
No formal/Primary	18	135	11.8
Secondary	12	104	10.3
Tertiary	2	23	8.0
Ethnicity			
Tumbuka	25	175	12.5
Chewa	2	51	3.8
Other (Tonga/Ngonde/Yao)	5	37	11.9

Distribution of positive culture results by demographic characteristics

The majority of isolates were recovered from female participants compared to males with 65.6%, (n = 32) and 34.4% (n = 32) in female and male patients respectively. Participants aged between 51 and 65 years had the highest prevalence (34.3%) of Urinary Tract Infections and the least prevalence was in the 19-35-year-age group with 9.4% of the isolates recovered. Most isolates (56.3%) were recovered from those who had no formal education or had only attained primary education with the least recovered from those with tertiary education (6.3%). In terms of marital status, 72% of the isolates were recovered from Married participants with the least isolated from those single/separated/divorced at 3%. Most Isolates were recovered from the Tumbuka subjects and the least from the Tonga/Ngonde/Yao with regards to Ethnicity with 78% and 16% from the Tumbukas and Tonga/Ngonde/Yao respectively.

Distribution of Bacteria isolates in Type 2 diabetic participants

A total of 32 (11%) bacterial uropathogens were isolated from positive urine culture ($\geq 10^5$ CFU/ml) on which identification was conducted. *E. coli* was the most recovered isolate with 43.8% followed by *Klebsiella* spp. (34.4%), *Enterobacter* spp. (12.5%), *Serratia* spp (6.2%) and 3.1% *Morganella* spp., as shown in **Figure 1**.

Antibiotic resistance pattern of isolates

The highest level of antibiotic resistance to one of any of the antibiotics utilized was observed in 57% (n = 14) of *E. coli* isolates, followed by *Enterobacter* spp., 25% (n = 4) resistance and *Klebsiella* spp with 18% (n = 11) resistant to antibiotics. Among all the isolates, *Morganella* spp and *Serratia* spp showed no resistance to any of the antibiotics utilized in the study. However, multi-drug (antibiotic) resistance (MDR) was observed in all the resistant *E. coli* isolates. The majority of the *E. coli* isolates were resistant to Ampicillin with only 14% of isolates being sensitive. However, the *E. coli* isolates were highly susceptible to Gentamicin with 100% sensitivity, followed by Ciprofloxacin with 86% sensitivity. Furthermore, at least half of the *E. coli* isolates were resistant to one or more of the 3rd generation cephalosporin antibiotics such as Ceftazidime and cefotaxime (with 43% and 50% sensitivity for the antibiotics respectively), as shown in **Table 3**.

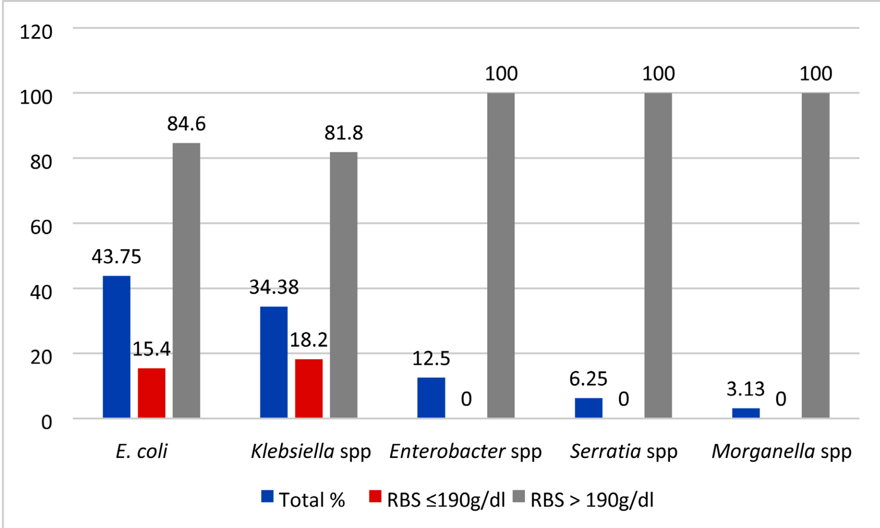

Figure 1. Percentage distribution of Bacteria isolates in type 2 diabetic subjects with a mean random Blood sugar (RBS) level of RBS ≤ 190 g/dl and >190 g/dl.

Table 3. Overall antibiotic susceptibility pattern of *E coli* isolates.

Antibiotic name	Number of samples (%)		n = 14
	Resistant	Susceptible	
Gentamicin (CN)	0 (0)	14 (100)	
Ampicillin (AMP)	12(86)	2 (14)	
Ciprofloxacin (CIP)	2 (14)	12 (86)	
Cefotaxime (CTX)	7(50)	7 (50)	
Ceftazidime (CAZ)	8 (57)	6(43)	
Ceftriaxone (CRO)	3 (21)	11 (79)	

Phenotypic beta-lactamase production in *E. coli* isolates

The Combined disc diffusion test showed that 63% (n = 8) of the *E. coli* isolates which were resistant to 3rd generation cephalosporins were positive for ESBL production. Thus, regarded as having the genes that code for enzymes (beta-lactamase) thus, having capability of producing one or more of the extended spectrum beta-lactamases (ESBL) that cause beta lactam antibiotic resistance including resistance to 3rd generation cephalosporins.

Association between UTIs and independent variables

In this study an overall UTIs prevalence of 11% (n = 294) was established. The prevalence was significantly higher in those with a mean random blood sugar (RBS) level of >190 g/dl (13.1%) than those with a mean RBS ≤ 190 g/dl (X^2, p value = 0.029). The prevalence of UTIs was also significantly higher in traders (29%, p value = 0.019) compared to the employed (16.6%), unemployed (7.2%), Artisans, and Students (11%). Equally, there was a significant prevalence of UTIs in those who had lived with diabetes between 5 and 10 years (17%, X^2, p value =

0.029) since diagnosis compared to those who had lived with Type 2 diabetes for less than 5 years.

Prevalence of UTIs was higher in female participants compared to males with 65.6% and 34.4% in female and male patients respectively. In terms of age groups; participants aged between 51 - 65 years had the highest prevalence of UTIs (34.3%) and the least prevalence was in the 19 - 35 years age group with 9.4% of the isolates recovered. Furthermore, most isolates (56.3%) were recovered from those who had no formal education or had only attained primary education with the least recovered from those with tertiary education (6.3%). Concerning marital status, 72% of the isolates were recovered from Married participants with the least isolated from those single/Separated/divorced at 3%. A majority of Isolates were recovered from the Tumbuka subjects and the least from the Tonga/Ngonde/Yao when it came to Ethnicity with 78% and 16% from the Tumbukas and Tonga/Ngonde/Yao respectively. However, age, gender, Level of education, ethnicity, and marital status were not significant contributors to the UTI prevalence.

Furthermore, at least half of the *E. coli* isolates were resistant to one or more 3rd generation cephalosporin antibiotics such as Ceftazidime and cefotaxime (with 43% and 50% sensitivity for the antibiotics respectively). However, these differences in resistance patterns between isolates from the various demographics and other categories assigned in this study were not statistically significant, as shown in Table 4.

Table 4. Association between UTI and age, gender, Glucose levels, duration of diabetes, level of education, ethnicity and marital status.

Socio-demographic Characteristics	Number of participants (%)			n = 294
	With UTIs	Without UTIs	Total	X^2: *p-Value*
Gender				
Male	11	124	135 (46)	0.159
Female	21	138	159 (54)	0.159
Average RBS				
≤190 g/dl	4	105	109 (37)	>0.05
>190 g/dl	28	157	185 (63)	0.002
Period with Diabetes				
<5 years	8	101	109 (37)	>0.05
5 - 10 years	20	100	120 (41)	0.029
>10 years	4	61	65 (22)	>0.05
Occupation				
Unemployed	15	191	206 (70)	>0.05
Employed	7	35	42 (14)	>0.05

Continued

Trader	8	20	28 (10)	0.019
Other (Artisan/Student)	2	16	18 (6)	>0.05
Age Groups (years)				
19 - 35	3	27	30 (10)	>0.05
36 - 50	10	75	85 (29)	>0.05
51 - 65	11	102	113 (38.5)	>0.05
>65	8	58	66 (22.5)	>0.05
Marital Status				
Married	23	181	204 (69.4)	>0.05
Widow/Widower	8	58	66 (22.4)	>0.05
Single/Divorced/Separated	1	23	24 (8.2)	>0.05
Level of education				
No formal/Primary	18	135	153 (52)	>0.05
Secondary	12	104	116 (39)	>0.05
Tertiary	2	23	25 (9)	>0.05
Ethnicity				
Tumbuka	25	175	200 (68)	>0.05
Chewa	2	51	53 (18)	>0.05
Other (Tonga/Ngonde/Yao)	5	37	42(14)	>0.05

4. Discussion

Prevalence of Urinary Tract Infections by demographic characteristics

The majority of isolates were recovered from female participants compared to males with 65.6% (n = 32) and 34.4% (n = 32) in female and male patients respectively. A study conducted by Gutema found gender to be a significant risk factor in the prevalence of urinary tract infections of bacterial etiology [19]. However, in this study, gender had no significant association with the prevalence of urinary tract infections in type 2 diabetes. This finding is supported by other research that found gender not to be a significant risk factor for the occurrence of urinary tract infections [5] [15] [20] [21]. This could be explained by previous research showing that women seek medical attention more often than males, thus, leading to higher recruitment of women for health-related studies [22]-[24]. This was also the case in this study with the majority of participants being female.

The prevalence of UTIs was significantly higher in those with a mean random blood sugar (RBS) level of >190 g/dl (13.1%) than in those with a mean RBS ≤ 190 g/dl. The prevalence of Urinary Tract Infections was also significantly higher in traders (29%, *p value* = 0.019) compared to the employed (16.6%), unemployed (7.2%), Artisans, and Students (11%). Equally, there was a significant prevalence of Urinary Tract Infections in those who had lived with diabetes between 5 and 10 years (17%, *P value* = 0.029) since diagnosis compared to those who had lived with type 2 diabetes for less than 5 years. This finding is comparable with other findings of a systematic review and meta-analysis conducted in Addis Ababa, Ethiopia [25]. This association is explained by the ability of microorganisms to thrive better in a hyperglycemic environment [26]. Furthermore, Diabetes' effects on the immune system include impaired immune responses within the hyperglycemic state of an individual [26]; lower production of interleukins in response to infections; reduced chemotaxis and phagocytic activity, immobilization of poly-morphonuclear leukocytes; glycosuria and gastrointestinal and urinary dysmotility [27]. Uncontrolled blood glucose levels result in shedding glucose in the urine, providing a conducive environment for bacteria growth in the urinary tract [9] [28].

Older (≥50 years) participants had a higher prevalence of Urinary Tract Infections with females aged 50 - 54 years having the highest prevalence of Urinary Tract Infections (35%) with the least prevalence being in the younger age groups under 35 years. These findings agree with those from Iran and Ethiopia that reported an increased prevalence of Urinary Tract Infections in older age groups among type 2 diabetic patients that is associated with poor control of diabetes as age advances [19] [28]. This was also the case in this study, as younger age groups and those with the least duration of diabetes had more controlled levels of blood glucose and lower prevalence of UTIs. However, there was no significant association between age, duration of diabetes, and UTIs prevalence, this is in line with other studies conducted in Ghana and Iran [11] [28]. Concerning education, most isolates (56.3%) were recovered from those who had no formal education or had only attained primary education with the least recovered from those with tertiary education. In terms of marital status, 72% of the isolates were recovered from Married participants with the least isolated from those single/Separated/divorced at 3%. A majority of Isolates were recovered from the Tumbuka subjects and the least from the Tonga/Ngonde/Yao when it came to Ethnicity with 78% and 16% from the Tumbukas and Tonga/Ngonde/Yao respectively. However, age, gender, Level of education, ethnicity, and marital status were not significant contributors to the UTI prevalence.

Antibiotic susceptibility pattern of *E. coli* and treatment considerations

The findings from this study showed a high prevelance of urinary tract infections coupled with high resistance rates to antibiotics commonly used for treatment, which is also similar to other studies conducted in sub-Saharan Africa [11]. Despite the prevalence and resistance trend being high in type 2 diabetes, the recommended first-line treatment and duration of antibiotics for urinary tract infections is similar to that of other patients [15]. Some studies, based on the findings that prevalence and antibiotic resistance is substantially higher in type 2 diabetics, have argued that patients with type 2 diabetes should receive a variation of antibiotics and longer antibiotic treatment than patients without diabetes mellitus for effective and successful therapy [29]. The choice of antibiotics in patients with type 2 diabetes also needs to consider possible drug interactions that could cause impaired glucose homeostasis [30]-[32]. Furthermore, dosage adjustment is also required for some antimicrobial agents in type 2 diabetes patients with renal impairment [33]. This study found gentamicin (84.2% susceptibility) and fluoroquinolones (84.2% to 86.8% susceptibility) effective against *E. coli.* Thus, these antibiotics could be utilized in treating UTIs of *E. coli* etiology. These findings align with findings from other studies carried out in southern Nigeria and Ethiopia with *E. coli* sensitivity ranging from 80% to 93% to fluoroquinolones and gentamicin [11] [34] [35]. On the other hand, a study conducted by Naqid observed an extreme susceptibility of uropathogenic *E. coli* to ciprofloxacin in Iraq [36], while high susceptibility of *E. coli* to gentamicin and cefotaxime were shown in a study conducted in Tunisia [37]. The highest level of antibiotic resistance to one of any of the antibiotics utilized was observed in *E. coli* isolates (57%; n = 14), followed by *Enterobacter* (25%; n = 4) resistance and *Klebsiella* spp. (18%; n = 11) resistant to antibiotics. Among all the isolates, *Morganella* spp and *Serratia* spp showed no resistance to any of the antibiotics utilized in the study. *E. coli* isolates were highly susceptible to gentamicin with 100% sensitivity, followed by ciprofloxacin with 86% sensitivity. However, multi-drug (antibiotic) resistance (MDR) was observed in all the resistant *E. coli* isolates. Most of the *E. coli* isolates were resistant to ampicillin, with only 14% being sensitive. Some *E. coli* isolates were resistant to one or more of the 3rd generation cephalosporin antibiotics such as ceftazidime and cefotaxime (with 43% and 50% sensitivity for the antibiotics respectively). The resistance could be attributed to overuse, abuse, poor compliance to treatment, and use of suboptimal drugs [38] [39] [40]. One of the major resistance mechanisms is by the production of ESBL enzymes that hydrolyze the antibiotics [41]. However, these differences in resistance patterns between isolates from the various demographic and other categories assigned in this study were not statistically significant.

ESBL-producing *E. coli* and multi-antibiotic resistance

The confirmatory disc diffusion test showed that *E. coli* isolates that were resistant to 3rd generation cephalosporins tested positive for ESBL production, thus regarded as having the genes that code for enzymes (beta-lactamases), therefore, having the capability of producing one or more of the extended-spectrum beta-lactamases that cause beta-lactam antibiotic resistance including resistance to 3rd generation cephalosporins. Similar findings were reported by Ugwu in southern Nigeria, where a tertiary care facility's outpatient urine samples contained a significant concentration of *E. coli* strains that produced an extended-spectrum band [35]. The high level (86%) found in this study is comparable with reports of 30% - 90% of ESBL-producing *E. coli* from various countries [42]-[45]. Additionally, high levels of resistance by *E. coli* were reported in systematic reviews by Carrillo-Larco in 2021 and Salari in 2022 [25] [46]. These reports particularly highlighted that resistance was linked to the production and utilization of ESBL enzymes [25]

[46]. These ESBL genes can be spread horizontally (carried on plasmids), making it easier for bacteria of different species to transmit resistance genes [47]. Because of this, empirical UTI therapy is difficult and has erratic results [48]. The results of other investigations in Ghana and Bangladesh, which showed higher multi-antibiotic resistance of up to 93% to routinely used antibiotics, are comparable to this study [11] [49]. Antibiotic usage and abuse, as well as the administration of less-than-ideal medications, have all been linked to the development of resistance [39] [40]. This might lead to greater treatment expenses, a bigger chance of complications, and a higher rate of morbidity and mortality.

The high rates of resistance by ESBL-producing *E. coli* (86% of isolates) are a serious concern [25]. Numerous studies from Sub-Saharan Africa have reported a high prevalence of ESBL-producing *E. coli* [42]-[44]. These observations tie in with the ones found in this study (86% ESBL *E. coli*), this entails a potential increase in ESBL and general MDR resistance with the increasing population of people with type 2 diabetes in Malawi. Therefore, it is critical to insist on antibiotic susceptibility testing before prescription. Curbing this serious threat to public health would need strategies to block the spread of resistant uropathogenic bacteria in both the lower-risk and higher categories of these type 2 diabetic individuals who have demonstrated a high prevalence of UTIs.

5. Conclusion and Recommendations

According to this study, type 2 diabetic patients had a significant prevalence of UTIs of bacterial etiology, particularly those with higher mean Random Blood Sugar levels (>190 g/dl). The phenotypic analysis found a concerning 62.5% ESBL-producing *E. coli.* Additionally, the ESBL-producing *E. coli* had high resistance levels to several antibiotics. This presents a serious public health threat that calls for well-informed solutions. Therefore, it is recommended that antibiotics be judiciously used by utilizing antimicrobial susceptibility testing before prescription, encouraging standard prescribing practices, and sufficient patient education on the use of antibiotics and the growing trend of antibiotic resistance. The findings of this study have demonstrated the significance of combining phenotypic methods with routine testing for antibiotic sensitivity to gain a deeper understanding of the basic components of antibiotic resistance in settings with limited resources. Thus, tests for common ESBL-producing strains are required.

6. Study Limitations

Due to resource constraints, ESBL tests were only done on *E. coli* isolates. Therefore, subsequent studies may focus on ESBL characterization of all uropathogens to establish a broader range of knowledge on bacteria that are resistant to antibiotics by coding for these enzymes.

Ethics Statement

The study project was authorized by MZUNIREC and issued a clearance certificate

(Ref No. MZUNIREC/DOR/23/34). Additionally, official approval was requested from Mzuzu Central Hospital. The participants were asked for their informed consent by declaring it in writing.

Funding

This research was supported by the Capacity Development of Applied Epidemiologists in the Eastern Africa Region (CDAE). This project is part of the European and Developing Countries Clinical Trials Partnership II (EDCTP2) Programme supported by the European Union. CDAE is jointly implemented by the African Population and Health Research Centre (APHRC), The Amref International University (AMIU) and the Jaramogi Oginga Odinga University of Science and Technology (JOOUST) and funded by the EDCTP2 (grant; CSA2020E-3139).

Acknowledgments

We express our gratitude to everyone who helped to make this work possible.

Author Contribution

Thomas Stuart Mughogho developed the concept and conducted analysis and interpretation of study findings. All co-authors contributed to the review and write-up of the manuscript.

Conflicts of Interest

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

References

[1] World Health Organization (2016) Global Report on Diabetes. https://www.who.int/publications/i/item/9789241565257

[2] International Diabetes Federation Diabetes (2019) IDF Diabetes Atlas. https://diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final-web.pdf

[3] Msyamboza, K.P., Mvula, C.J. and Kathyola, D. (2014) Prevalence and Correlates of Diabetes Mellitus in Malawi: Population-Based National NCD STEPS Survey. *BMC Endocrine Disorders*, **14**, Article No. 41. https://doi.org/10.1186/1472-6823-14-41

[4] Toniolo, A., Cassani, G., Puggioni, A., Rossi, A., Colombo, A., Onodera, T. and Ferrannini, E. (2019) The Diabetes Pandemic and Associated Infections: Suggestions for Clinical Microbiology. *Reviews in Medical Microbiology*, **30**, 1-17. https://doi.org/10.1097/MRM.0000000000000155

[5] Sajid, M., Akash, H., Rehman, K., Fiayyaz, F., Sabir, S. and Khurshid, M. (2020) Diabetes-Associated Infections: Development of Antimicrobial Resistance and Possible Treatment Strategies. *Archives of Microbiology*, **202**, 953-965. https://doi.org/10.1007/s00203-020-01818-x

[6] Martins, M., Boavida, J.M., Raposo, J.F., Froes, F., Nunes, B., Ribeiro, R.T., Macedo, M.P. and Penha-Gonçalves, C. (2016) Diabetes Hinders Community-Acquired Pneumonia Outcomes in Hospitalized Patients. *BMJ Open Diabetes Research and Care*, **4**, e000181. https://doi.org/10.1136/bmjdrc-2015-000181

[7] Unnikrishnan, R. and Misra, A. (2020) Infections and Diabetes: Risks and Mitigation with Reference to India. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, **14**, 1889-1894. https://doi.org/10.1016/j.dsx.2020.09.022

[8] Vignera, S.L., Condorelli, R.A., Cannarella, R., Giacone, F., Mongioi, L.M., Cimino, L., Defeudis, G., Mazzilli, R. and Calogero, A.E. (2019) Urogenital Infections in Patients with Diabetes Mellitus: Beyond the Conventional Aspects. *International Journal of Immunopathology and Pharmacology*, **33**, 1-6. https://doi.org/10.1177/2058738419866582

[9] Woldemariam, H.K., Geleta, D.A., Tulu, K.D., Aber, N.A., Legese, M.H., Fenta, G.M. and Ali, I. (2019) Common Uropathogens and Their Antibiotic Susceptibility Pattern among Diabetic Patients. *BMC Infectious Diseases*, **5**, 1-10. https://doi.org/10.1186/s12879-018-3669-5

[10] Youssouf Kani Elmi, S., Saber Ashour, M., Zakaria Alsewy, F. and Fawzy Abd El Moez Azzam, N. (2021) Phenotypic and Genotypic Detection of Extended Spectrum Β-Lactamases among Escherichia Coli and Klebsiella Pneumoniae Isolates from Type 2 Diabetic Patients with Urinary Tract Infections. *African Health Sciences*, **21**, 497-504. https://doi.org/10.4314/ahs.v21i2.3

[11] Adu-Poku, M. and Addo, M.G. (2020) Antimicrobial Susceptibility Profile of Urinary Tract Pathogenic Infections in Diabetic Patients Attending a Health Facility in Kumasi, Ghana. *International Journal of Pathogen Research*, **4**, 37-45. https://doi.org/10.9734/ijpr/2020/v4i230109

[12] Asamoah-Boaheng, M., Sarfo-Kantanka, O., Tuffour, A.B., Eghan, B. and Mbanya, J.C. (2019) Prevalence and Risk Factors for Diabetes Mellitus among Adults in Ghana: A Systematic Review and Meta-Analysis. *International Health*, **11**, 83-92. https://doi.org/10.1093/inthealth/ihy067

[13] Grandy, S., Fox, K.M. and Hardy, E. (2013) Prevalence and Recurrence of Urinary Tract and Genital Infections among Adults with and without Type 2 Diabetes Mellitus in the General Population: A Longitudinal Cohort Study. *Journal of Diabetes Research & Clinical Metabolism*, 1-8.

[14] Nigussie, D. and Amsalu, A. (2017) Prevalence of Uropathogen and Their Antibiotic Resistance Pattern among Diabetic Patients. *Turk Uroloji Dergisi*, **43**, 85-92.

[15] Nitzan, O., Elias, M., Chazan, B. and Saliba, W. (2015) Urinary Tract Infections in Patients with Type 2 Diabetes Mellitus: Review of Prevalence, Diagnosis, and Management. *Diabetes, Metabolic Syndrome and Obesity*, **8**, 129-136. https://doi.org/10.2147/DMSO.S51792

[16] Almugadam, B.S., Ibrahim, M.K., Liu, Y., Chen, S., Wang, C., *et al.* (2021) Association of Urogenital and Intestinal Parasitic Infections with Type 2 Diabetes Individuals: A Comparative Study. *BMC Infectious Diseases*, **21**, Article No. 20. https://doi.org/10.1186/s12879-020-05629-9

[17] Alemu, M., Belete, M.A., Gebreselassie, S., Belay, A. and Gebretsadik, D. (2020) Bacterial Profiles and Their Associated Factors of Urinary Tract Infection and Detection of Extended Spectrum Beta-Lactamase Producing Gram-Negative Uropathogens among Patients with Diabetes Mellitus at Dessie Referral Hospital, Northeastern Ethiopia. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, **13**, 2935-2948. https://doi.org/10.2147/dmso.s262760

[18] Clinical & Laboratory Standards Institute: CLSI Guidelines (2019) CLSI M100-ED29: 2019 Performance Standards for Antimicrobial Susceptibility Testing. 29th Edition.

[19] Gutema, T., Weldegebreal, F., Marami, D. and Teklemariam, Z. (2018) Prevalence, Antimicrobial Susceptibility Pattern, and Associated Factors of Urinary Tract Infections

among Adult Diabetic Patients at Metu Karl Heinz Referral Hospital, Southwest Ethiopia. *International Journal of Microbiology*, **2018**, Article 7591259. https://doi.org/10.1155/2018/7591259

[20] Abu-Ashour, W., Twells, L.K., Valcour, J.E. and Gamble, J.M. (2018) Diabetes and the Occurrence of Infection in Primary Care: A Matched Cohort Study. *BMC Infectious Diseases*, **18**, Article No. 67. https://doi.org/10.1186/s12879-018-2975-2

[21] Anis, A. and Chaudhary, A. (2018) Urinary Tract Infections Caused by ESBL Producing *E. coli* in Diabetes Mellitus: Alternative Approaches for Treatment and Management. *International Journal of Pharma Professional's Research*, **11**, 119-132. https://ijppr.humanjournals.com/wp-content/uploads/2018/02/11.Anis-Ahmad-Chaudhary.pdf

[22] Bertakis, K.D., Azari, R., Helms, L.J., Callahan, E.J. and Robbins, J.A. (2000) Gender Differences in the Utilization of Health Care Services. *The Journal of Family Practice*, **49**, 147-152

[23] Ek, S. (2015) Gender Differences in Health Information Behaviour: A Finnish Population-Based Survey. *Health Promotion International*, **30**, 736-745. https://doi.org/10.1093/heapro/dat063

[24] Olanrewaju, F.O., Ajayi, L.A., Loromeke, E., Olanrewaju, A., Allo, T. and Nwannebuife, O. (2019) Masculinity and Men's Health-Seeking Behaviour in Nigerian Academia. *Cogent Social Sciences*, **5**, Article 1682111. https://doi.org/10.1080/23311886.2019.1682111

[25] Salari, N., Karami, M.M., Bokaee, S., Chaleshgar, M., Shohaimi, S., Akbari, H. and Mohammadi, M. (2022) The Prevalence of Urinary Tract Infections in Type 2 Diabetic Patients: A Systematic Review and Meta-Analysis. *European Journal of Medical Research*, **27**, Article No. 20. https://doi.org/10.1186/s40001-022-00644-9

[26] Chang, C.H., Wang, J.L., Wu, L.C., Chuang, L.M. and Lin, H.H. (2019) Diabetes, Glycemic Control, and Risk of Infection Morbidity and Mortality: A Cohort Study. *Open Forum Infectious Diseases*, **6**, ofz358. https://doi.org/10.1093/ofid/ofz358

[27] Carey, I.M., Critchley, J.A., Dewilde, S., Harris, T., Hosking, F.J. and Cook, D.G. (2018) Risk of Infection in Type 1 and Type 2 Diabetes Compared with the General Population: A Matched Cohort Study. *Diabetes Care*, **41**, 513-521. https://doi.org/10.2337/dc17-2131

[28] Mehrabi, M., Salehi, B., Rassi, H. and Dehghan, A. (2020) Evaluating the Antibiotic Resistance and Frequency of Adhesion Markers among Escherichia Coli Isolated from Type 2 Diabetes Patients with Urinary Tract Infection and Its Association with Common Polymorphism of Mannose-Binding Lectin Gene. *New Microbes and New Infections*, **38**, Article 100827. https://doi.org/10.1016/j.nmni.2020.100827

[29] Hendriks-Spoor, K.D., Wille, F.L., Doesschate, T.T., Dorigo-Zetsma, J.W., Verheij, T.J.M. and van Werkhoven, C.H. (2022) Five Versus Seven Days of Nitrofurantoin for Urinary Tract Infections in Women with Diabetes: A Retrospective Cohort Study. *Clinical Microbiology and Infection*, **28**, 377-382. https://doi.org/10.1016/j.cmi.2021.06.034

[30] Chan, J.C., Cockram, C.S. and Critchley, J.A. (1996) Drug-Induced Disorders of Glucose Metabolism. Mechanisms and Management. *Drug Safety*, **15**, 135-157. https://doi.org/10.2165/00002018-199615020-00005

[31] Ray, C., Wu, V.C., Wang, C., Tu, H. and Huang, Y. (2021) Hypoglycemia Associated with Drug-Drug Interactions in Patients with Type 2 Diabetes Mellitus Using Dipeptidylpeptidase-4 Inhibitors. *Frontiers in Pharmacology*, **12**, 1-8. https://doi.org/10.3389/fphar.2021.570835

[32] May, M. and Schindler, C. (2016) Clinically and Pharmacologically Relevant Interactions of Antidiabetic Drugs. *Therapeutic Advances in Endocrinology and Metabolism*, **7**, 69-83.

[33] Munar, M.Y. and Singh, H. (2007) Drug Dosing Adjustments in Patients with Chronic Kidney Disease. *American Academy of Family Physicians*, **75**, 1487-1496.

[34] Seifu, W.D. and Gebissa, A.D. (2018) Prevalence and Antibiotic Susceptibility of Uropathogens from Cases of Urinary Tract Infections (UTI) in Shashemene Referral Hospital, Ethiopia. *BMC Infectious Diseases*, **18**, Article No. 30. https://doi.org/10.1186/s12879-017-2911-x

[35] Ugwu, M.C., Shariff, M., Nnajide, C.M., Beri, K., Okezie, U.M., Iroha, I.R. and Esimone, C.O. (2020) Phenotypic and Molecular Characterization of β-Lactamases among Enterobacterial Uropathogens in Southeastern Nigeria. *The Canadian Journal of Infectious Diseases & Medical Microbiology*, **2020**, Article 5843904. https://doi.org/10.1155/2020/5843904

[36] Naqid, I.A., Balatay, A.A., Hussein, N.R., Saeed, K.A., Ahmed, H.A. and Yousif, S.H. (2020) Antibiotic Susceptibility Pattern of *Escherichia coli* Isolated from Various Clinical Samples in Duhok City, Kurdistan Region of Iraq. *International Journal of Infection*, **7**, 3-7. https://doi.org/10.5812/iji.103740

[37] Daoud, N., Hamdoun, M., Hannachi, H., Gharsallah, C., Mallekh, W. and Bahri, O. (2020) Antimicrobial Susceptibility Patterns of *Escherichia coli* among Tunisian Outpatients with Community-Acquired Urinary Tract Infection (2012-2018). *Current Urology*, **14**, 200-205. https://doi.org/10.1159/000499238

[38] Ayukekbong, J.A., Ntemgwa, M. and Atabe, A.N. (2017) The Threat of Antimicrobial Resistance in Developing Countries: Causes and Control Strategies. *Antimicrobial Resistance and Infection Control*, **6**, Article No. 47. https://doi.org/10.1186/s13756-017-0208-x

[39] Salam, M.A., Al-Amin, M.Y., Salam, M.T., Pawar, J.S., Akhter, N., Rabaan, A.A. and Alqumber, M.A.A. (2023) Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare*, **11**, Article 1946. https://doi.org/10.3390/healthcare11131946

[40] Ahmed, I., Rabbi, B. and Sultana, S. (2019) Antibiotic Resistance in Bangladesh: A Systematic Review. *International Journal of Infectious Diseases*, **80**, 54-61. https://www.sciencedirect.com/science/article/pii/S1201971219300086 https://doi.org/10.1016/j.ijid.2018.12.017

[41] Teklu, D.S., Negeri, A.A., Legese, M.H., Bedada, T.L., Woldemariam, H.K. and Tullu, K.D. (2019) Extended-Spectrum Beta-Lactamase Production and Multi-Drug Resistance among *Enterobacteriaceae* Isolated in Addis Ababa, Ethiopia. *Antimicrobial Resistance & Infection Control*, **8**, Article No. 39. https://doi.org/10.1186/s13756-019-0488-4

[42] Erb, S., D'Mello-Guyett, L., Malebo, H.M., Njee, R.M., Matwewe, F., Ensink, J., Hinic, V., Widmer, A. and Frei, R. (2018) High Prevalence of ESBL-Producing *E. Coli* in Private and Shared Latrines in an Informal Urban Settlement in Dar es Salaam, Tanzania. *Antimicrobial Resistance & Infection Control*, **7**, Article No. 3. https://doi.org/10.1186/s13756-017-0292-y

[43] Gaviria, L.P., Montsant, L., Azuaje, C., González-Díaz, A., Horcajada, J.P., Limón, E., Viñas, M., Espinal, P. and Fusté, E.A. (2022) Descriptive Analysis of Urinary ESBL-Producing-*Escherichia coli* in Cerdanya Hospital. *Microorganisms*, **10**, Article 488. https://doi.org/10.3390/microorganisms10030488

[44] Jia, P., Zhu, Y., Li, X., Kudinha, T., Yang, Y., Zhang, G., Zhang, J., Xu, Y. and Yang,

Q. (2021) High Prevalence of Extended-Spectrum Beta-Lactamases in *Escherichia coli* Strains Collected from Strictly Defined Community-Acquired Urinary Tract Infections in Adults in China: A Multicenter Prospective Clinical Microbiological and Molecular Study. *Frontiers in Microbiology*, **12**, Article 663033. https://doi.org/10.3389/fmicb.2021.663033

[45] Mahmud, Z.H., Kabir, M.H., Ali, S., Moniruzzaman, M., Imran, K.M., Nafiz, T.N., Islam, M.S., Hussain, A., Hakim, S.A.I., Worth, M., Ahmed, D., Johnston, D. and Ahmed, N. (2020) Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Drinking Water Samples from a Forcibly Displaced, Densely Populated Community Setting in Bangladesh. *Frontiers in Public Health*, **8**, Article 228. https://doi.org/10.3389/fpubh.2020.00228

[46] Carrillo-Larco, R.M., Anza-Ramírez, C., Saal-Zapata, G., Villarreal-Zegarra, D., Zafra-Tanaka, J.H., Ugarte-Gil, C. and Bernabé-Ortiz, A. (2021) Type 2 Diabetes Mellitus and Antibiotic-Resistant Infections: A Systematic Review and Meta-Analysis. *Journal of Epidemiology and Community Health*, **76**, 75-84. https://doi.org/10.1136/jech-2020-216029

[47] Darphorn, T.S., Bel, K., Koenders-van Sint Anneland, B.B., *et al.* (2021) Antibiotic Resistance Plasmid Composition and Architecture in *Escherichia coli* Isolates from Meat. *Scientific Reports*, **11**, Article No. 2136. https://doi.org/10.1038/s41598-021-81683-w

[48] Castanheira, M., Simner, P.J. and Bradford, P.A. (2021) Extended-Spectrum β-Lactamases: An Update on Their Characteristics, Epidemiology and Detection. *JAC-Antimicrobial Resistance*, **3**, dlab092. https://doi.org/10.1093/jacamr/dlab092

[49] Mirnezami, M., Ranjbar, R., Niakan, M. and Ahmadi, M.H. (2020) Frequency of Antimicrobial Resistance and Class 1 and 2 Integrons in *Escherichia Coli* Strains Isolated from Urinary Tract Infections. *Iranian Journal of Pharmaceutical Research*, **19**, 282-287.