Study of probiotic effect on Staphylococcus epidermidis and Staphylococcus haemolyticus isolated from urinary tract catheters

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ABSTRACT

Catheter associated urinary tract infection consider one of the most common source of hospital acquired infections. A total of 188 samples, from various urinary tract catheterized patients, a total 116 samples revealed bacterial growth. Most infections of urinary tract catheter were more common in patients of ages above 50 years and whose catheterization period exceeded six days. the affectivity of antimicrobial agent of *Lactobacillus* spp. *Lactobacillus acidophilus* (*L acidophilus*)besides *Lactobacillus casie*(*L casie*) was detected by well diffusion method. *L casie* showed high level of ability to inhibition growth of *S epidermidis* and *S haemolyticus* than *L acidophilus.* Highest inhibition zone is indicated by bacterial cell broth $P<0.05$ between 19mm and 20mm for *L acidophilus* and *L casie,* respectively, while larger inhibition zone was observed by cell free supernatant (11mm) and (12mm) for *Lactobacillus acidophilus* and *Lactobacillus casie,* respectively.

Keywords: probiotic, Staphylococcus epidermidis, urinary tract catheters

Introduction:

Catheter associated urinary tract infections (CAUTIs) is a kind that's more resonating of hospital acquired infection (Gravel *et al*., 2007). Pathological microbes enter the bladder by tip contamination at the time of insertion with urethral flora such as *Staphylococcus epidermidis* (*S epidermidis*) and *Staphylococcus haemolyticus* (*S haemolyticus*) or apart from bacteria gaining access to the catheter's exterior or interior, catheterized patients' bladders contain more urine, increasing the risk of bacteruria (Barford and Coates, 2009). In addition, Becker *et al.* (2014) demonstrated that *S epidermidis* and *S haemolyticus* were classified as normal flora of the skin and mucous membrane, but now they are considered as one of major hospital acquired pathogens, especially associated with device related infections particularly in the immune-compromised patients and the chronic exhausted patient who needs the long-term central venous access.Tambyah and Maki (2000) mentioned that some symptoms i.e. chills, fever, leakage of urine around catheter, pressure of catheter , pain and discomfort in lower back or stomach are associated with CAUTIs; if the patients left without treatment and catheter remain for long period, this leads to kidney damage, pyelonephritis and even septicemia. Miriam *et al*. (2012) reported that a live microorganisms (M.O) like *Lactobacillus, Enterococcus,*

Bifidobacterium, and *Saccharomyces cerevisiae* which are normal flora of human, expected to have health benefit and save effected when took or applied to the body, that termed by probiotic. Probiotic M.O beneficially affects human and can inhibit *Staphylococcus* spp. and other infections of human via a number of works including increasing uro-epithelial barrier function and increased of the immune response by signal few antigen presenting cells to immune system cells. There is also on evidence that probiotics can prevent colonization of pathogens. This can be done via mechanisms, such as down regulation of virulence factors of *Staphylococcus* that is responsible for biofilm formation and inhibition of pathogen adherence (Piqué *et al*., 2019) .Prabhurajeshwar and Chandrakanth (2019) demonstrated that regulatory action of probiotic is carried out by species of Lactobacillus that yield antibacterial substances, such as lactic and additional organic acids (lactic and citric acids), hydrogen peroxide (H2O2) bacteriocins, and others which are low-molecular weight proteins or peptides have biologically active that prevent the growth of a diversity of pathogenic bacteria. Probiotic strains must not compete with the M.O of the normal flora; instead they must interrelate in symbiosis (da Silva Sabo et al., 2020). In addition*, L acidophilus* produces some antimicrobial substances, such as acidophilin, bacterial peptidesand. These are all antibacterial to pathogenic, besides *Lactobacillus* possesses high capacity in the inhibition and treatment of urogenital infections. Moreover, producing bacteriocins described by its effect on cellular membranes instability producing pore in membrane or deprivation of bacterial DNA via DNAse enzyme(Al-Mathkhury and Assal, 2012). Patients favor medicine with minimum or no side effects for treatment of their disorder thus probiotics suggest such an alternative, being living, non- harmful organisms, they have a Generally Recognized As Safe (GRAS) designation, indicating that they are extremely safe (Jankovic, 2008). Lactose intolerance, diarrheal disorders, allergies, lowering serum cholesterol, reducing the risk of carcinogenicity and mutagenicity, and immune system activation are all conditions that probiotic microorganisms are used to treat and prevent. (Thantsha *et al*., 2012). As well as, Shiel *et al*. (2004) mentioned that probiotics have been shown to have beneficial benefits not only in the stomach, but also in other body parts. When taken parenterally, probiotics, for instance, are identified to have anti-inflammatory actions. Lactose absorption issues (also known as lactose-intolerance or lactose-indigestion) are caused through the failure to hydrolyze lactose due to a deficiency of the enzyme -D-galactosidase (lactase). Lactose that has not been digested makes its way to the colon, where lactose fermenters dwell and break it down (Buller and Grand, 1990; Adams and Moss, 2000). Fermentation of lactose in colon leads to high levels of glucose in blood and hydrogen gas in breath (Thantsha *et al*., 2012).Lactobacillus spp. bulgaricus and Streptococcus thermophilus which are found in conventional yoghurt cultures and probiotic strains produce -D-galactosidase which improves lactose tolerance (Adams and Moss, 2000). Moreover, Mclean and Rosenstein (2000) mentioned that during the late 80's, The first *Lactobacillus* spp. strain with a probiotic was identified. Using in vitro interference test models, *Lactobacillus* strains that affect UTI pathogens, bacterial vaginosis BV pathogens, and Candida albicans have been described later (Atassi et al., 2006; and Kaewsrichan et al., 2006). The effect probiotic *in vivo* has largely been studied using a few specific strains and the experiments have mostly been made on women with BV(Foschi *et al*., 2017). One study was consisted of 40 women with revealed BV infections, the majority of which had earlier been treated for BV with oral antibiotics for five days, half of the participants were given vaginal capsules containing the probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14, while the other half received metronidazole vaginal gel. The probiotic group had a much superior effect, as measured by a lower Nugents score after 30 days (Rönnqvist, 2007).

The probiotic is more safe and beneficial than antibiotic, the probiotic inhibited the growth of unwanted M.O without risk of developing growth of resistance pathogens or interfere

with human normal flora; Antibiotics are often used, which lead to the growth of multidrug resistant microorganisms (Moh et al., 2021). The consumption of live M.O has a positive effect on human intestinal micro flora, longevity and improve human health, the consumption of lactobacilli for a long period saves human exposure, with very few insignificant public health problems has directed most lactobacillus strains to be classified as «generally recognized as safe»(GRAS). Moreover, the European food Safety Authority (EFSA) have arranged a number of Lactobacillus spp. for Qualified Presumption of Safety(QPS) status based on their safety estimation characteristic (Pradhan et al., 2020).

Methods:

A total 188 isolates collected from various urinary catheterized patients, samples collection process were included both gender; males and females in all ages and different disease cases from hospitalized patients.After lifting the urinary tract catheter from the patients, indwelling catheters were cut about 6cm from the tip of that adjacent the bladder and urinary tract by sterile scissors and transported to laboratory under sterile condition (Kirmusaoglu *et al*., 2017). Then, several media, including blood, mannitol salt and MacConkey agars, were used to culture isolates which are prepared as touched on earlier at the beginning of the chapter, and incubated at 37ºC for 24hrs. in aerobic conditions. The isolates were examined for their morphological shape (size, colour, pigments, and haemolytic activity on blood agar). Mannitol salt agar regarded as a selective to identify staphylococci, and showed the capacity of isolates to ferment mannitol suger, XLD agar also used to detect H_2S production by some genus of bacteria such as *Proteus*. All plates were incubated at 37 ºC for 24hrs.

For detection probiotic affectivity against *S epidermidis* and *S haemolyticus* isolates two methods were used:

Well diffusion method: In this method, all *Lactobacillus* spp. were cultured in MRSB (10^7CFU/ml) at 37°C with 5% CO_2 for 24hrs, and used as the broth-culture-bacteria (BCB). In addition, staphylococci isolates were grown in broth culture and incubated at 37°C for 24hrs.In addition, cell free supernatant (CFS) for *Lactobacillus* spp. was obtained by centrifuging the culture at 10000 rpm/15mnts., then clarified the supernatant through a filter paper of 0.22µm pore size (NOUROUZI *et al*., 2004).

Agar well diffusion method: The well diffusion method was performed on the surface of nutrient agar plate, and streaked with pathogenic bacterial broth culture. Wells with 6mm diameter were prepared by using a cork borer of 6mm diameter. Each well was filled with 75µl of CFS, and marked properly with the isolates' names. After that, all plates were incubated at 37°C for 24 hrs in the presence of CO2. Zone diameter of inhibition (ZDI) values around each wells were recorded in mm, and inferred as less active with ZDIs ≤10 mm, moderately active ZDIs (11–14) mm and highly active when ZDIs ≥15 mm (Tagg and McGiven, 1971; Halder *et al*., 2017)(Figure1).

Figure 1: Impact of *L casie* and *L acidophillus* against *S epidermidis* and *S haemolyticus* isolates

Statistical analyses

All data were subjected to statistical analysis using Statistical-Package-for- Social-Science (SPSS, version 25.0) for windows. Besides Chi Square was used for all results with significant level ($P \leq 0.05$).

Results and Discussion:

The most common source of hospital-acquired infections is indwelling medical equipment, insertion of catheter provided suitable environment for patients flora colonization and biofilm creation. In the current study, samples were displayed positive bacterial growth in 116(61.7%). These results may be resulted from frequency used of urinary tract catheter to even unnecessary cases in some times and provided a suitable environment onto bacteria to formation of biofilm that made it more aggressive and resistance. And may lead to increased incidence of CAUTIs. Jacobsen *et al*. (2008) mentioned that the occurrence of CAUTIs was 21%-50% of patients and agreed with what was found by Mohammad (2012) who showed that bacterial infection accounted for 70.9% of CAUTIs.The most common bacteria isolated in the current study were *S epidermidis* 20(10.63%) and *S haemolyticus* 19(10.20%). Furthermore, Naqid *et al*. (2020) in Duhok province in Iraq got slightly different results with *S epidermidis* (4.3%) and *S haemolyticus* (5.7%) respectively. Few differences may be returned to differences in the geographical region, or differences in the kind of samples.

Effect of probiotic on the *S epidermidis* and *S haemolyticus*

The results in the present study with probiotic bacteria against *S epidermidis* and *S haemolyticus* isolated from urinary tract catheter showed variance of inhibition ability ranging in case of cell free CFS supernatant from 8-12 mm and in case of bacterial culture broth BCB from 10-20mm. The *L casie* showed high significant ($P<0.03$), ($P<0.01$) ability to inhibit growth of *S epidermidis* 60% an *S haemolyticus* 63%, as illustrated in the Table(1)

Table 1: Antimicrobial effect of *L casie* and *L acidophilus* against *S epidermidis* and *S haemolyticus* isolates

Bacteria	***L casie***	***L acidophilus***
	No.(%)	**No.(%)**
S epidermidis	12(60)	11(55)
S haemolyticus	12(63.2)	10(52.6)

***P*<0.05**

Thus, as lactic acid bacteria showed results on the other pathogenic bacteria. Shaaban *et al*. (2020) in Egypt found that *L casie* has ability against 60% of Proteus mirabilis. *L casie* expressed inhibition zone of 9-12mm for staphylococci isolates when used CFS well diffusion method. On other hand, BCB expressed larger inhibition zone than that observed in CFS, which was 13-20mm against staphylococci isolates, as illustrated in Table(2).

Table 2: Antimicrobial effect of *L casie* and *L acidophilus* against *S epidermidis* and *S haemolyticus* isolates according to Zone diameter of inhibition.

BCB						
Bacteria	***L casie***			***L acidophilus***		
	Active No.(%)	Moderate active No.(%)	Less active No.(%)	Active No.(%)	Moderate active No.(%)	Less active No.(%)
S epidermidis	9(75)	3(25)	0(0)	6(54.55)	4(36.36)	1(9.09)
S haemolyticus	8(66.67)	4(33.33)	0(0)	4(40)	5(50)	1(10)
CFS						
Bacteria	***L casie***			***L acidophilus***		
	Active No.(%)	Moderate active No.(%)	Less active No.(%)	Active No.(%)	Moderate active No.(%)	Less active No.(%)
S epidermidis	0(0)	5(41.67)	7(58.33)	0(0)	3(27.27)	8(72.73)
S haemolyticus	0(0)	5(41.67)	7(58.33)	0(0)	4(40)	6(60)

***P*<0.05**

The high inhibition zone by BCB may go beyond to large antimicrobial substance in the well besides in addition to bacterial cell itself that plays a significant role in the inhibition of pathogenic bacteria. Other local studies on the antagonist effect of *L acidophilus* on pathogenic bacteria have reached decisions that are somewhat compatible with the current study, such as Al-Asady *et al*. (2020) who found that *L acidophilus* had ability to inhibit *S epidermidis* in (73.3%). Also, Al-Azzawi *et al*. (2020) mentioned that the ability of this bacterium to inhibit *P aeruginosa* was 61.1%.

Conclusion:

Urinary tract catheters are an auxiliary source for biofilm formation and transmission of infection.Opportunistic bacteria *S epidermidis* and *S haemolyticus* found as most common isolated bacteria followed by pathogenic bacteria *E coli* and *Klebsiella* spp. and other types of bacteria in small proportions. *Lactobacillus* spp., such as *L casie* and *L acidophilus* expressed significant activity against *S epidermidis* and *S haemolyticus*. Moreover, *L casie* showed high effectiveness compared to *L acidophilus*. The efficiency of probiotics to inhibit bacteria is close to that of antibiotics. However, it is not dangerous in terms of side effects.

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