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ISOLATION AND IDENTIFICATION OF BACTERIA FROM IRAQI WOMEN WITH RECURRENT URINARY TRACT INFECTION

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Abstract

The current study was carried out to identify the most prevalent bacterial species among Iraqi women suffered from recurrent urinary tract infections (rUTI). Eighty mid-stream urine specimens were collected from Iraqi women referring to different hospitals in Baghdad governorate. Forty specimens were taken from rUTI female patients which represent the patient group. Another forty mid-stream urine specimens were obtained from healthy women; thereby considered as the control group. All grown colonies were primarily identified via morphological and biochemical tests. The identification was confirmed using VITEK 2 compact system. The results showed that *Escherichia coli* was a predominant isolate (42.5%) followed by *Staphylococcus aureus* (22.5%), *Staphylococcus epidermidis* (12.5%), *Proteus mirabilis* (10%) and (5%) for each *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.
***Keywords*:** Recurrent urinary tract infections, Iraqi women, bacteria

Introduction

Urinary tract infections (UTI) are among the most prevalent forms of primary and acute care infections seen (Madeo and Johnson, 2015). Markedly, Flower *et al.* (2014) indicated that the UTI will adversely affect the quality of life for women.

The recurrent urinary tract infection (rUTI) as the occurrence of a urinary tract infection with symptoms that happen during at least three times in the same year followed by previous symptoms of urinary tract infection (Giarenis and Robinson, 2016). rUTI is one of the most common diseases affecting women around the world. Women have a 50% higher chance of developing a UTI (Griebling *et al.*, 2005) in their lives with a 25% chance of recurring infection (Scholes *et al.*, 2000).

rUTI in women is a common phenomenon before and after menopause. The patients' history of recurrence must be recognized and examined thoroughly. Thus, the risk factor should be identified and treating them accordingly, even though antibiotics are one of the most used methods to control these infections (Abdullatif *et al.*, 2014).

In Iraq, Al-Kuraishi *et al.* (2013) stated that the prevalence of rUTI in Wasit governorate was 11.8% and the most frequent causative agent was *E. coli*. Al-Mendelawy (2010) reported that 10.3% of Iraqi children had rUTI. Al-naimi and Abbas (2016) indicated that 74% of Iraqi leukemic patients were presented with rUTI and *E. coli* is the predominant etiological agent. Likewise, Alhamdany (2018) indicated that E. coli was the main isolated bacteria in patients with rUTI and Diabetes Miletus from Babylon governorate.

On the other hand, *Klebsiella*, *Enterobacter*, and *Proteus* species, and enterococci uncommonly cause uncomplicated cystitis and pyelonephritis (Nimri, 2010). Another study was explained that *E. coli* represent the most common causative agent of UTI (74.6%), followed by *Klebsiella* spp. (11.7%), *Staphylococcus saprophyticus* (6.4%), and *Pseudomonas aeruginosa* (2.2%) (Safar *et al.*, 2009).

To our best knowledge, this is the first work investigated the prevalence of rUTI in Iraqi women. Other studies were conducted either on a sample of both sex patients or patients with underlying diseases.

Materials and Methods

Specimen collection

Around forty mid-stream urine specimens were collected from Iraqi women referring to different hospitals in Baghdad. All were suffered from rUTI that represents the case (patient) group. An extra forty mid-stream urine were collected from apparently healthy Iraqi women; hence considered as the control group. All specimens were collected from November 2018 to April 2019.

Isolation and identification of bacteria

Isolation of bacteria from mid-stream urine specimens was performed depending on routine laboratory techniques. All specimens were cultured on blood agar for primary isolation and detecting hemolysis pattern. The plates were observed for colony morphology. The developed colonies on blood agar were cultured on a group of culture media such as MacConky agar, Eosin methylene blue, and Mannitol salt agar (HiMedia, India). All plates were incubated aerobically for 24 h. at 37°C. Subsequently, Gram-stain characteristics and relevant biochemical tests were carried out following the procedures described by Harley (2016). IMViC tests (Indole, methyl red, Voges-Proskauer, citrate utilisation), urease and triple sugar iron (TSI) were achieved for all Gram-negative isolates. Furthermore, catalase and oxidase were achieved for all isolates in the current study; whereas, Coagulase was performed for cocci. Thereafter, identification was confirmed by the VITEK 2 compact system.

Results and Discussion

The results of identification were recorded in Table 1.

Table 1: Results of growth of all isolates on culture media and biochemical tests (no. = 40 isolates)

Isolate Cultural / Biochemical Test	*E. coli*	*P. auroginosa*	*K. pneumonia*	*P. mirabilis*	*S. aureus*	*S. epidermidis*
Hemolysis	β	β	γ	γ	β	γ
Lactose fermentation	LF	LNF	LF	LNF	NA	NA
Eosin methylene blue agar	+ (green metallic sheen)	+ colourless	+ colourless	+ colourless	NA	NA
Mannitol fermentation	NA	NA	NA	NA	F	NF
Indole	+	-	-	-	-	-
Methyl red	+	-	-	-	+	+
Vogas Proskauer	-	-	+	+	+	-
Citrate utilization	+	+	+	-	-	-
TSI	A/A + -	K/K - -	A/A+ -	A/A ++, K/A ++	-	-
Urease	-	+	+	+	-	-
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	-	-	-
Coagulase	-	-	-	-	+	-
Mast staph	-	-	-	-	+	-

LF= lactose fermenter, LNF= lactose non-fermenter, NA= nonapplicable .

Figure 1 shows the percentage of all isolates collected from clinical specimnes in the present work. Only 17 (42.5%), 9 (22.5%), 5 (12.5%), 4 (10%), 2 (5%), 2 (5%) identified as *E. coli*, *S. aureus*, *S. epidermidis*, *P. mirabilis*, *P. aeruginosa*, and *K. pneumoniae*, respectively.

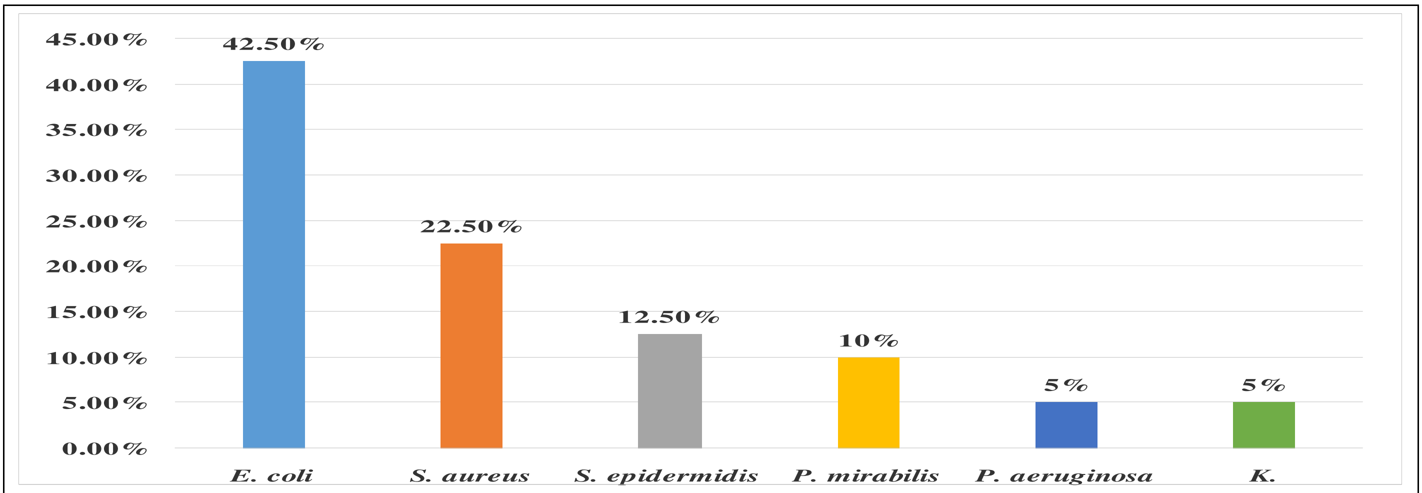

Fig. 1: The percentage of bacterial isolates collected from specimens of the current study.

Similarly, close results were recorded by Tamadonfar *et al*. (2019) who have claimed that the rUTIs are commonly caused by *E. coli* that led to the initial infection. Our results are also corresponded with; Kodner *et al*. (2010) and Ana *et al*. (2015) who have ensured that UTI are common infections caused by *E. coli* followed by *K. pneumoniae*, *P. mirabilis*, *Enterococcus faecalis* and *S. saprophyticus,* in the other word *K. pneumoniae*, *P. aeruginosa*, *Proteus*, and other organisms are more common in patients with certain risk factors for complicated urinary tract infections.

In Iraq, the current findings are in an agreement with Al-naimi *et al*. (2016) who have recorded (31.82 %) of rUTI cases caused by *E. coli* followed, *K. pneumonia*e (22.2 %) and the remaining percentage caused by different bacterial isolates. Meanwhile, the results of Martin *et al*. (2019) supported the present study results, who have explained the most prevalent bacterial uropathogen was *E. coli* (41.9%) followed by *S. aureus* (31.4%). In addition, the results of the current study are agreement with Alhamdany (2018) who have noticed the preidominant isolate in rUTI was *E. coli* (55%).

Such dominance of *E. coli* is owing to a variety of virulence factors of *E. coli* have been identified, such as endotoxins in all strains, adhesins (pili) and capsule present in some strains were associated with UTIs and colonization factors (Bien *et al*., 2012). Besides, there is a variety of virulence-associated factors possessed by *E. coli* that assist bacteria to adhere to injuring and invade host tissue such as toxins, adhesins, invasins and siderophores. The presence and number of factors related to virulence predict virulence *in vivo* (Johnson *et al*., 2005).

Elevated prevalence of *E. coli* in women may be due to the closeness of the anus to the vagina. The inherent virulence of *E. coli* is accountable for this high possibility of urinary tract colonization and UTIs in females such as its capability of adhering to the urinary tract, also the association with other microorganisms moving from perineum areas contaminated with faecal microbes to the warmest and most humid place of female genitalia (Andabati *et al*., 2010).

Many uropathogens were encoded urease enzyme, including *P. mirabilis* (Armbruster *et al*., 2012), *K. pneumoniae* (Podschun *et al*., 1998) and *P. aeruginosa* (Visca *et al*., 1992). This enzyme was very important for colonization and causing UTIs (Li *et al*., 2002) owing to urease activity to degrade urea into ammonia and carbon dioxide (Griffith *et al*., 1976), this process leads to elevate urine pH, production of calcium crystals (apatite) and magnesium ammonium phosphate (struvite) precipitate in urine and on catheters (Armbruster *et al*., 2012). Importantly, ammonia accumulation becomes toxic to the uroepithelial cells, causing direct damage to the tissue (Coker *et al*., 2000).

The *P. mirabilis* urease was one of the important and essential enzymes for the colonization of the bladder and kidneys and promotes the formation of stones (Armbruster *et al*., 2012; and Jacobsen *et al*.,2011). The *P. mirabilis* urease is induced by urea and is constitutively expressed during growth in urine (Kosikowska *et al*., 2011).

Our results disagreed with Al-Mendalawi (2010) who have reported that the mix infection of *S. aureus* and *P.auroginosa* were an uncommon cause of rUTI and represents (0.7 %) of urinary isolates. Moreover, Alhamdany (2018) recorded that *S.aureus* and *K. pneumoniae* were responsible for about 6.3% of rUTI cases and *P. mirabils, P.auroginosa* were 20% and 12.4% respectively.

Likewise, Alshabi *et al*. (2019) have recorded that the prevalence of UTI in pregnant women for *S. aureus* was 10.52%. Previous studies have linked the increasing Staphylococcal UTIs to increased use of instrumentation such as bladder catheters (Iregbu *et al*., 2013).

The present results disagreed with Fadhel *et al*. (2013) as they demonstrated that the prevalence of staphylococci from UTI in respect to *S. epidermidis* is higher than *S. aureus* 55.5% (10 out of 18) and 26.6% (8 out of 30), respectively. This variation may be returned to the misuse of antibiotics which makes the bacterial isolates more virulent and invasive and the difference in awareness between societies.

However, these results are reported by Melaku *et al*. (2012) who have claimed that the percentage of Gram-negative and Gram-positive were 52.6% and 47.4%, respectively. Also, the same authors explained that *E. coli*, *K. pneumoniae*, *P. aeruginosa*, were the dominant Gram-negative isolates, on the other hand, the percentage of *S. aureus* species was 36.3%. In other words, *E. coli* from UTI and *S. aureus* from surgical wounds were predominant isolates in UTI cases because these isolates showed high resistance to common antibiotics.

The present results were compatible with Alshabi *et al*. (2019) who have observed that the frequency of Gram-negative isolates is more as compared to the Gram-positive isolates, and the most predominant microorganism is *E. coli (*73.68%*)* followed by *Pseudomonas* (10.52 %).

On the other hand, current results are disagreeing with Weekes *et al*. (2015) who have reported that the Gram-negative bacteria cause 90% of UTI cases while Gram-positive bacteria cause only 10% of the cases. Earlier studies in Uganda 2011, 2015, and Nigeria 2016 reported high rates of *S. aureus* (22.5%), (43.7%), and (28%), respectively (Mwaka *et al*., 2011; Odoki *et al*., 2015 and Ekwealor *et al*., 2016).

The pattern may differ from place to place and from time to time, but it is observed that *E. coli* in most studies conducted in different regions of the world. Besides, *E. coli* represents the most frequent UTI-causing uropathogen in pregnant women or clinical settings.

The current results agreed with Hadi *et al*. (2014) who have recorded that the Gram-negative bacteria cause 81.3 % of UTI cases and Gram-positive bacteria cause only 18.7% of the cases in Basra governorate. In Duhok governorate, Mahde *et al*. (2015) demonstrated that the prevalence of Gram-negative and Gram-positive bacteria in UTI patients was 52.48 % and 47.51%, respectively.

Moreover, Alhamdany (2018) reported that the Gram-negative and Gram-positive in rUTI patients reached 93.7% and 6.3 % respectively in Babylon governorate. On the other hand, the current results disagreed with Abdullah (2019) who have found that the Gram-positive and Gram-negative percentage in patients with urinary tract infection in Duhok governorate were 53 and 47%, respectively.

This difference can be explained by geographical variation, the misuse of antibiotics that cause multidrug-resistant bacteria that in turn participate in the distribution of bacteria.

References

Abdullah, I.M. (2019). Multiple drugs resistance among urinary tract infection patients in Duhok city-kurdistan region Iraq. Duhok Medical Journal. 13(1): 22-31.

Abdullatif, A.; Kamran, A.; Iftikhar, Z.; Muhammad, S.K. and Prokar, D. (2014). Recurrent urinary tract infections in women. Int Urogynecol J. doi 10.1007/s00192-014-2569-5.

Alhamdany, M.H.A. (2018). Antibiotic Susceptibility of Bacteria Isolated from Patients with Diabetes Mellitus and Recurrent Urinary Tract Infections in Babylon Province, Iraq. Medical Journal of Babylon, 15(1): 63-68.

Alhamdany, M.H.A. (2018). Antibiotic Susceptibility of Bacteria Isolated from Patients with Diabetes Mellitus and Recurrent Urinary Tract Infections in Babylon Province, Iraq. Medical Journal of Babylon, 15(1): 63-68.

Al-Mendalawi, M.D. (2010). Urinary tract infection in Iraqi children. Saudi J Kidney Dis Transpl., 21: 961-963.

Al-naimi, I.A. and Abbas, M.A. (2016). Recurrent Urinary Tract Infections in Iraqi leukemic patients. International Journal of Advanced Research, 4(3): 1433-1439.

Al-Naimi, I.A. and Abbas, M.A. (2016). Recurrent Urinary Tract Infections in Iraqi leukemic patients. International Journal of Advanced Research, 4(3): 1433-1439.

Alshabi, A.M.; Alshahrani, M.S.; Alkahtani, S.A. and Akhtar, M.S. (2019). Prevalence of urinary tract infection and antibiotic resistance pattern in pregnant women, Najran region, Saudi Arabia. African Journal of Microbiology Research. 13(26): 407-413.

Ana, L. Flores-Mireles.; Jennifer, N.W.; Michael, C. and Scott, J.H. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 13(5): 269–284.

Andabati, G. and Byamugisha, J. (2010). Microbial aetiology and sensitivity of asymptomatic bacteriuria among ante-natal mothers in Mulago hospital, Uganda. Afr Health Sci. 10(4): 349–352.

Armbruster, C.E. and Mobley, H.L. (2012). Merging mythology and morphology: the multifaceted lifestyle of Proteus mirabilis. Nat Rev Microbiol. 10(11): 743-54.

Bien, J.; Sokolova, O. and Bozko, P. (2012). Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. Int J Nephrol. 681473.

Coker, C.; Poore, C.A.; Li, X. and Mobley, H.L. (2000). Pathogenesis of Proteus mirabilis urinary tract infection. Microbes Infect. 2(12): 1497-505.

Ekwealor, P.A.; Ugwu, M.C.; Ezeobi, I.; Amalukwe, G.; Ugwu, B.C.; Okezie, U.; Stanley, C. and Esimone, C. (2016). Antimicrobial Evaluation of Bacterial Isolates from Urine Specimen of Patients with Complaints of Urinary Tract Infections in Awka, Nigeria. International Journal of Microbiology. 1-6.

Fadhel, A.N.; Abureesha, R.A. and Al-azzawi, R.H. (2013). Prevalence of *S.epidermidis* and *S. aureus* and their biofilm ability among Iraqi patients suffering from urinary tract infection Iraqi Journal of Science, 54(3): 547-552.

Flower, A.; Bishop, F. and Lewith, G. (2014). How women manage recurrent urinary tract infections: an analysis of postings on a popular web forum. BMC Fam Pract., 15: 162.

Giarenis, I. and Robinson, D. (2016). Lower urinary tract infections. In: Luesley DM, Kilby MD, Obstetrics & Gynaecology: An Evidence-based Text for the MRCOG. 3rd edn. CRC Press, London: Chapter 97: 773-9.

Griebling, T.L. (2005). Urologic diseases in America project: trends in resource use for urinary tract infections in women. J Urol. 173(4): 1281–1287.

Griffith, D.P.; Musher, D.M. and Itin, C. (1976). Urease The primary cause of infection-induced urinary stones. Invest Urol., 13(5): 346–350.

Hadi, A.M.; Sheri, F.H. and Jaccob, A.A. (2014). Urinary Tract Infection Prevalence and Antibiotic Resistance A Retrospective Study in Basra Governorate, Iraq. Al-Mustansiriyah Journal of Pharmaceutical Sciences (AJPS), 14(2): 129-135.

Harley, J.P. (2016). Laboratory Exercises in Microbiology. 10th ed. McGraw-Hill Higher Education. New York.

Ibrahim, M.A. (2019). Multiple drugs resistance among urinary tract infection patients in Duhok city-kurdistan region Iraq. Duhok Medical Journal, 13(1): 22-31.

Iregbu, K. and Nwajiobi-Princewill, P. (2013). Urinary tract infections in a tertiary hospital in Abuja, Nigeria. African Journal of Clinical and Experimental Microbiology. 14(3): 169-173.

Jacobsen, S.M. and Shirtliff, M.E. (2011). Proteus mirabilis biofilms and catheter-associated urinary tract infections. Virulence, 2(5): 460-465.

Johnson, J.R.; Kuskowski, M.A.; O'Bryan, T.T.; Colodner, R. and Raz, R. (2005). Virulence genotype and phylogenetic origin in relation to antibiotic resistance profile among Escherichia coli urine sample isolates from Israeli women with acute uncomplicated cystitis. Antimicrobial Agents and Chemotherapy, 49(1): 26-31.

Kodner, C.M. and Thomas, G.E.K. (2010). Recurrent urinary tract infections in women: diagnosis and management. Am Fam Physician, 82(6): 638-43.

Kosikowska, P. and Berlicki, L. (2011). Urease inhibitors as potential drugs for gastric and urinary tract infections: a patent review. Expert Opin Ther Pat., 21: 945–957.

Li, X.; Zhao, H.; Lockatell, C.V.; Drachenberg, C.B.; Johnson, D.E. and Mobley, H.L. (2002). Visualization of Proteus mirabilis within the matrix of urease-induced bladder stones during experimental urinary tract infection. Infect Immun. 70(1): 389-94.

Madeo, M. and Johnson, P. (2015). Testing for urinary tract infection in non-catheterised patients. Journal of Community Nursing. 29(1): 22-31.

Mahde, S.A.A.; Nashwan, M.R.; Ibrahim, N.R.; Hussein, A.A.T. and Amer, A.B. (2015). Urinary Bacterial Profile and Antibiotic Susceptibility Pattern among Patients with Urinary Tract Infection in Duhok City, Kurdistan Region, Iraq. Int. J. Pure Appl. Sci. Technol., 30(2): 54-63.

Martin, O.; Adamu, A.A.; Julius, T.; Josephat, N.M.; Eddie, W.; Charles, D.K.; Ezera, A. and Joel, B. (2019). Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda. International Journal of Microbiology. Volume 2019, Article ID 4246780, 8.

Melaku, S.; Gebre-Selassie, S.; Damtie, M. and Alamrew, K. (2012). Hospital acquired infections among surgical, gynaecology and obstetrics patients in Felege-Hiwot referral hospital, Bahir Dar, northwest Ethiopia. Ethiop Med J., 50(2): 135-44.

Mwaka, D.; Mayanja-Kizza, H.; Kigonya, E. and Kaddu-Mulindwa, D. (2011). Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda. Afr Health Sci., 11(2): 182-189.

Nimri, L. (2010). Community-acquired urinary tract infections in a rural area in jordan: predominant uropathogens, and their antimicrobial resistance. Webmed Cent Microbiol, 1: 1-10.

Odoki, M.; Bazira, J.; Moazam, M.L. and Agwu, E. (2015). Health-point survey of bacteria urinary tract infections among suspected diabetic patients attending clinics in Bushenyi district of Uganda, Special Bacterial Pathogens Journal (SBPJ). 1(1): 0005–0009.

Podschun, R. and Ullmann, U. (1998). Klebsiella spp as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 11: 589–603.

Safar, F.; Mohammad, Y.A.; Reza, G.; Behrooz, N. and Ailar, N. (2009). Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. International Journal of Infectious Diseases. 13(2):140-144.

Scholes, D.; Hooton, T.M.; Roberts, P.L.; Stapleton, A.E.; Gupta, K. and Stamm, W.E. (2000). Risk factors for recurrent urinary tract infection in young women. J Infect Dis. 182(4):1177–1182.

Tamadonfar, K.O.; Omattage, N.S.; Spaulding, C.N.; Hultgren, S.J. (2019). Reaching the end of the line: urinary tract infections. Microbiol Spectrum. 7(3):0014-2019.

Visca, P.; Chiarini, F.; Mansi, A.; Vetriani, C.; Serino, L. and Orsi, N. (1992). Virulence determinants in Pseudomonas aeruginosa strains from urinary tract infections. Epidemiol Infect. 108(2): 323-336.

Weekes, L.M. (2015). Antibiotic resistance changing management of urinary tract infections in aged care. Med J Aust., 203(9): 352.