

Isolation and Characterization of Bacteria Associated with Urinary Tract Infection in Diabetics and Non-diabetics Women

Gullan O. Rahman, Gulala R. Khidher, Ayad H. Hasan and Aqeel I. Gheni

Department of Medical Microbiology, Faculty of Science and Health, Koya University, Kurdistan Region – F.R. Iraq

Abstract—Urine samples were collected from Dr. Khalid General Hospital and student's accommodation in Koya city from November 2017 to February 2018. Mid-stream urine samples were obtained from a total of 60 patients; the samples were collected in equal number from diabetic and non-diabetic women (1:1). Three different species of bacteria were diagnosed by conventional methods, and antibiotic sensitivity test was carried out by agar disc diffusion. *Escherichia coli* was isolated in 10/30 (33%) in diabetics versus 15/30 (50%) in non-diabetics. *Klebsiella pneumoniae* were isolated in 6/30 (20%) in diabetics and 3/30 (10%) in non-diabetics. *Enterococcus* spp. were isolated in 4/30 (13%) in diabetics and 2/30 (7%) in non-diabetics. On the one hand, *E. coli*, *K. pneumoniae*, and *Enterococcus* spp. in both groups showed resistance to ampicillin and norfloxacin. On the other hand, ciprofloxacin and chloramphenicol were the most effective antibiotic against all bacterial species in both groups. The aims of this study were to determine the differences in microbiological characteristic of urinary tract infection between diabetic and non-diabetic women and then investigate their antibiotic sensitivity.

Index Terms—Antimicrobial resistance, Urinary tract infection in diabetic women, Urinary tract infection.

I. INTRODUCTION

Urinary tract infections (UTIs) are the second common infection in human that can be caused by various pathogenic microbes [1]. Based on the site of the infection, UTIs could be divided into three types: Cystitis (bladder infection), pyelonephritis (kidney infection), and bacteriuria (the existence of bacteria in the urine) [2].

Worldwide, about 150 million people are diagnosed with UTI annually most of them are women [3]. It is being reported that 60% of practice visited women have had UTI at

some time in their life [4]. Patients with diabetics are another group of people who are vulnerable to UTI, which most of them developing kidney disease that leading to end-stage renal disease [5].

Variable factors that have been proposed to develop UTIs generally in women and specifically in diabetic patients include pelvic anatomy (shorter distance between anus and urethra), a high glucose concentration in the urine that might stimulate the pathogenic bacteria to overgrow and virulence factors that possessed by invaded bacteria [6,7]. Other conditions such as vaginitis, age, instrumentation of the urinary system, control of metabolic, and diabetic cystopathy might allow pathogenic bacteria to have access to urinary system and cause infection [8,9].

To establish a successful infection, the pathogenic bacteria need to adhere to the epithelial cell of urinary tract, colonize, and multiply, then spread to the nearby tissue and organs. This infection could be symptomatic or asymptomatic [10]. *Escherichia coli* are the most common bacterial pathogens that cause UTIs in non-diabetic and diabetic women, followed by *Staphylococcus saprophyticus*, *Enterococcus*, *Klebsiella*, *Enterobacter*, and *Proteus* species are less common causes [7,11].

Widespread use of antibiotics, lack of infection prevention, and limitation of control program are the reasons behind developing multidrug resistant by pathogenic bacteria in UTI patients [1,12]. Other reasons are improper use of antibiotics, unnecessary antibiotic prescription, and incorrect dose to be used [13].

The aims of this study were to isolate and identify the causative bacterial species of UTI in diabetic and non-diabetic women and then determine their antibacterial sensitivity patterns.

II. MATERIALS AND METHODS

A. Specimen Collection

A total of 60 specimens of clean-catch midstream urine samples were collected from two groups of adult women how have UTI. The first group of the samples was collected from diabetic women and the second group was collected from non-diabetics. Clean-catch midstream technique has many advantages. It is simple to apply, cheap, can be conducted anywhere and keeping the patient's away from any risk of

introducing bacteria into the bladder by catheterization [14]. The samples were transferred within 1 h of collection to the laboratory. All the diabetic urine specimens (30/60) were collected from Dr. Khalid General Hospital in Koya city, and the non-diabetic urine specimens were collected from Dr. Khalid General Hospital and student's accommodation of Koya University. The specimens' collection was conducted from November 2017 to February 2018.

B. Bacterial Isolation and Identification

Standard methods for urine culture were performed by inoculating culture media (Blood agar containing 5% sheep blood, MacConkey agar, and Eosin methylene blue agar) with a known volume of urine to workout CFU/mL. Urine cultures were incubated overnight aerobically at 37°C [15]. Bacterial identification was based on standard Gram stain procedure, then observed under oil immersion objective lens ($\times 100$), cultural characteristics such as colony morphology and biochemical tests [16].

C. Antibiotic Susceptibility Test

Standardized disc diffusion method was used for all isolates to determine their sensitivity against ampicillin (AMP 25 μ g), nalidixic acid (NAL 30 μ g), azithromycin (AZM 15 μ g), chloramphenicol (CHL 10 μ g), streptomycin (STR 25 μ g), norfloxacin (NOR 10 μ g), ciprofloxacin (CIP 10 μ g), tetracycline (TET 10 μ g), penicillin (PEN 10 μ g), bacitracin (BAC 10 μ g) (Bioanalyse company) on antibiotic media [17].

III. RESULTS AND DISCUSSION

A. Bacterial Isolation and Identification

The identity of isolated bacteria was confirmed using cultural characteristic and biochemical tests. Three different species of bacteria were isolated from the total of 60 specimens. *E. coli* was the most causative bacteria for UTI in both groups at ratio of 33% (10/30) in diabetics and 50% (15/30) in non-diabetics, followed by *Klebsiella pneumoniae* at ratio of 20% (6/30) in diabetics and 10% (3/30) in non-diabetics. *Enterococcus* spp. were isolated at lowest ratio from the groups of the study, which was 13% (4/30) in diabetics and 7% (2/30) in non-diabetics (Fig. 1).

The results are in agreement with other previous studies, which stated that *E. coli* is the most prevalent causative agent

of UTI [3,8,18], followed by *K. pneumoniae* and *E. faecalis* in most cases [19]. There are many factors that are putting *E. coli* in the top of the bacteriuria hierarchy. First, since *E. coli* is a part of gastrointestinal tract normal flora, and the distance between urethra and anus is too short. Hence, *E. coli* could get advantage from that and have access to urinary system, then cause infection. Second, *E. coli* have many different virulence factors that initiate the infection and then empower it. Of these, outer cellular appendages, fimbriae that enable *E. coli* to attach to epithelial cells of urinary system and colonize there [9,20,21]. Aerobactin, an active iron chelation system, is another virulence factor employed by *E. coli* to gain iron in poor-iron environment such as urinary system [22-24]. Fimbriae and aerobactin system are associated together in *E. coli* isolates from UTI [25]. Other virulence factors that associated with aerobactin-producing *E. coli* isolates and enhance their ability to cause UTI are capsular polysaccharide (K antigens), which blocks opsonization and has antiphagocytic and anticomplementary activities, and serum resistance [26]. Other virulence factors such as hemolysin, which lyses erythrocytes of all mammals, and cytotoxic necrotizing factor urges *E. coli* to cause UTI at less extend [27].

B. Antibiotic Susceptibility Test

To determine the antibiotic-resistant patterns, all bacterial isolates were exposed to different types of antibiotic that belonged to various antibiotic families (Table I).

Antibacterial susceptibility patterns of commonly used 10 antibiotics were different according to the origin of bacterial isolates and the mechanism of the antibiotics action.

Bacterial isolates from diabetic patients were sensitive to most tested antibiotics. There were mainly sensitive to chloramphenicol and ciprofloxacin; however, all *E. coli* isolates were sensitive to streptomycin and all *K. pneumoniae* isolates were sensitive to nalidixic acid and norfloxacin. *Enterococcus* spp. showed 100% resistance to the most of used antibiotics against 100% resistance to norfloxacin and azithromycin.

Non-diabetic bacterial isolates showed a reasonable sensitive to most of used antibiotics, although was lower than diabetic bacterial isolates. Both *E. coli* and *Enterococcus* spp. showed high level of resistance (when $>50\%$ of the samples were resistant to the antibiotic considered as high level of resistance) against norfloxacin, ampicillin, and streptomycin, in addition to azithromycin for *E. coli*. and *K. pneumoniae* was resistance to norfloxacin (Table I).

Although chloramphenicol and ciprofloxacin were from different antibiotic family with different mechanisms of action, they found to be the most effective antibiotic against all bacteria isolates in both groups (diabetic and non-diabetic). The bactericidal action of chloramphenicol, which belongs to chloramphenicol family is inhibiting the peptidyl transferase activity of the bacterial ribosome that prevents protein chain elongation and then shut down bacterial protein synthesis [28,29]. Ciprofloxacin, which belongs to fluoroquinolones family, prevents bacterial DNA replication and repairs by inhibiting topoisomerases II and IV [30].

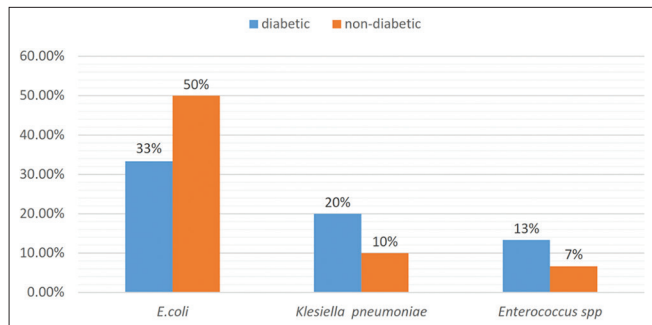


Fig. 1. Bacterial distribution in diabetic and non-diabetic urinary tract infections.

TABLE I ANTIBIOTIC RESISTANCE PERCENTAGE OF ISOLATED BACTERIAL SPECIES IN BOTH DIABETIC AND NON-DIABETIC UTIS

Bacterial species	Patient's group	Resistance percentage to									
		BAC	PEN	NAL	CHL	CIP	NOR	TET	AMP	STR	AZM
<i>E. coli</i>	Diabetic	23	25	50	0	0	40	30	50	0	22
	Non-diabetic	27	20	50	40	40	100	50	80	54	63
<i>K. pneumoniae</i>	Diabetic	44	33	0	0	0	0	50	67	25	33
	Non-diabetic	50	25	25	50	50	74	25	50	25	50
<i>Enterococcus</i> spp.	Diabetic	0	50	0	0	0	0	100	0	0	100
	Non-diabetic	35	40	60	25	20	100	50	80	63	25

BAC: Bacitracin, PEN: Penicillin, NAL: Nalidixic acid, CHL: Chloramphenicol, CIP: Ciprofloxacin, NOR: Norfloxacin, TET: Tetracycline, AMP: Ampicillin, STR: Streptomycin, AZM: Azithromycin, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*

All bacterial isolates in both groups of study were showed high rate of resistance against ampicillin and norfloxacin. Ampicillin is a semi-synthetic derivative of penicillin that inhibits the third and last stage of bacterial cell wall synthesis by binding to specific penicillin-binding proteins located inside the bacterial cell wall. Hence, resistance to ampicillin is simply basically mediated by β -lactamase that inactivates the antibiotic by hydrolyzing the β -lactam ring [31]. Norfloxacin is a synthetic fluoroquinolone that prevents DNA synthesis by inhibiting bacterial DNA gyrase. Hence, resistance to norfloxacin would be through gaining mutation in the target genes (*gyrA* and *gyrB*) that encoding gyrase and increasing levels of multidrug efflux pump AcrAb, thereby reducing the effective intracellular drug concentration [32].

In many countries, such as Kurdistan, antibiotics are available over the counter without a prescription. Hence, individual can purchase any antibiotic with the quantity that she/he likes. This lack of antibiotic regulation leads to be abused and overused, which is forcing bacteria to develop resistance against it by modifying the antibiotic structure or its target. Resistance can spread in bacterial community and transfers to another species by several ways such as transformation, conjugation, and transduction [33]. However, sensitivity to some of used antibiotics in this study may return to the limitation of using them as a first agent in the UTI treatment in Kurdistan Region - Iraq.

IV. CONCLUSION

Our results showed that *E. coli* is the most common isolate in diabetic and non-diabetic UTIs, followed by *K. pneumoniae* and *Enterococcus* spp. ciprofloxacin can be used to treat UTI in diabetic or non-diabetic [34].

V. ACKNOWLEDGMENT

We would like to thank the department of medical microbiology for providing the required materials to carry out the work. We would like also to extend our thanks to the Faculty of Science and Health and Koya University.

REFERENCES

[1] R. Daza, J. Gutierrez and G. Piedrola, "Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract

infections," *International Journal of Antimicrobial Agents*, vol. 18, pp. 211-215, Sep, 2001.

[2] B. Foxman, "Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs," *Dis Mon*, vol. 49, pp. 53-70, Feb, 2003.

[3] A. Al-Asoufi, A. Khlaifat, A. A. Tarawneh, K. Alsharafa, M. Al-Limoun and K. Khleifat, "Bacterial quality of urinary tract infections in diabetic and non diabetics of the population of Ma'an province, Jordan," *Pakistan Journal of Biological Sciences*, vol. 20, pp. 179-188, 2017.

[4] S. Nys, T. van Merode, A. I. Bartelds and E. E. Stobberingh, "Urinary tract infections in general practice patients: Diagnostic tests versus bacteriological culture," *Journal of Antimicrobial Chemotherapy*, vol. 57, pp. 955-958, May, 2006.

[5] L. M. Muller, K. J. Gorter, E. Hak, W. L. Goudzwaard, F. G. Schellevis, A. I. Hoepelman, R. Meiland, S. E. Geerlings, "Increased risk of common infections in patients with Type 1 and Type 2 diabetes mellitus," *Clinical Infectious Diseases*, vol. 41, pp. 281-288, Aug. 1, 2005.

[6] L. E. Nicolle, "Urinary tract infection in geriatric and institutionalized patients," *Current Opinion in Urology*, vol. 12, pp. 51-55, Jan, 2002.

[7] A. Ronald, "The etiology of urinary tract infection: Traditional and emerging pathogens," *The American Journal of Medicine*, vol. 113, Suppl 1A, pp. 14S-19S, Jul. 8, 2002.

[8] O. Nitzan, M. Elias, B. Chazan and W. Saliba, "Urinary tract infections in patients with Type 2 diabetes mellitus: Review of prevalence, diagnosis, and management," *Diabetes, Metabolic Syndrome and Obesity*, vol. 8, pp. 129-36, 2015.

[9] E. J. Boyko, S. D. Fihn, D. Scholes, L. Abraham and B. Monsey, "Risk of urinary tract infection and asymptomatic bacteriuria among diabetic and nondiabetic postmenopausal women," *American Journal of Epidemiology*, vol. 161, pp. 557-564, Mar. 15, 2005.

[10] B. W. Trautner, R. A. Hull and R. O. Darouiche, "*Escherichia coli* 83972 inhibits catheter adherence by a broad spectrum of uropathogens," *Urology*, vol. 61, pp. 1059-1062, May, 2003.

[11] M. H. Saber, L. Barai, J. A. Haq, M. S. A. Jilani and J. Begum, "The pattern of organism causing urinary tract infection in diabetic and non diabetic patients in Bangladesh," *Bangladesh Journal of Medical Microbiology*, vol. 4, pp. 6-8, 2010.

[12] G. C. Schito, K. G. Naber, H. Botto, J. Palou, T. Mazzei, L. Gualco, A. Marchese, "The ARESC study: An international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections," *International Journal of Antimicrobial Agents*, vol. 34, pp. 407-413, Nov, 2009.

[13] J. Frère and S. Rigali, "The alarming increase in antibiotic-resistant bacteria," *Drug Targets Review*, vol. 3, pp. 26-30, 2016.

[14] W. E. Stamm, G. W. Counts, K. R. Running, S. Fihn, M. Turck and K. K. Holmes, "Diagnosis of coliform infection in acutely dysuric women," *The New England Journal of Medicine*, vol. 307, pp. 463-468, Aug. 19, 1982.

[15] J. Vandepitte, J. Verhaegen, K. Engbaek, P. Rohner, P. Piot and C. C. Heuck, *Basic Laboratory Procedures in Clinical Bacteriology*, 2nd ed. Switzerland: World Health Organization Geneva, 2003.

- [16] G. H. John, *Bergey Manual of Determinative Bacteriology*, 9th ed. Baltimore: Williams & Wilkins, 1994.
- [17] CLSI, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 13th ed. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.
- [18] R. Funfstuck, L. E. Nicolle, M. Hanefeld and K. G. Naber, "Urinary tract infection in patients with diabetes mellitus," *Clinical Nephrology*, vol. 77, pp. 40-48, Jan, 2012.
- [19] H. Z. Hamdan, E. Kubbara, A. M. Adam, O. S. Hassan, S. O. Suliman and I. Adam, "Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan," *Annals of Clinical Microbiology and Antimicrobials*, vol. 14, p. 26, Apr, 21 2015.
- [20] M. Bonadio, E. Boldrini, G. Forotti, E. Matteucci, A. Vigna, S. Mori, O. Mori, "Asymptomatic bacteriuria in women with diabetes: Influence of metabolic control," *Clinical Infectious Diseases*, vol. 38, pp. e41-e45, Mar. 15, 2004.
- [21] J. Bien, O. Sokolova and P. Bozko, "Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage," *International Journal of Nephrology*, vol. 2012, p. 681473, 2012.
- [22] A. Bindereif and J. B. Neilands, "Aerobactin genes in clinical isolates of *Escherichia coli*," *Journal of Bacteriology*, vol. 161, pp. 727-735, Feb, 1985.
- [23] A. Bagg and J. B. Neilands, "Molecular mechanism of regulation of siderophore-mediated iron assimilation," *Microbiology Review*, vol. 51, pp. 509- 518, Dec, 1987.
- [24] K. W. Yun, H. Y. Kim, H. K. Park, W. Kim and I. S. Lim, "Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children," *Journal of Microbiology, Immunology and Infection*, vol. 47, pp. 455-61, Dec, 2014.
- [25] S. H. Jacobson, M. Hammarlind, K. J. Lidfeldt, E. Osterberg, K. Tullus and A. Brauner, "Incidence of aerobactin-positive *Escherichia coli* strains in patients with symptomatic urinary tract infection," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 7, pp. 630-634, Oct, 1988.
- [26] J. Z. Montgomerie, A. Bindereif, J. B. Neilands, G. M. Kalmanson and L. B. Guze, "Association of hydroxamate siderophore (aerobactin) with *Escherichia coli* isolated from patients with bacteremia," *Infection and Immunity*, vol. 46, pp. 835-838, Dec, 1984.
- [27] R. P. Rennie and J. P. Arbuthnott, "Partial characterisation of *Escherichia coli* haemolysin," *Journal of Medical Microbiology*, vol. 7, pp. 179-188, May, 1974.
- [28] O. Jardetzky, "Studies on the mechanism of action of chloramphenicol. I. The conformation of chloramphenicol in solution," *Journal of Biological Chemistry*, vol. 238, pp. 2498-508, Jul, 1963.
- [29] P. S. Stewart and J. W. Costerton, "Antibiotic resistance of bacteria in biofilms," *Lancet*, vol. 358, pp. 135-138, Jul. 14, 2001.
- [30] M. Le Bel, "Ciprofloxacin: Chemistry, mechanism of action, resistance, antimicrobial spectrum, pharmacokinetics, clinical trials, and adverse reactions," *Pharmacotherapy*, vol. 8, pp. 3-33, 1988.
- [31] D. M. Livermore, "Beta-Lactamases in laboratory and clinical resistance," *Clinical Microbiology Reviews*, vol. 8, pp. 557-584, Oct, 1995.
- [32] S. K. Morgan-Linnell, L. B. Boyd, D. Steffen and L. Zechiedrich, "Mechanisms accounting for fluoroquinolone resistance in *Escherichia coli* clinical isolates," *Antimicrob Agents Chemother*, vol. 53, pp. 235-241, Jan, 2009.
- [33] G. Stotzky, M. A. Devanas and L. R. Zeph, "Methods for studying bacterial gene transfer in soil by conjugation and transduction," *Advances in Applied Microbiology*, vol. 35, pp. 57-169, 1990.
- [34] S. M. Aswani, U. Chandrashekar, K. Shivashankara and B. Pruthvi, "Clinical profile of urinary tract infections in diabetics and non-diabetics," *Australasian Medical Journal*, vol. 7, pp. 29-34, 2014.