Biocontrol of Clinical Bacteria Infecting Urinogenital System by Probiotic Bacteria

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Bacteria are considered the dominant microorganisms, which cause urinary tract infection (UTI) of both male and female. The development of multi-drug resistance phenomenon in bacteria leads to the necessity of finding an alternative remedy. Probiotics are suggested as a suitable and appropriate solution. In this study, cell free supernatant CFS of Enterococcus faeciumNM1 is examined for its inhibitory effect against clinical urogenital bacterial strains and Candida albicans isolated from urinary tract infection infections. CFS obtained from these probiotic bacteria showed a promising antibacterial and anticoncidial results. E. faeciumNM1 possessed capability to be used as a probiotic against medical bacteria isolated from urogenital infections.

Keywords: Urogenital infections, Probiotics, Multi-drug resistance, Inhibitory effect.

Probiotics are beneficial that have provided health benefits when consumed. They are used to suppress carcinogenesis, reduce cholesterol level of blood serum, prevent bacteria-associated diarrhea, inhibit adhesion of pathogenic bacteria to epithelial tissues of human systems, and were also used to improve innate and acquired immunity, gastric function(Abdel-Shafi et al., 2014; Agerholm-Larsen et al., 2000 and Turgis et al., 2013).

An inflammatory syndrome caused by microorganism’s invasion of the urinary tract. The microorganism’s infection can affect a variety of patients ranging from healthy women to men and children according to their immunity. Most uncomplicated and complicated cases can be treated easily by antimicrobial agents and demand longer treatment periods in more complicated (Harry et al., 1996).

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One or more structures in the urinary tract become infected after bacteria overcome its strong natural defenses, most cases of UT’s bacterial infection that can multiply at the opening of the urethra and migrate to the bladder (known as the ascending route) (Harry et al., 1996 and Jackson, 2006). (Al-Sharif & Al-Run, 2008) reported that UTI is a wide definition that includes different clinical manifestations such as:

a) Asymptomatic bacteriuria: Bacteriuria with no symptoms.
b) Uncomplicated urinary tract infection: Infection with a normal, unobstructed genitourinary tract with least instrumentation.
c) Complicated urinary tract infection: Infection in men or pregnant women patient with presence of foreign body (urinary catheter, stone).
d) Relapse: Recurrence of bacteriuria with the same microorganism and implies failure to eradicate infection within seven days of therapy.
e) Reinfection: Recurrence of bacteriuria with a new microorganism, 80% of recurrent infections are due to reinfection. It is difficult to differentiate from relapse when infection occurs with the same species.
f) Cystitis: Infection of the bladder and inflammatory syndrome with dysuria, urgency, and supra-pubic tenderness.
g) Pyelonephritis: Acute or chronic bacterial infection of kidney characterized by flank pain, tenderness and fever.
h) Urethritis: Lower urinary tract inflammation with or without bacterial infection.
i) Prostatitis: Bacterial infection, inflammation and pain affecting on the prostate gland.
j) Probiotics (derived from Latin and Greek) means “for life” is defined according to FAO/WHO as: Live microorganisms had health benefit on the host.

Some Lactobacillus sp. and Enterococcus faecium have ability to prevent the attachment of some pathogenic bacteria to the intestinal mucosa, that’s why, they are used as probiotics. Enterococci can resist the growth of pathogenic bacteria since they have the ability to lower the pH and produce organic acids especially, lactic acid and hydrogen peroxide, in addition to their capability to produce inhibitory substances called bacteriocins (Enan et al., 2015). The intention of probiotics uses to control and inhibit the urogenital bacteria due to the resistance of many bacteria to antimicrobial agents and the development of the phenomenon of multidrug resistance bacteria (MDR bacteria).

This work aimed to preventing some clinical bacteria infecting the urinogenital system by the probiotic Enterococcus faecium and their cell free supernatant (CFS).
Materials and Methods

**Bacterial strains and culture media**

The probiotic bacterium *Enterococcus faecium NM*₂ was kindly provided by Prof. Dr. Gamal Enan, Faculty of Science, and Zagazig University. It was propagated and subculture on De Man, Rogosa and Sharpe (MRS, Oxoid) medium. It produced inhibitory activity as described by Enan *et al.*, (2015). The inhibitory substances were due to acids and bacteriocins which designated as Enterocin.

The sensitive microorganisms used were isolated from urogenital patients admitted to Fakous General Hospital, Al-Sharkia, Egypt. From both males and females at different ages, four isolates were obtained and designated *P. aeruginosa* (1U), *S. aureus* (2U), *K. pneumonia* (4U) and *E. coli* (6U). Another four bacterial isolates of urogenital origin were kindly provided by Prof. Dr. Ahmed Anwar Shahin from Faculty of Medicine, AL-Qasr Al-Aini. They were also designated, *Shigella* (31U), *C. albicans* (32U), *Salmonella paratyphiB* (33U) and *Proteus vulgaris* (34U).

**Preparation of cell free supernatant (CFS)**

*Enterococcus faecium NM*₂, the producer of inhibitory substances, as grown in De Man, Rogosa and Sharpe broth for 16h at 37°C. CFS was obtained by centrifuging the culture (10.000 x g for 15 min at 4 °C) then the supernatant was collected.

**Inhibition test of microbial pathogens**

Erlenmeyer flasks of 250 ml, each containing 100 ml BHI broth, were inoculated by 2.0x 10⁴ CFU/ml of *E. faecium NM*₂ and about 2.0x 10⁴ CFU/ml of each bacterial pathogen used. In another experiment MRS broth inoculated by *E. faecium NM*₂ (by 2.0x 10⁴ CFU/ml) was inoculated also by about 2.0x 10⁴ CFU/ml of the *C. albicans*. Another series of 250 ml Erlenmeyer flasks, each containing 100 ml Brain Heart Infusion (BHI) broth, were inoculated either by 2.0x 10⁴ CFU/ml of each bacterial pathogen or by 2.0x 10⁴ CFU/ml of *C. albicans* used; and was treated by 1% v/v of CFS. The inoculated and treated Erlenmeyer flasks containing both test samples and controls were incubated at 37°C for 3 days (bacterial samples) or 6 days (Candidal test). After suitable time intervals 1 ml aliquots were removed, serially diluted and plated on to agar plates containing the selective and specific agar media for enumeration of each microbe used (Oxoid). These specific agar media were inoculated by sample dilutions and incubated at 37°C for 48h. Then CFU/ml was obtained as described previously for similar experimental conditions (Enan, 2000 and Enan *et al.*, 2000).

**Results and Discussion**

It was essential to test the sensitivity of uropathogens to different types of antibiotics. Results are given in Table 1.

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TABLE 1. Antibiotic susceptibility profile of selected clinical bacterial isolates.

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<thead>
<tr>
<th>Bacterial isolates</th>
<th>Tested antibiotics</th>
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<tr>
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<td>IPM</td>
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<td>1U</td>
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<td>33U</td>
<td>S</td>
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<tr>
<td>34U</td>
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It was shown that all the bacterial isolates except *P. vulgaris* (isolate no 34U) were nearly resistant to all antibiotics except for Imipenem (IPM) while *P. vulgaris* was sensitive for Gentamicin (CN) and Amikacin (AK). Table 1 illustrates that *C. albicans* (isolate no 32U) is completely resistant to all antibacterial agents. These were interesting results to evaluate bacterial isolates inhibition of urogenital system by the probiotic bacterium *E. faecium* NM₂.

The evaluation study of antibacterial activity CFS of *E. faecium* NM₂ against the 8 urogenital pathogens are showed in Fig. 1-8. Urogenital bacterial pathogens alone in control experiments grew vigorously and viable cells was 4-5 log cycles increase within 96 h in other samples which treated with CFS of *Enterococcus faecium* NM₂ thoroughly with urogenital pathogens, urogenital pathogens growth was decreased by 2 log cycles within 12hr and 48 or 72 h in case of *S. aureus* (isolate 2U), growth declined to 9.5x10⁵ within 12h by 2 log cycles while *E. coli* (isolate 6U), growth declined to 7.5x10⁵ by 2 log cycles within 96 h of incubation.

The growth of *p. aeruginosa* (isolate 1U) treated with NM₂ declined to 6.6x10⁷ by 2 log cycles within 48h, *K. pneumonia* (isolate 4U) treated with NM₂ decreased by 2log cycles to 8.1x 10⁷ within 72h. In case of Shigella (isolate 31U) and *P. vulgaris* (isolate 34U), growth decreased by 2 log cycles within 72h, while in *S. paratyphi B* (isolate 33U), viable cells decreased by 2 log cycles within 48h to 3.8x10⁵*C. albicans* (isolate 32U) which proved to be resistant to all antibacterial agents decreased by 2 log cycles to 6.1x 10⁵ within 72h. Results are illustrated in the following Fig. (1-8).

Urinary tract infection is infectious diseases most of people during the span of their lifetime (Hoberman & Wald, 1997). The type of infection can cause serious consequences if remains untreated (Pezzlo, 1988). Although several different microorganisms are involved in causing urinary tract infection, including fungi and viruses, the role of bacteria in causing these infections can’t be ignored (Bonadio et al., 2001).

Fig. 1. Inhibition of *S. aureus* (code 2U) by CFS *E. faecium* NM<sub>2</sub>.

Fig. 2. Inhibition of *E. coli* (code 6U) by CFS of *E. faecium* NM<sub>2</sub>.
Fig. 3. Inhibition of *P. aeruginosa* (code 1U) by CFS of *E. faecium NM*.

Fig. 4. Inhibition of *K. pneumonia* (code 4U) by CFS of *E. faecium NM*.
Fig. 5. Inhibition of *Shigella* (code 31U) by CFS *E. faecium NM₂*.

Fig. 6. Inhibition of *P. vulgaris* (code 34U) by CFS of *E. faecium NM₂*. 

Fig. 7. Inhibition of *S. paratyphi* B (code 33U) by CFS of *E. faecium* NM2.

Fig. 8. Inhibition of *C. albicans* (code 32U) by CFS of *E. faecium* NM2.

Treatment of urinary tract infection patients is often started empirically. Therapy is based on doing of urine culture to recognize the pathogen responsible for such injury and its susceptibility to different antibiotic agents. However, because of the evolving antibiotic resistance phenomenon, an urgent necessity to face such problem (Lim et al., 2009).

*Enterococcus faecium* is a lactic acid bacterium that had antibacterial effect in vitro. It’s a gram positive bacterium that has good microbiological characteristics such as short generation time and bacteriocin production (Lewenstein et al., 1979) and also has effect in gastrointestinal disorders (Salminen & Deighton, 1992).

*Enterococcus faecium* NM2 isolated from urine of healthy people decreased the growth of urogenital pathogen used in this study qualitatively. This was proven in other studies due to the action of bacteriocins. Hence, *E. faecium* NM2 may be used as an appropriate probiotic (Enan et al., 2015) and can reduce pH to pH 3.8 after 20h. (Zakaria, 2013).

Certain characters should be available for any bacterial organism to be used as probiotic adjuvant such as production of inhibitory acids and proteins, production of H₂O₂, adherence to genital surfaces and good growth and survival in presence of antimycotic and antibacterial agents. *E. faecium* NM2 possess most of these criteria; so, it used as probiotic to prevent the urogenital pathogens (Ronnqvist et al., 2005).

Gram positive and negative bacteria are inhibited by the production of antimicrobial substances, such as organic acids, hydrogen peroxide, reuterin and bacteriocins (Silva et al., 1987; Vandenbergh, 1993; Meurman et al., 1995 and O’Hara et al., 2007). CFS has antimicrobial substances may induce an antagonistic action to pathogens (Balcazar et al., 2007). The accumulation of such metabolites such as short chain fatty acids can reduce the pH which can inhibit the growth of clinical pathogens.

Cell free supernatant has an antibacterial activity against urinary tract infection pathogens as it contains organic acids that decrease the pH where uropathogens can’t survive (Sawa et al., 2009 and Enan et al., 2014). These acids may acidify cytoplasm of susceptibility bacteria leading to collapsing proton gradient which causes bacterial death (Tharmaraj & Shah, 2009). Because of *E. faecium* NM2 probiotic characters showed previously and so, in our work, this organism could be used as probiotic bacteria (Enan et al., 2015).

**References**


Egypt. J.Microbiol. 51 (2016)


(Received 10/11/2016; accepted 5 / 1 / 2017)
The antimicrobial activity of Enterococcus faecium NM2 for the treatment of urinary tract infections (UTI) was assessed in this study. The cell-free supernatant (CFS) of Enterococcus faecium NM2 showed antibacterial activity against urinary tract infection (UTI) clinical isolates of Enterococcus faecium NM2 and Candida albicans. The antibacterial activity of Enterococcus faecium NM2 CFS against UTI clinical isolates of Enterococcus faecium NM2 and Candida albicans showed promising results. This study suggests that Enterococcus faecium NM2 CFS could be a potential alternative to antibiotics in the treatment of UTI.