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)

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

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

Figure 1 shows the percentage of all isolates collected from clinical specimens 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.

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 agreement with Al-naimi et al. (2016) who have recorded (31.82 %) of rUTI cases caused by E. coli followed, K. pneumoniae (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 predominant 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. aeruginosa* 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. mirabilis*, *P. aeruginosa* 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**


