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Research article

Prevalence and Antibiotic Susceptibility of *Enterococcus faecalis* Isolated from Urine Samples of Iraqi Women with Urinary Tract Infections

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ABSTRACT

Enterococcus faecalis is one of the most important pathogens associated with urinary tract infections (UTIs) in women. The literature on the incidence of UTI infections caused by *E. faecalis* in Iraq is limited. The increasing antibiotic resistance of this bacterium is one of the main challenges facing clinicians. This study aims to investigate the prevalence of *E. faecalis* UTIs in Iraq and to assess the response pattern to three commonly used antibiotics in Iraq: nitrofurantoin, ampicillin, and ceftriaxone. A total of 83 urine samples were collected from women suffering from UTIs. Morphological and biochemical methods were used to identify the isolates, and VITIK II technology was employed to confirm bacterial identification. The microdilution in microtiter plates was used to measure the susceptibility of *E. faecalis* to the three antibiotics. The results showed that the rate of UTI caused by *E. faecalis* was moderate, at 12.04%. The resistance to ampicillin was 100%, while resistance to nitrofurantoin was 50%. The response to ceftriaxone was also moderate. In conclusion, the study found that the incidence of UTI with *E. faecalis* among patients was moderate, and the effectiveness of nitrofurantoin was 50%.

Keywords: Ampicillin, Ceftriaxone, *Enterococcus faecalis*, Minimum Inhibitory Concentrations, Nitrofurantoin, UTIs.

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1. INTRODUCTION

Urinary tract infections (UTIs) are a common problem for women everywhere, impacting both health and costs. *Escherichia coli* is the primary pathogen that usually causes UTIs [1]. Another bacterium, *Enterococcus faecalis*, is becoming a greater concern, particularly in infections acquired in the community or hospitals [2]. *E. faecalis* typically lives harmlessly in the gut and urinary system, but it can cause trouble when it gets the chance to cause infection or invade the host tissue. Its ability to form biofilms and resist antibiotics makes treating these infections a big challenge. It's important to be aware of these developments in UTI treatment [3]. *E. faecalis* bacteria appear in UTIs at varying rates depending on the location. This variation can be attributed to factors such as healthcare quality, antibiotic use, and individual health conditions [4]. In Iraq, UTIs are a serious health concern, but there is limited data on how common *E. faecalis* is among women there. Understanding more about these bacteria is crucial, as they are associated with more frequent infections and serious complications, such as kidney infections and chronic kidney problems, particularly for individuals with other health issues [5].
The treatment of *E. faecalis* infections is becoming increasingly challenging due to its intrinsic resistance to several antimicrobial agents, including cephalosporins and low levels of aminoglycosides

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as well as its capacity to acquire resistance through horizontal gene transfer. Moreover, the inappropriate or empirical use of antibiotics has accelerated the emergence of multidrug-resistant (MDR) strains. Therefore, regular surveillance of local antibiotic susceptibility patterns is essential for guiding appropriate therapy and preventing the spread of resistant strains [6]. In Iraq, antibiotic resistance is a growing public health problem, partly due to the unregulated use of antibiotics and the absence of updated national antimicrobial stewardship programs [7]. Reports on the resistance patterns of *E. faecalis* from urinary isolates in Iraqi women remain uncommon, making it difficult for clinicians to adopt evidence-based treatment strategies. Hence, region-specific studies are urgently needed to provide baseline data on prevalence and resistance profiles.

This study aims to investigate the prevalence of *E. faecalis* bacteria in urine samples from Iraqi women with urinary tract infections (UTIs). It also examines how these bacteria respond to specific antibiotics. The antibiotics tested include nitrofurantoin, ampicillin, and cefotaxime. These are all frequently used to treat UTIs in Iraq. By determining the effectiveness of these treatments against urinary *E. faecalis*, we aim to refine treatment recommendations and mitigate the spread of antibiotic-resistant strains in the region. This information is crucial for both healthcare providers and patients in ensuring better health outcomes.

2. MATERIALS and METHODS

2.1. Isolation and identification

The 83 midstream urine samples were collected from inpatients with UTIs at Babylon Teaching Hospital in Babylon, Iraq. The clinical samples were obtained aseptically. All patients had discontinued antibiotic treatment 72 hours before sample collection and provided consent to participate. The study was approved by the Ethics Committee of the Department of Biology, College of Science, University of Baghdad. The urine samples were immediately transferred to the clinical lab.

The standard method of Parameswarappa *et al.* (2013) was used to identify the *E. faecalis* isolated from urine samples. The VITEK 2 fluorescence-based identification system (ID-GNB card) was employed to confirm the species of the isolated bacteria. The isolates were preserved for short-term storage by streaking onto nutrient agar slants and plates, incubated at 37°C, and then stored at 4°C for up to one week. For long-term preservation, the isolates were suspended in nutrient broth containing 20% glycerol (Sigma-Aldrich) and stored at –25 °C.

2.2. Standard inoculum

The standard inoculum of bacterial suspension was prepared by washing the overnight growth (grown at 37 °C in MHB) of bacteria with sterile phosphate-buffered saline (PBS; 0.1 M, pH 7.2) using centrifugation at 5,000 g for 10 min (Beckman Coulter, Brea, USA), and the optical density of bacterial suspension was adjusted to 0.05 at 600 nm using spectrophotometer (Bioevopeak, Jinan, China).

2.3. Minimum inhibitory concentrations (MIC)

The microtiter plate technique of Al-Mutalib & Zgair (2023) was followed to measure the MICs of cefepime, nitrofurantoin, and cefotaxime against ten *E. faecalis* (E.f.1, E.f.2, E.f.3, E.f.4, E.f.5, E.f.6, E.f.7, E.f.8, E.f.9, and E.f.10) isolates. Two milligrams of the above antibiotics were prepared in Muller-Hinton broth (MHB, HiMedia, India). From the above stock solution, two-fold dilutions (100 µL) were prepared in the U-shape microtiter plate using MHB (HiMedia). Six microliters of the standard inoculum of *E. faecalis* were added to each well. MHB and bacterial isolate (1st control), MHB alone (2nd control), and various double dilutions of antibiotics only (3rd control) were used. The plates were incubated at 37 °C for 18 h. The MIC was measured by checking the lowest antibiotic concentration that completely inhibits growth [9].

2.4. Statistical analyses

The statistical analysis was conducted, and the graphs were generated utilizing Origin v. 8.6 software (OriginLab, Northampton, USA). The data were presented as means ± standard error (M ± SE). The student t-test was used to check the group differences.

3. RESULTS

The present study found a moderate incidence rate of urinary tract infections caused by *E. faecalis* (12.04%). Bacterial isolates from UTIs were identified using biochemical tests and confirmed with the VITIK 2 system. Table 1 shows the MICs of three antibiotics (nitrofurantoin, ampicillin, and ceftriaxone). The CLSI breakpoints were used to interpret the results. The findings indicated that all isolates were resistant to ampicillin, and 50% were resistant to nitrofurantoin. Although CLSI does not provide interpretation guidelines for ceftriaxone against ten *E. faecalis* isolates, as the breakpoints for ceftriaxone against *E. faecalis* are not available from CLSI.

Table 1. Minimum inhibitory concentrations of three antibiotics, nitrofurantoin, cefotaxime, and cefepime, against ten clinical isolates of *E. faecalis* (E.f.1, E.f.2, E.f.3, E.f.4, E.f.5, E.f.6, E.f.7, E.f.8, E.f.9, and E.f.10) isolated from urine samples of patients with UTIs.

No of Isolates	Nitrofurantoin (µg/mL)	Ampicillin (µg/mL)	Ceftriaxone (µg/mL)
E.f.1	15.6 (S)	61.5 (R)	15.6
E.f.2	15.6 (S)	31.25 (R)	31.25
E.f.3	31.2 (R)	7.8 (R)	31.25
E.f.4	15.6 (S)	125 (R)	62.5
E.f.5	15.6 (S)	125 (R)	15.6
E.f.6	62.5 (R)	61.5 (R)	15.6
E.f.7	31.2 (R)	125 (R)	31.25
E.f.8	15.6 (S)	250 (R)	15.6
E.f.9	250 (R)	500 (R)	250
E.f.10	125 (R)	250 (R)	125

4. DISCUSSION

UTIs are a major global public health concern, especially for women, who are more vulnerable due to their anatomy [10]. There is increasing concern about *E. faecalis* as a pathogenic cause of UTI. This study examined how frequently *E. faecalis* appears in urine samples from Iraqi women with UTIs and assessed the effectiveness of three common antibiotics, nitrofurantoin, ampicillin, and ceftriaxone, against these bacteria. The findings

indicated that *E. faecalis* was present in approximately 12.04% of the samples. Notably, all tested strains were resistant to ampicillin, posing a significant challenge to antibiotic treatment.

The observed prevalence rate of 12.04% in this study is lower than global reports, indicating that *E. faecalis* is a significant cause of UTIs, particularly in complicated or recurrent cases [11]. Several regional studies report different prevalence rates. For example, studies in the Middle East and Asia found a higher prevalence of *E. faecalis* than what we found in our study [11,12]. However, this prevalence is higher than rates reported in the United States of America (USA), where *E. faecalis* causes about 5.3% of UTIs in community settings [13]. The difference may be due to variations in healthcare infrastructure, infection control practices, antibiotic use, and patient populations. In Iraq, self-medication and over-the-counter access to antibiotics may contribute to the higher prevalence and the selection pressure for resistant strains.

The complete resistance of *E. faecalis* to ampicillin in this study is a particularly concerning finding. Traditionally, *E. faecalis* has been considered susceptible to β-lactam antibiotics, especially ampicillin plus ceftriaxone, which is often a fine drug for treating enterococcal UTIs [14]. The emergence of high-level ampicillin resistance may indicate the dissemination of β-lactamase-producing strains or alterations in penicillin-binding proteins, as documented in recent literature [15]. Similar high resistance rates have been reported in neighboring countries, where indiscriminate use of β-lactams has contributed to the rapid evolution of resistant enterococcal clones. The finding has significant clinical implications, as ampicillin-resistant *E. faecalis* may necessitate the use of more toxic or expensive alternatives such as vancomycin or linezolid, increasing the burden on healthcare systems [16].

Nitrofurantoin has demonstrated moderate effectiveness, with approximately half of the tested strains being resistant. It has long been a preferred oral treatment for uncomplicated UTIs, including those caused by *E. faecalis* [17]. However, the rise in resistance observed in this study may be linked to its frequent use as a first-line treatment in community healthcare. Resistance in enterococci often results from mutations in specific genes that diminish the effectiveness of the drug [18]. Notably, the resistance rates in this study are higher compared to those reported in studies from Europe and North America [19]. This highlights the need for continued local surveillance, as patterns of antibiotic resistance can differ greatly between regions.

In this study, ceftriaxone, a third-generation antibiotic, showed only a moderate effect against *E. faecalis*, a type of bacteria. It's essential to recognize that Enterococcus species, such as E. faecalis, are naturally resistant to cephalosporins due to the way their penicillin-binding proteins function [20]. This moderate response might be due to issues in the lab or the presence of mixed infections, since cephalosporins are not typically recommended for treating enterococcal infections [21]. However, testing for ceftriaxone could still be useful in complicated urinary infections involving other bacteria, though it should not guide treatment for infections caused solely by E. faecalis.

This study highlights important considerations for managing UTIs and antibiotic use in Iraq. The high resistance to ampicillin and moderate resistance to nitrofurantoin indicate that we should revise our treatment guidelines. Physicians should consider conducting urine tests and checking for bacteria before initiating treatment, especially in patients with complicated or recurrent infections. Furthermore, there is a critical need for programs that promote responsible antibiotic use to help reduce misuse [22]. Educating the public about the dangers of self-medication and the importance of completing prescribed treatments is essential to prevent further resistance from developing [23].

The presence of *E. faecalis* in the urine of patients with UTIs is more common than previously recognized, raising important concerns for accurate diagnosis. Often, routine urine tests focus on detecting Gram-negative bacteria, leaving enterococcal infections overlooked. As *E. faecalis* becomes more involved in UTIs, laboratories should use reliable techniques such as VITEK II or MALDI-TOF, as this study suggests. Accurate identification is crucial to ensure appropriate treatment and to monitor resistance patterns that impact public health policies and practices [24]. Comparing this study's findings with international data shows that antimicrobial resistance varies significantly across regions. Das et al. (2020) found that 44.73% *E. faecalis* and 63% *E. faecium* were MDR [25], while nitrofurantoin is effective against *E. faecalis* in many areas; Tang et al. (2013) found that Fosfomycin is more effective against vancomycin-resistant enterococci (VRE) [26]. The Enterococci, w*ith a* 50% resistance rate observed here in Iraq, present significant challenges. The widespread ampicillin resistance indicates the potential spread of resistant strains in bacterial infection cases, emphasizing the need for further research to understand transmission and develop effective control measures.

It's important to acknowledge some limitations of this study. With only 83 urine samples and focusing exclusively on women, the results may not be applicable to other groups, such as men, children, or patients with catheter-related UTIs. Additionally, the research examined just three antibiotics. Including others, such as vancomycin, linezolid, and fluoroquinolones, could provide a broader understanding of resistance patterns. Nonetheless, this study provides valuable baseline data for Iraq, where research on *E. faecalis* UTIs is limited. This study found that *E. faecalis* is quite common among Iraqi women with UTIs, and there are concerning trends with antibiotic resistance, especially to ampicillin and nitrofurantoin. It highlights the need for continuous local monitoring, careful use of antibiotics, and effective stewardship programs to tackle the growing issue of antimicrobial resistance. Doctors should exercise caution when prescribing treatments and prioritize the use of culture-guided approaches. There's also a call for more research with larger groups to better understand resistant *E. faecalis* strains in Iraq.

5. Conclusion

This study sheds light on how common *E. faecalis* is as a cause of UTIs in Iraqi women, with a prevalence rate of about 12.04 %. It's concerning that this bacterium shows a high level of antibiotic resistance. They are completely resistant to ampicillin and have a 50% resistance to nitrofurantoin. This means that treating these infections is becoming increasingly challenging. Even ceftriaxone, which is commonly used, isn't as effective due to the bacteria's inherent resistance. These results underscore the importance of ongoing monitoring of antibiotic resistance, improved management of antibiotic use, and treatment based on laboratory cultures. It's also important to address issues like self-medication and encourage the responsible use of antibiotics. Looking ahead, larger studies that explore how resistance works would be beneficial in informing more effective treatment options.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

The study was carried out after receiving ethical approval from the ethics committee of the Ministry of Health, Iraq, with serial number MoH/1014/00448, 10th October, 2023

Author contributions

Kassa T: Resources; Supervision; Validation; Roles/Writing, Writing - review & editing.

Rasheed HJ: Investigation; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; and Writing - review & editing.

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