The Use of Cranberry Juice Cocktail as an Anti-adherence Agent for the Control of Chronic Urinary Tract Infection in Catheterized Geriatric Patients

by

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The Use of Cranberry Juice Cocktail as an Anti-adherence Agent for the Control of Chronic Urinary Tract Infection in Catheterized Geriatric Patients

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Abstract:

In vitro and *in vivo* assays have demonstrated that cranberry juice and cranberry juice cocktail, a derivative beverage, are powerful inhibitors of bacterial adherence to uroepithelia cells, indicating the possible use of the juice for the treatment or prevention of urinary tract infection. This study looks at the capacity of cranberry juice cocktail (CJC) to induce anti-adherence activity in the urine of a compromised population. Factors which increase the risk of infection of the urinary tract include sex, in which females are at higher risk, old age, residence in extended care facilities, indwelling urethral catheterization, and decreased functional capacity. Eight elderly female subjects, all catheterized and nursing home residents at various levels of decreased functional capacity were given 4 ounces of CJC daily for a period of twelve weeks. Urine specimens were collected on three days in the week prior to the first ingestion of CJC and then 24 hours, 48 hours, and 72 hours after

the beginning of cranberry juice administration, and then weekly for the next twelve weeks of daily CJC consumption. The urine specimens were used as the reaction medium for adherence assays of bacteria isolated from the individual patients to healthy donor uroepithelia cells. Mean bacterial adherence in the presence of the base-line control urine was compared to the mean bacterial adherence in the presence of urine collected throughout the remainder of the study.

Significantly more anti-adherent urine after CJC consumption was demonstrated in four of the eight patients in comparison to the base-line controls. Average reductions of bacterial adherence of 62%, 46%, 42%, and 28% were observed in the presence of urine collected during the twelve weeks of CJC ingestion for these four patients. Two more of the subjects exhibited average increases in the anti-adherence activity of their urine during the period of CJC ingestion, but not to significant levels.

A common pattern was observed in the induction of significant anti-adherence activity for three of the four patients with significantly anti-adherent urine in which the activity was not obvious until four weeks after the initiation of CJC consumption. The effect observed beginning on the fourth week was very strong and remained at the same approximate level for the remainder of

the study.

The results of this study provide evidence that cranberry juice or derivatives of the juice could be very useful for the reduction of UTI in this high risk population.

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I. Introduction:

Urinary tract infection:

Urinary tract infection (UTI) is defined quantitatively as the presence of bacteriuria, which is 10^5 bacteria or greater per ml of a fresh-void midstream urine specimen and pyuria. Some authors consider 10^3/ml bacteria as clinically significant if the specimen is carefully collected.[28] UTIs are divided into infections of the upper urinary tract, which are those urinary structures superior to the bladder including the ureters and kidneys resulting in pyelonephritis (kidney inflammation), and infections of the lower urinary tract in which the urethra and bladder are infected causing urethritis and cystitis. Infection of the upper urinary tract usually occurs as a progression from infection of the lower urinary tract in an ascending fashion.

A UTI can be symptomatic or asymptomatic. Symptomatic infection of the lower urinary tract may include dysuria, urgency, and frequency of urination, and for infections of the upper urinary tract the symptoms may also include fever, chills, flank pain, and tenderness.[3] Urinary tract infection by urea splitting bacteria such as *Proteus* and *Klebsiella* can cause the formation of infectious stones and lead to severe

renal damage.[50] These organisms can also cause the blockage of urinary catheters in catheterized individuals through the build up of urinary crystals leading to very dangerous bacteremia and septicemia.[21] It is generally agreed that symptomatic UTI should be treated promptly and appropriately with antimicrobial medication.

Asymptomatic bacteriuria (ABU) is common in the elderly in which large numbers of bacteria multiply in the urine without the production of symptoms. Treatment of ABU, especially in the elderly, is of questionable value. While some investigators have related ABU to decreased renal function,[11] others have found no correlation.[1] Urinary tract infection, both symptomatic and asymptomatic, has been associated with increased mortality in the elderly,[60,43] though it is unclear if the bacteriuria is the cause of the increased mortality or if there are underlying causes making these individuals more susceptible to UTI and increasing mortality. Other investigators have found no correlation between bacteriuria and mortality.[37,38] In an attempt to clarify the validity of treating ABU in the elderly, Nicolle et al[37] did a study of 50 institutionalized bacteriuric women who were assigned randomly to antibiotic therapy or no therapy over a one year period. There was no

significant difference in morbidity or mortality between the two groups. The group receiving therapy actually had a trend toward decreased survival, and reinfection with increasingly antimicrobial-resistant organisms was common in comparison with the untreated group. The elderly population is also more susceptible to the damaging effects of antimicrobials due to impairments of metabolism and secretion of these substances leading to serum levels high enough to cause further renal damage, and increased risk of adverse interactions between the antimicrobials and other medications.[50] The consensus on the issue of treating asymptomatic bacteriuria is that the risks are greater than the benefits and therefore it is reasonable to treat only symptomatic infection.

There are several factors that predispose individuals to urinary tract infection. Micturition with complete bladder emptying is considered the most important defense mechanism[54] and conditions that impair urinary flow are the most prevalent predisposing factors, including: hyperplasia, catheterization, instrumentation, and neurogenic disorders.[66] Sexual activity[36] and menopause[46] are also thought to be associated with increased risk of infection. However, in most cases infection occurs as

a primary condition in otherwise healthy people.[66] For unknown reasons UTIs are often recurrent in individuals. There are two theories concerning recurrent infection,[66] The *host-susceptibility theory* states that UTIs occur more frequently in some because of special predisposing factors, and the *random-colonization theory* states that changes occur in host resistance following the first incidence of infection. Most of the literature relates increased risk of UTI to predisposing determinants. Other factors that increase the risk of UTI include sex and age, with young girls and old women being the highest risk groups not otherwise predisposed. Also, concurrent disease, living arrangements, nosocomial transmission, and urethral catheterization substantially increase the risk of infection.

Increased risk of UTI in women:

UTI is observed much more frequently in women than men. In the younger population the ratio of bacteriuric women to bacteriuric men is about 30:1, and in the elderly this ratio decreases to around 2 or 3:1,[6] but is still indicative of a substantially increased risk of UTI in women. The factors predisposing women to UTI more than men are thought to

be due primarily to differences in the route of infection. The urethra in women is short and close to the bacteriologically colonized vagina and perineum. In the pathogenesis of infection in women bacteria colonize the periurethral area and then the urethra before gaining access to the bladder and upper urinary tract.[63,66] The risk in women is thought to be increased by sexual activity[36] and menopause. Raz and Stamm[46] showed that there was a significant decrease in the incidence of UTI in postmenopausal women who received intravaginal estrogen treatment compared to women who received placebo. The reason for this activity is unknown. Stamey and Sexton[68] have shown that some women appear to be more susceptible to UTI than others as indicated by significantly more receptors for pathogenic bacteria than healthy controls, and significantly more vaginal carriage of Enterobacteriaceae.

Prevalence of UTI in the Elderly:

The epidemiology of UTI in the elderly is much different than that of the rest of the population both pathologically and statistically. The most common cause of bacteriuria in the elderly is acute pyelonephritis,[14] whereas symptoms of the lower urinary tract are most often seen in the

younger population.[3,50] Bacterial infections of the urinary tract are second only to respiratory infections in the elderly.[75] The prevalence of UTI in the elderly is greatly increased when compared to the young to middle-aged in which only 5% of women and less than 0.1% of men are bacteriuric.[25] In single survey studies it has been found repeatedly that at least 20% of women and 10% of men over the age of 65 years are bacteriuric.[3,5,9,24,57,60] When these studies are extended longitudinally, the percentage of elderly people reporting bacteriuria in at least one of several surveys done in a series over periods from one to five years increases substantially. In three surveys at six month intervals by Boscia et al[7] of 184 women and 76 men over the age of 68, the percentage of individuals infected in at least one of the three surveys was 30% of women and 11% of men. Upon doing an extension of this longitudinal study to include 865 women over 68 covering a five year period in which surveys were taken every six months, the cumulative percentage of women bacteriuric in at least one of the surveys was 33%.[3] Interestingly, only 4.9% of these women were bacteriuric at each survey, showing a lower than expected prevalence of recurrence in the same individuals.[3] In a two-survey study covering a five-year interval of 101 women and 87

men over 65 years old, another group found that 38% of the women and 15% of the men were bacteriuric in at least one of the two surveys.[60] Another study including two surveys separated by one year of 231 women and 121 men over age 70 found that 44% of the women and 28% of the men were bacteriuric in at least one of the two surveys.[24] The prevalence of UTI in the elderly increases with institutionalization or hospitalization,[1,9,61] and concurrent disease.[50] Studies have also found increases in the incidence of infection with age:[9] 20% of the women and 3% to 5% of the men surveyed between 65 and 70 years old were bacteriuric, whereas people over 80 were found to have an increased incidence in women to between 23% and 50% and 20% of men. Other studies have found no change in incidence over age 70 in the same living arrangements.[3,7] UTI is more difficult to diagnose in the elderly because pulmonary and gastrointestinal signs dominate the clinical picture[3] plus symptoms such as lethargy, confusion, anorexia, and incontinence cause missed or delayed diagnosis.[50] A study by Gleckman et al[18] indicated that diagnosis was initially missed in 21% of the cases of UTI surveyed in the elderly.

There are many factors involved in the increased incidence of UTI

in the elderly. Powers et al[44] attempted to identify antecedent factors for the development of UTI in the elderly including: prior cerebrovascular accident, decreased functional status, decreased mental status, bladder catheterization, and prior antibiotic use. The most prominent factor is thought to be incomplete emptying of the bladder upon micturition in the elderly due to prostatic disease in men, bladder prolapse in women, and neurogenic bladder in either sex.[3,50,54,60] Pathogenesis is also increased in elderly women due to fecal and urinary incontinence causing an increase in the size of inoculum entering the periurethral area.[3,50] Some studies have shown an increased susceptibility of the uroepithelia of the elderly to adherence by pathogenic bacteria,[47] while a later study demonstrated increased adherence to the uroepithelia of elderly men versus young men only, not for women.[56] Urine does have some antimicrobial properties[62] which would be diminished in the elderly due to declined renal function, including: low pH, osmolality extremes, and high urea content. Orskov et al[42] showed that Tamm-Horsfall protein (THP), the most abundant glycoprotein in urine, binds and coats the type 1 pili of gram-negative bacteria, possibly acting as a host defense mechanism to block the binding of bacteria to the uroepithelia cells.

This protein has been shown to be decreased in the elderly.[55] The risk factors of increased institutionalization and hospitalization and a greater need for indwelling urethral catheterization are also extremely important in the elderly.

Increased prevalence of UTI in extended care facilities:

Substantial increases in the prevalence of UTI in the elderly are seen when they live in care facilities such as nursing homes and hospitals. Single survey studies indicate that at any one time 25% of elderly women and 20% of elderly men in extended care facilities are bacteriuric as compared to around 20% of women and 10% of men in the overall elderly population.[1,9,61] In one of the previously mentioned studies[3] 33% of the population surveyed was bacteriuric in at least one six-month survey over a five year period, this cumulative incidence increased to 57% when only the portion of these women living in nursing homes was considered. Bacteriuria is thought to be the most common nosocomial infection in the United States. [3,31] The higher rate of UTI in this population is attributable to the greater level of debilitation of these people with more perineal soiling, incomplete bladder emptying,[3] more

frequent catheterization,[20] plus increased exposure to environmental and therapeutic risk factors such as antimicrobial resistant bacterial strains.

Catheterization and UTIs:

Another factor that greatly increases the risk of UTI in the elderly is indwelling urethral catheterization which is the most frequent cause of nosocomial infection.[32] In a study by Warren et al[71] it was found that once a catheter is in place the risk of UTI increases to 5% to 10% per day causing virtually all patients with catheters in place for weeks and months to become bacteriuric. Nine percent of nursing home patients are catheterized,[20] playing a substantial role in this population's increased risk of UTI. Catheter-associated infection and sepsis is very difficult to treat and prevent,[64,51] and results in measurable morbidity and mortality.[43]

Nickel[32] describes the insertion and leaving of a Folly Catheter *in situ* as a bridge between the outside world and the sterile bladder, along which bacteria can travel by one of three mechanisms: Bacteria can be pushed into the bladder during instrumentation, but this should account for only about 2% of infections with good sterile technique. Retrograde

intraluminal spread of the bacteria can occur, beginning at the drainage spigot of the collecting bag, then into the bag itself and along the internal surface of the drainage system and the catheter. Bacteria can also colonize the inner surface of the catheter via a disconnected catheter drainage tube junction. The retrograde intraluminal mechanism of entry is thought to be the most common route of bacterial entry to the catheterized urinary tract.[33,34] Bacteria form a thick, coherent biofilm composed of an extensive anionic matrix of exopolysacharides produced by the bacteria that extends throughout the drainage system and into the catheter.[32] Migration of the bacteria from the urethral meatus along the outside of the catheter has also been shown to be a major but slower pathway for the entry of bacteria.[34] Upon reaching the tip of the Foley catheter the bacteria must then adhere to the bladder wall before a symptomatic UTI can occur.[33] Along with bacterial entry, this adherence is also enhanced by the catheter in that it can disrupt the bladder's natural defenses against bacterial adherence by not completely draining and flushing the bladder as micturition does, allowing a small amount of urine to always remain in the bladder in which bacteria can multiply.[32] A serious danger in catheter-associated infection occurs with urease

producing bacteria such as *Proteus mirabilis* and *Klebsiella* spp. These bacteria split urea to form struvite crystals that can complex with other urine components such as Tamm-Horsfall glycoprotein and obstruct the catheter. Obstruction of the catheter can lead to very dangerous bacteremia and septicemia.[21] Bacteria within the biofilm coating a catheter system are conferred with a degree of antibiotic resistance most likely due to poor penetration of the antibiotic into the biofilm.[35] Antibiotic therapy has been shown to inhibit bacterial adherence to the bladder wall in an experimental model,[41] but in any form of long-term Foley catheterization antibiotics would be counterproductive as the bacteriuria would eventually occur anyway, and the organisms would most likely have developed multiple resistence to further antimicrobial therapy.[32]

Microbiology of Urinary Tract Infection:

Escherichia coli is the most common cause of urinary tract infection causing up to 90% of infections in younger adults and around 75% of infections in the elderly.[8,70] Other organisms are also found frequently infecting the elderly such as *Proteus, Klebsiella, Enterobacteria, Serratia,*

Pseudomonas, and enterococci.[70] The greater prevalence of non-*E. coli* strains in the elderly is thought to result from the greater amount of hospitalization and institutionalization of this population leading to greater exposure.[68] Also, increased obstruction, increased catheterization, and increased antimicrobial use[3] in the elderly increase the incidence of infection with non-*E.coli* strains. Infection with multiple organisms is also found much more often in the elderly. Nicolle et al[37] reported that multiple organisms were isolated in 14% to 30% of UTIs in the elderly population that they surveyed.

The adherence of bacteria to the uroepithelium is the first and most important factor in urinary tract infection. The adherence of *E. coli* to the uroepithelium allows them to overcome clearing mechanisms of the urinary tract and nutrient deprivation resulting in growth advantages and increased toxicity.[74] Ofec et al.[40] suggested that type 1 fimbriae act as adhesins in the initiation of UTI. Type 1 fimbriae are filamentous appendages found on many strains of *E. coli* and other gram negative bacteria such as *Klebsiella* and *Pseudomonas.* Bacteria isolated from infections of the lower urinary tract are more likely to express type 1 pili than other virulence factors such as type P pili.[19] Type 1 pili are

identified because they cause hemagglutination of guinea pig erythrocytes. This activity is blocked by the addition of D-mannose, and is referred to as mannose-sensitive hemagglutination (MSHA). Type 1 pili are expressed in *E. coli* in a phase variant manner in which pili expression can be turned on or off. Hultgren et al.[22] found that after the introduction of type 1 piliated *E. coli* into the bladders of mice, nonpiliated variants were expelled in the urine and piliated variants were associated with the bladder uroepithelium for as long as 14 days after inoculation. They also showed that treatment with antiserum specific to type 1 pili prior to inoculation significantly reduced or completely blocked bladder colonization. In a human study of 41 bacteriuric adults it was found that 76% (31 of 41) of the urine specimens obtained contained bacteria expressing type 1 pili, and 78% (27 of 31) of the type 1 piliated bacteria were associated with exfoliated epithelia cells.[26] Pretreatment of type 1 fimbriated *E. coli* with 2.5% D-mannose has been shown to completely block bacterial adherence to human uroepithelia cells, while pretreatment of the epithelia cells with mannose had no effect on bacterial adherence.[48] This suggests that mannose residues occur in the receptors for type 1 fimbriae. Type 1 pili have also been

shown to contribute to the ability of *Klebsiella pneumoniae* to infect the urinary tract.[30] Mobley et al.[31] suggested that the persistence of *E. coli* in the catheterized urinary tract may be dependent on the expression of type 1 pili and not type P pili or other virulence factors. Expression of type P fimbriae has also been associated with the infectivity of *E. coli* in the urinary tract. *E. coli* carrying the *pap* DNA sequences express type P fimbriae. It has been found that P fimbriated *E. coli* bind to GalαGalβ disaccharide moieties contained on P blood group antigens.[40] Strains of *E. coli* expressing type P fimbriae have been isolated from patients with upper UTIs[23] and it has been shown that people expressing the P_1 blood group have a risk of recurrent pyelonephritis 11 times greater than the population at large.[29] P fimbriation was also found to be more prevalent in cystitis patients (53%) than in healthy controls (35%).[65] Strains of *E. coli* expressing both type 1 and type P fimbriae have also been isolated from bacteriuric patients.[26] *Klebsiella* species have also been shown to express type 1 fimbriae[30] and type III fimbriae or mannose resistant /Klebsiella-like hemagglutinins (MR/K-HA) which have been correlated with the persistence of *Providencia stuartii* infection in catheter-associated UTI.[32] *Proteus mirabalis* also expresses type III (MR/K-HA) fimbriae as

well as mannose resistant /Proteus-like hemagglutinins (MR/P-HA) or type IV fimbriae[15] which have been associated to acute pyelonephritis.[15,53]

Host Defense Against Urinary Tract Infection:

Although urine is considered a good culture medium for bacteria it does have some antibacterial properties including: low pH, osmolality extremes, high urea content, high organic acid concentrations, and bactericidal prostatic secretions.[62] Urinary tract infection, especially pyelonephritis is accompanied by both local and systemic immune responses. Glauser et al.[17] found that antiinflammatory agents render animals highly susceptible to UTI. The inflammatory response to UTI includes the secretion of cytokines and the release of inflammatory cells into the urine.[10] Specific immunity has been considered a major host defense mechanism against UTI, but its role is questionable because patients with known cellular or humoral immunodeficiencies rarely have recurrent urinary tract infections.[66] Reduced secretory IgA has been reported in people susceptible to UTI, and the urine of people infected and producing increased secretory IgA was shown to block bacterial

adherence to uroepithelia to a greater extent than the urine of uninfected control subjects.[67] Studies have also shown that local immunization into the bladder with bacteria decreased adherence from 4.5% of the infecting inoculum adhering in control animals to only 0.5% adherence in immunized animals.[69] The most important indigenous defense against UTI is micturition with complete emptying of the bladder which flushes pathogens from the urinary tract. It is believed that bacterial clearance by micturition is enhanced by Tamm-Horsfall protein (THP), the most prominent urinary glycoprotein, which is thought to entrap bacteria for clearance from the urinary tract. Orskov et al.[42] demonstrated that type-1 fimbriated *E. coli* are trapped by THP preventing there adherence to urinary tract cells. Electron microscopic studies indicated that THP forms a pseudocapsid around type-1 fimbriated bacteria, but not around bacteria without type-1 fimbriae.[27] Other studies indicated that type-1 fimbrial mediated bacterial adherence could be inhibited by THP in a dose dependent manner[12] and THP concentrations greater than 30μg/ml inhibited bacterial adherence and less than this amount enhanced adherence.[13] Sobota and Apicella[58] demonstrated that the removal of THP from the urine induced a substantial increase in adherence

compared with urine containing the THP when the urine was used as the reaction media for bacterial adherence assays to human uroepithelia cells.

Effect of Cranberry Juice Cocktail on Bacterial Adherence to Uroepithelia:

Cranberry juice has been attributed with the ability to decrease or prevent urinary tract infections for many years. It was originally thought that cranberry beverages acted by decreasing the pH and increasing the concentration of organic acids such as hippuric acid in the urine to bacteriostatic levels,[17] but this view was later found to be unsubstantiated by a number of investigators.[59,49] After many years of speculation concerning the value of cranberry juice in the prevention and treatment of urinary tract infection Sobota[59] demonstrated that urine collected after the consumption of cranberry juice could block the adherence of *E. coli* to uroepithelial cells when this urine was used as the medium for a bacterial adherence assay. The urine from 15 of 22 subjects who had previously drank 15 ounces of CJC exhibited a significant bacterial-adherence blocking effect when compared to control urine collected prior

to CJC consumption. A similar effect was also demonstrated in mice whose water supply was replaced by CJC for 14 days when compared to a control group of mice.[59] *In vitro* studies by Sobota demonstrated that fresh squeezed cranberry juice as well as the cocktail blocked the adherence of *E. coli* to epithelia cells by up to 97% when used directly, and still exhibited about 30% blockage even at dilutions of 1:100.[59] Zafriri et al.[73] later demonstrated that CJC could inhibit the adherence of *E. coli* to yeast, tissue culture cells, erythrocytes, and mouse peritoneal macrophages. This group also demonstrated that the CJC was an effective blocker of both type 1 and type P fimbriated strains of *E. coli*. Sobota[59] had demonstrated previously that CJC caused a 75% or greater decrease in the adherence of 60% of 77 uropathogenic strains of *E coli* tested.

Clinical testing of Cranberry Juice Cocktail:

The first clinical trial of CJC use for the reduction of UTI was in 1968 by Prodomas et al..[45] Sixty patients with UTIs including 44 women and 16 men were given 16 ounces of CJC per day for 21 days and the clinical progression of their infections were analyzed. Thirty-two of these

patients exhibited a positive clinical response, 12 showed moderate improvement, and the remaining 16 showed no improvement. Within six weeks from the end of the treatment with CJC, 61% (27 of 44) of those whom had shown improvement suffered a recurrent infection. This incidence of recurrence was thought to be indicative of a blocking effect by the CJC on the ability of bacteria to cause infection. This study served as an early indicator of the possible value of CJC for use in the reduction of UTI.

Avorn et al.[2] recently performed a carefully designed and well controlled study of 153 elderly women. The subjects were randomly assigned to consume 300ml/day of either CJC or a placebo which looked and tasted identical to CJC for a period of six months. Measures were taken to avoid the intake of any other cranberry product by the subjects. Monthly samples of clean-void urine were collected, upon which standard urinanalysis, bacterial culture, and antibiotic sensitivity testing was performed. Bacteriuria (10^5 bacteria/ml) was found in 28.1% of the urine samples collected from the placebo group, but in only 15% of the specimens collected from the group ingesting CJC. The mean proportion of all of the urine specimens from each individual that was bacteriuric-

pyuric in the placebo group was 26.1% and that of the group receiving CJC was only 16.5%. Also, they found that the rate of change of urine from being bacteriuric-pyuric to an uninfected state on a month to month basis in the placebo group was only 0.28 and 0.54 in the group receiving CJC. This indicates that the bacteriuric individuals receiving CJC were only one quarter as likely to have an infection the next month in comparison to the placebo group. Another indicator of the effectiveness of CJC was that during the study women in the placebo group were treated with antimicrobials 16 times for UTIs, and in the group receiving CJC only 8 treatments were reported.

In a study performed in this laboratory by Wollet[72] the anti-adherence activity of urine was examined following CJC consumption by 3 groups of individuals. The test groups included a control group of 25 individuals with no reported infection, 19 patients who were classified as chronic UTI sufferers, and 6 patients who were classified as chronic sufferers but who had been placed on 4 ounces of CJC per day for two years prior to the initiation of the study. A sample of urine from each of these subjects, with or without CJC added *in vitro*, was tested for its ability to block bacterial adherence to uroepithelia cells. The mean

adherence of bacteria to uroepithelia cells incubated in the urine of the 6 patients on CJC was 6.44 ± 1.81 bacteria per cell, that of the 19 chronic sufferers not on CJC was 12.69 ± 1 .59 bacteria per cell, and for the control group it was 21.22 ± 4.24 bacteria per cell. After addition of CJC, increased anti-adherence activity was observed in the urine from the control and untreated chronic group but not in the urine of the group previously treated with CJC, suggesting previous saturation of the specimens with the anti-adherent element of the CJC in this group.

The Current Study:

In the present study we examined the effects of 4 ounces of CJC per day over a twelve week period on the urine of severely compromised, catheterized women ranging in age from 76 to 92 years. The subjects were all residents of the Beeghly Oaks nursing home in Youngstown, Ohio. All but one of these patients had been treated for from 1 to 6 symptomatic UTIs in the previous year. We wanted to determine the effectiveness of CJC in increasing the anti-adherent activity in the urine of this very high risk group, and how effective the induced protection might be against the various bacteria isolated from these patients.

II. Materials and Methods:

Patient Selection:

All of the subjects used in this study were incontinent catheterized women, ranging in age from 76 to 92 years who were patients of the Beeghly Oaks nursing home. Each patient had a history of urinary tract infection defined as averaging three or more UTIs per year, and most had recurrent infections in the year prior to the study: Patient 1 had four infections, patient 2 had no infections in the year just prior to the study but had suffered from chronic UTI for several years previous to the study, patient 3 had four infections in the previous year, patient 4 had one infection in the previous year and a history of chronic UTI, patient five had 5 infections in the previous year and entered the study with an infection, patient 6 had one infection in the previous year plus a history of chronic infection, patient 7 had three infections in the previous year and entered the study bacteriuric, and patient 8 had seven infections in the previous year and entered the study with an active infection. All patients chosen gave written consent for their involvement in this study.

Cranberry Juice Administration and Specimen Collection:

The study covered a 13 week period from February 8, 1992 until May 10, 1992, including one week before the administration of cranberry juice cocktail (CJC) and 12 weeks in which the patients were given 4 ounces of CJC each morning. Cranberry juice cocktail (*Ocean Spray Corporation*) which contains about 33% cranberry juice plus sweeteners such as high fructose corn syrup and added vitamin C was used because it is the most palatable and commonly consumed form of cranberry juice. In the week prior to administration of the CJC, specimens of urine were collected from each patient on three days, Monday, Wednesday, and Friday. The adherence data from these three specimens was combined account for slight day to day variances in anti-adherence activity. Urine was collected by the nursing staff of Beeghly Oaks and refrigerated immediately. Administration of CJC began on the second Monday of the study. Specimens were collected 24 hours (Tuesday), 48 hours (Wednesday), and 72 hours (Thursday) after the beginning of cranberry juice administration, and then on every Monday for the remaining eleven weeks of the study. On the days of collection the specimens were transported to the laboratory and centrifuged for 10 minutes at 4,500*g* to

remove the patients cells and the cleared urine was stored at -20°C.

Bacteria Isolation:

On the day of collection aliquots of the specimens were plated on MacConkey's agar. Isolated colonies from the first week of the study (before CJC) were replated on nutrient agar and genus and species identified using API 20E strips (*Fisher Scientific,* Pittsburgh, Pa / US). After isolation and identification 15% glycerol cultures were prepared and stored at -70°C to be used for the patient's adherence studies. An adherence study was done on each isolate of patient number four from which two isolates were obtained. The bacterial isolates from each patient included the following: Patient 1, *Proteus mirabilis*; Patient 2, *Pseudomonas aeruginosa*; Patient 3, *Escherichia coli*; Patient 4, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*; Patient 5, *Pseudomonas aeruginosa*; Patient 6, *Klebsiella pneumoniae*; Patient 7, *Pseudomonas aeruginosa*; and Patient 8, *Escherichia coli.*

Adherence Assay:

The uroepithelia cells from the individual patients could not be

used in this study as it was found in most cases that the cells were very fragile and fragmented during the isolation procedure. Uroepithelia cells from a healthy female donor were collected and used in the study. Investigators have reported day-to-day variability in the receptivity of uroepithelia cells to bacterial adherence caused by various factors such as menstruation.[18] To reduce the effects of this variability the uroepithelia cells used to assay all of the specimens of a particular patient were isolated from one specimen of donor urine collected over several hours. For each experiment ten milliliters of donor urine was centrifuged at 4,500g for 10 minutes to pellet the exfoliated uroepithelia cells and the supernatant was discarded. To avoid variations in bacterial adherence tendencies due to factors such as phase variation of fimbrial expression, the bacteria used to assay an individual patient's specimens were obtained from one 48-hour static culture. Bacteria was collected from 2-ml ($\approx 10^9$ bacteria) of the static culture for each specimen assayed. To avoid damaging the donor uroepithelia cells from to much agitation, 2 ml of patient urine was first added to the bacteria pellet and the bacteria resuspended with a vortex mixer. This bacteria suspension was then poured over the pellet of donor cells and resuspended by

vortexing for approximately ten seconds. Negative controls were produced for each patient specimen by suspending a uroepithelia pellet in two milliliters of specimen urine without the addition of bacteria. All specimens were then incubated for 30 minutes in a 37°C water bath. Following incubation the specimens were briefly agitated with a vortex mixer to resuspend the cells and poured into disposable beakers. The samples were then extracted using a 2ml disposable syringe. A filter holder containing an 8μm nucleopore filter was fitted to the syringe and the sample pushed gently through the filter. The sample was then washed 2x with 50 ml of deionized water to remove unattached bacteria. The filters were then removed from the filter holders and placed cell side down onto microscope slides to dry. After the filters had dried they were pealed off the slide leaving the cells adhered to the glass surface. The slides were then gram stained and scored.

Scoring:

Using a light microscope, fifty cells were scored on each slide for the number of gram-negative rods adhering to each cell. A mean adherence was determined for each specimen and for the negative

control of each specimen. The negative controls were used to ensure that the donor cells and the patient specimens were not previously contaminated with bacteria. Base-line control data for each set of patient specimens were produced from the data prior to the initiation of CJC therapy. The data was compared by one-way ANOVA to determine significant changes in bacterial adherence.

III. Results:

Decreases in Bacterial Adherence:

Urine from four of the eight patients studied was significantly more anti-adherent after several weeks of daily cranberry juice cocktail ingestion in comparison to the base-line controls, and two additional patient urines showed an effect on adherence though statistically insignificant. For ease of discussion the eight patients were arbitrarily numbered 1 through 8. The two assays of patient 4 urine were denoted as 4K for the assay with the *Klebsiella pneumoniae* isolate and 4P for the assay with the *Pseudomonas aeruginosa* isolate. A significant reduction in bacterial adherence was observed in adherence assays of the urine from patient 3 (see Figure 3 and Table 4) which showed a 46% decrease from the base-line control in average bacterial adherence over the twelve weeks of CJC ingestion (Table 1), patient 4P (see Figure 5 and Table 6) exhibited a 28% average decrease in adherence (Table 1), a 62% decrease in average adherence was observed for patient 5 (see Figure 7 and Table 7), and patient 8 (see Figure 10 and Table 10) which had a 42% decrease. Also, in the assays of patients 4K (see Figure 4 and Table 5) and 6 (see Figure 8 and Table 8) though not significant

statistically, a decrease in adherence observed for most of the urine specimens collected after CJC ingestion began as compared to the base-line controls. A plot of the adherence for these two patients showed that by the end of the study a trend of decreased bacterial adherence was approaching significant levels. The assays of urine from Patients 1, 2, and 7 exhibited either no significant change or slight increases in bacterial adherence (see Figures 1, 2, and 9, and Tables 2, 3, and 9).

Adherence Tendencies Over Time:

For three of the four patients exhibiting significantly more anti-adherent urine at the end of the study, a definite pattern was observed in the induction of the anti-adherence activity. Although an increase in the anti-adherence capacity of the urine was evident for these three patients soon after initiation of CJC ingestion the effect was variable and did not become significant until the fourth week of juice ingestion and beyond. The urine of these patients after four weeks exhibited a profound increase in its ability to block bacterial adherence (Figures 3, 5, and 8). The average bacterial adherence observed in the adherence assays before week four of CJC consumption for these three patients was

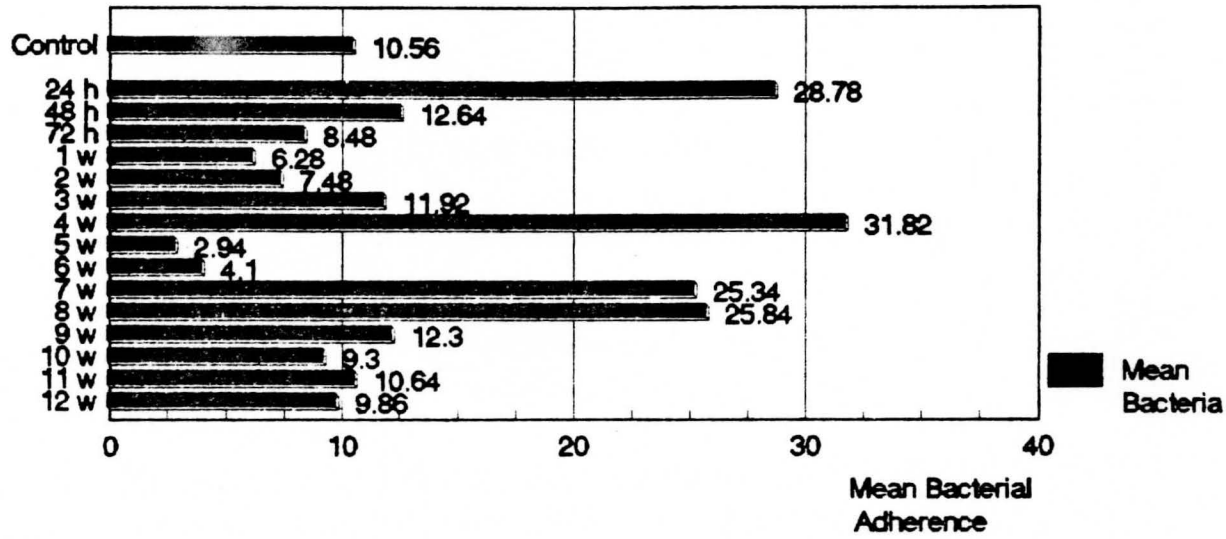
10.87, 13.06, and 12.33 bacteria/cell (base-line controls were 13.11, 20.69, and 12.66 bacteria/cell, respectively). The average bacterial adherence observed for urine collected after four weeks was 4.24, 4.54, and 3.95 bacteria/cell respectively. These values represent decreases in adherence of bacteria incubated with post four week specimens compared to the first three weeks of CJC ingestion of 61%, 65%, and 68%, and decreases of 68%, 78%, and 69% when compared to the base-line controls. The increase in anti-adherent activity was not a gradual progression but appeared to increase dramatically at week four and then continue at about the same level for the remainder of the study. In the fourth patient exhibiting significantly more anti-adherence activity, patient 4P, the increased activity was first observed after 72 hours (Figure 5). Although 10 of the 12 urine samples taken from this point on exhibited significantly more anti-adherence than the base-line control, the drop in bacterial adherence in the assays of these samples was not nearly as marked as in the urine of the three patients for whom the effect was delayed until week four. The average drop in bacterial adherence was 7.29 bacteria/cell after ingestion of the juice from patient 4 with the *P. aeruginosa* isolate.

Adherence Tendencies of the Different Strains of Bacteria:

Both isolates of *Escherichia coli* and two of the four *Pseudomonas aeruginosa* isolates were blocked significantly from adhering to uroepithelia by post-CJC urine. *Klebsiella pneumoniae* isolated from both patients 4K and 6 exhibited decreased adherence in the presence of post-CJC urine from these patients, but the decreases were not significant. Figure 6 compares the trends of adherence of the two bacterial isolates from patient 4, *K. pneumoniae* and *P. aeruginosa* that were both assayed using patient 4 urine. The trends for these two isolates are similar but with a greater reduction in adherence against the *Pseudomonas* strain that was blocked to significant levels versus the *Klebsiella* isolate that was not.

Figure 1: Bacterial adherence data for Patient 1. Bacterial isolate: *Proteus mirabilis.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

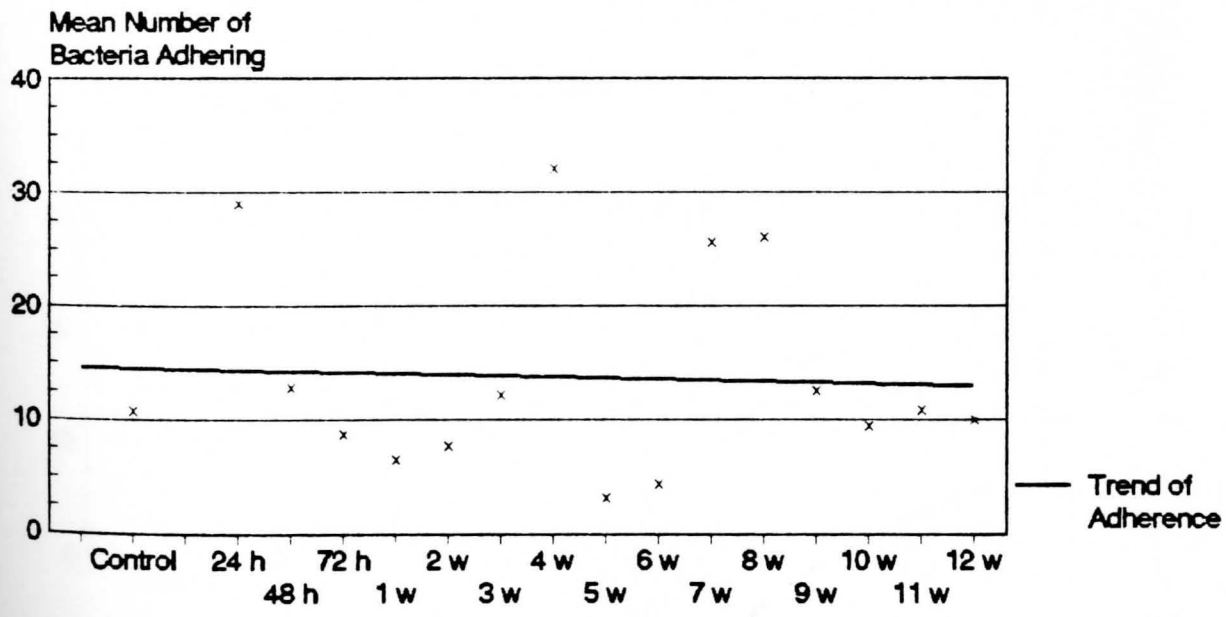
A) Patient 1: Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

PATNT-1

B) Patient 1: Trend of Bacterial Adherence to Uroepithelia

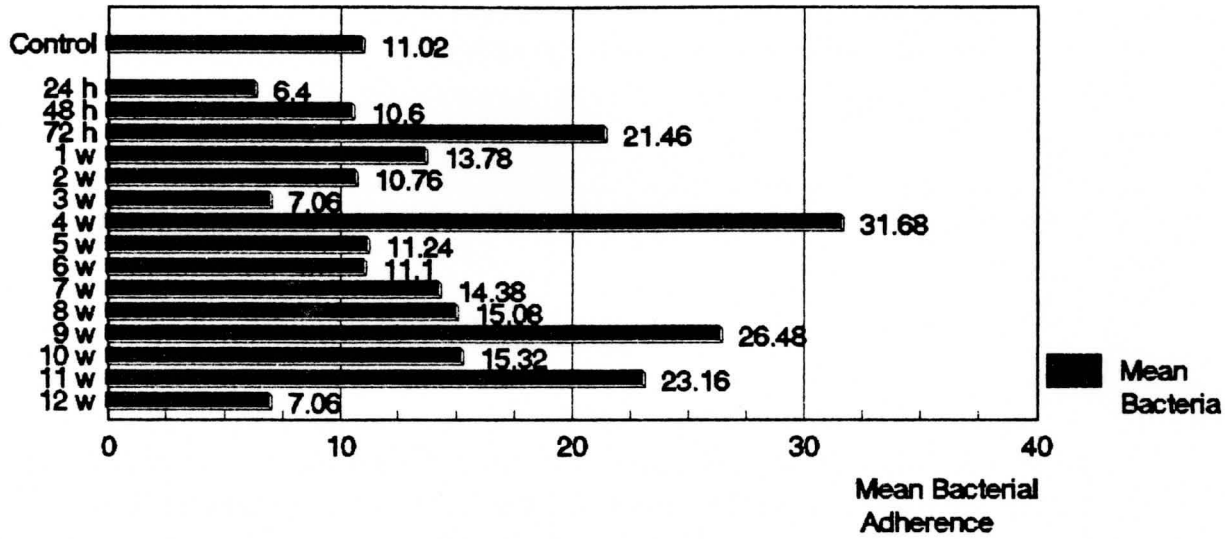


KeyChart 2000

1-TREND

Figure 2: Bacterial adherence data for Patient 2. Bacterial isolate: *Pseudomonas aeruginosa.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

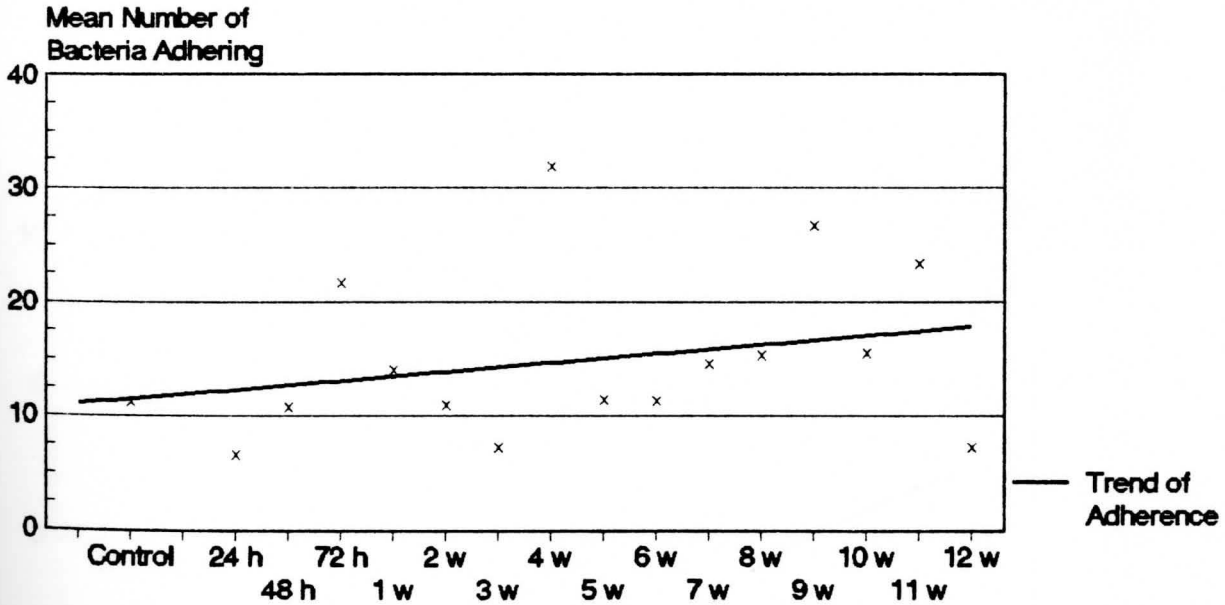
A) Patient 2: Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

PATNT-2

B) Patient Two: Trend of Bacterial Adherence to Uroepithelia

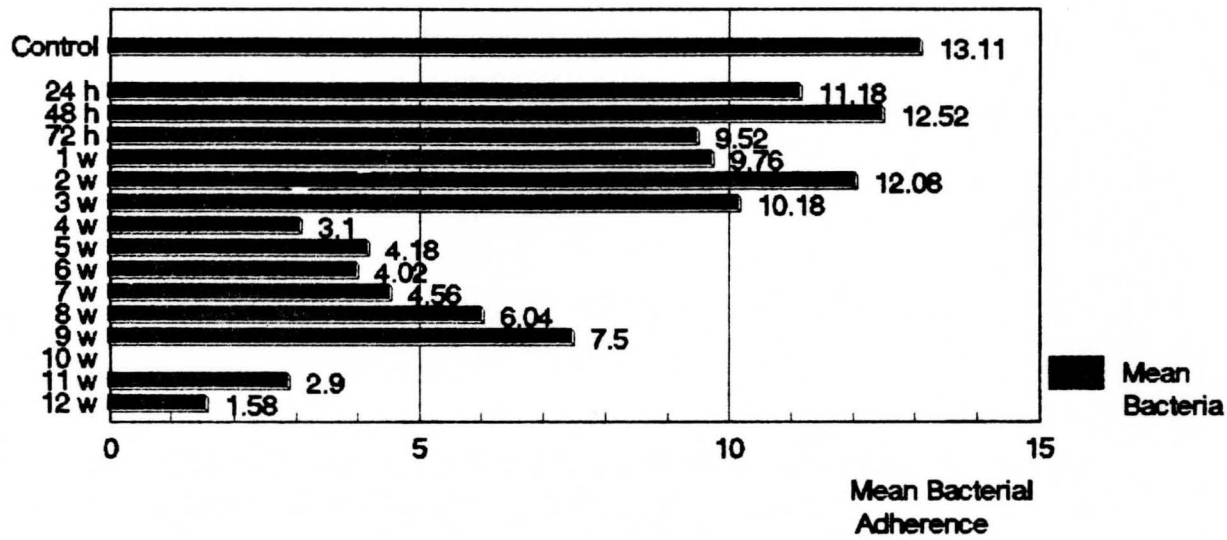


KeyChart 2000

2-TREND

Figure 3: Bacterial adherence data for Patient 3. Bacterial isolate: *Escherichia coli.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

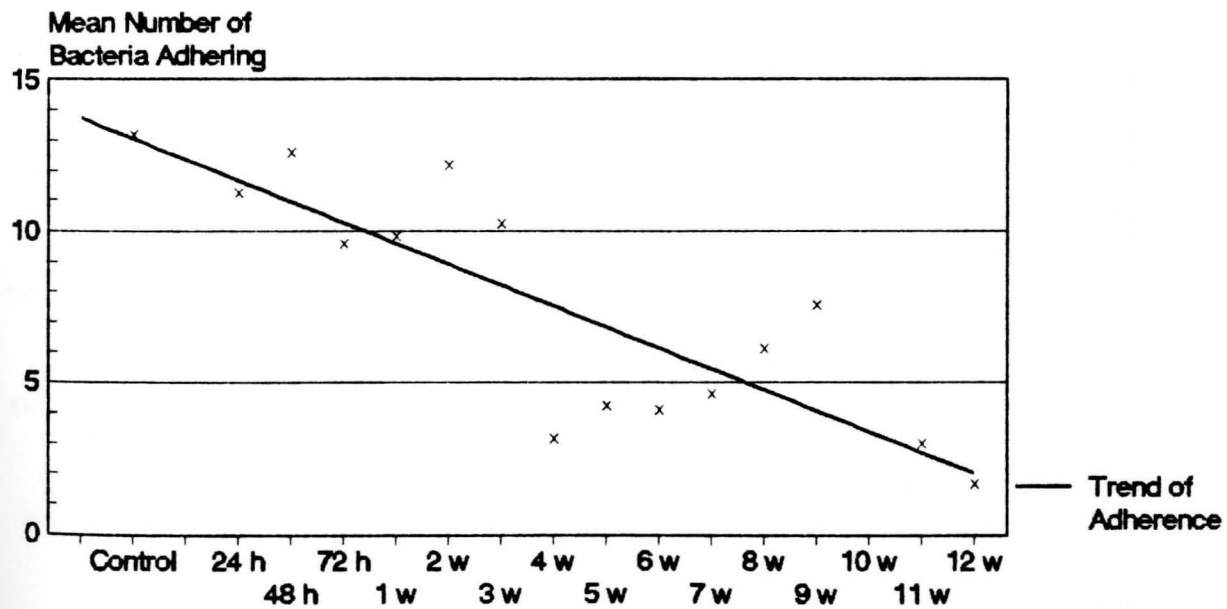
A) Patient 3: Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

PATNT-3

B) Patient 3: Trend of Bacterial Adherence to Uroepithelia

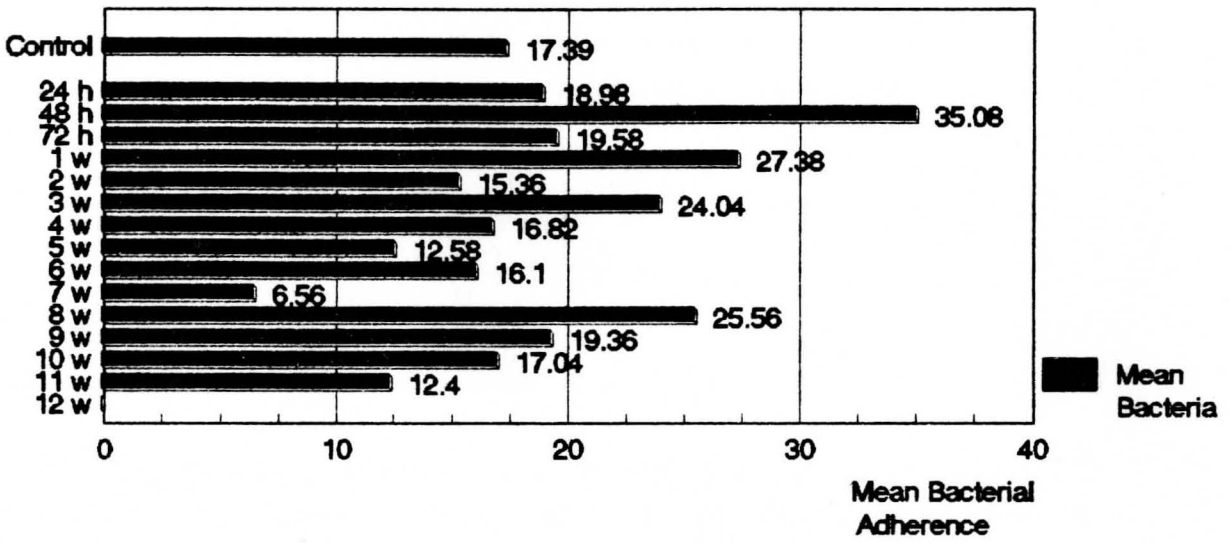


KeyChart 2000

3-TREND

Figure 4: Bacterial adherence data for Patient 4K. Bacterial isolate: *Klebsiella pneumoniae.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

A) Patient 4K: Mean Bacterial Adherences to Uroepithelia



B) Patient 4K: Trend of Bacterial Adherence to Uroepithelia

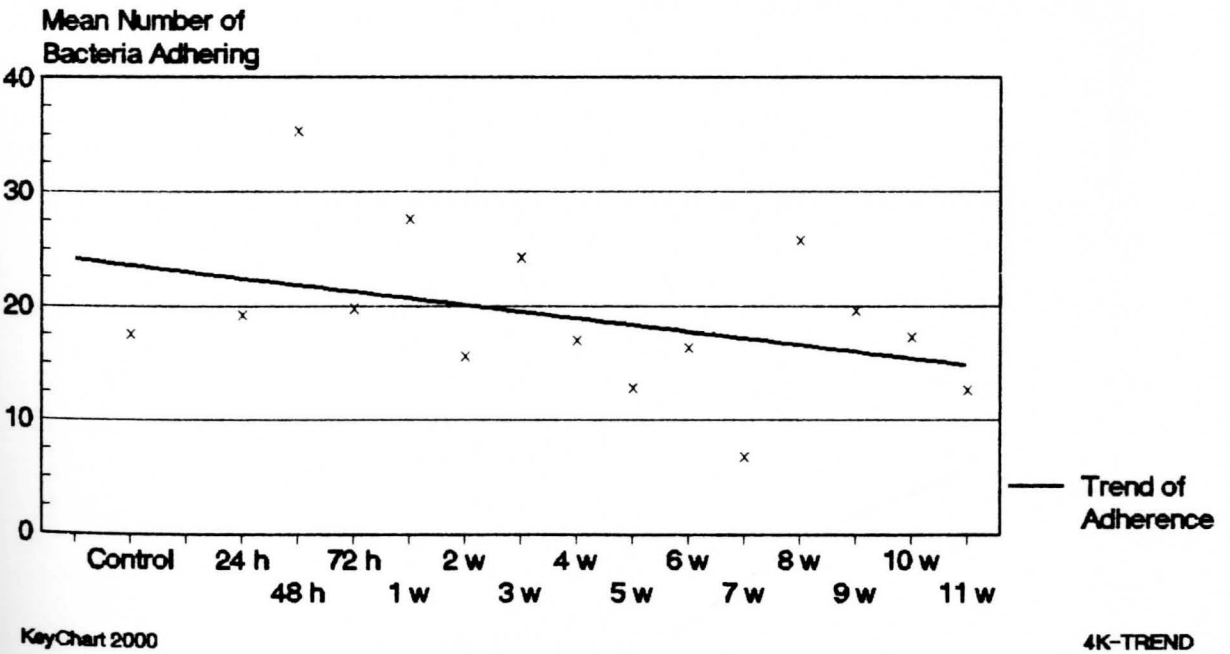
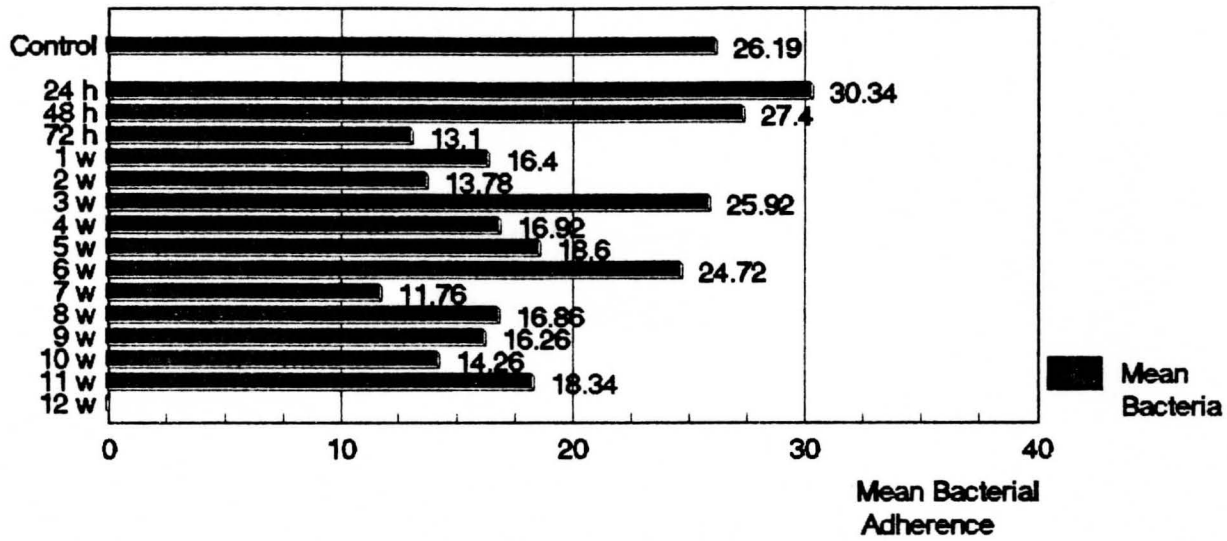


Figure 5: Bacterial adherence data for Patient 4P. Bacterial isolate: *Pseudomonas aeruginosa.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

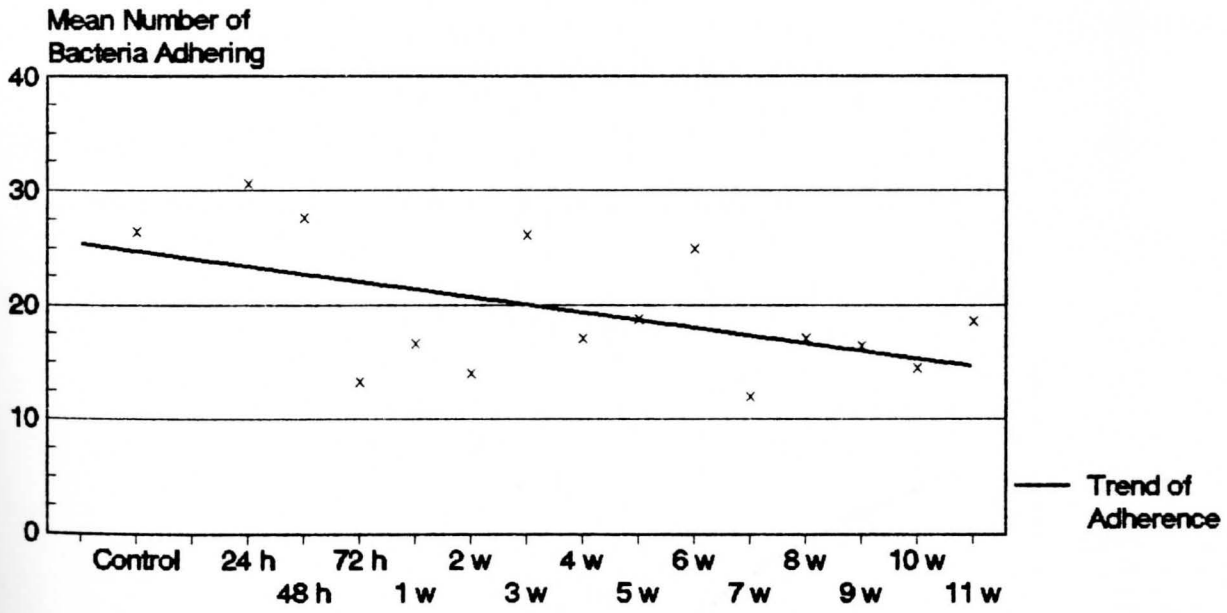
A) Patient 4P: Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

PATNT-4P

B) Patient 4P: Trend of Bacterial Adherence to Uroepithelia



KeyChart 2000

4P-TREND

Figure 6: Comparison of the trends of adherence of the *Klebsiella pneumoniae* and the *Pseudomonas aeruginosa* isolated from Patient 4 using Patient 4 urine as the adherence medium. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

Comparison of Trends of Two Isolates from Patient Four

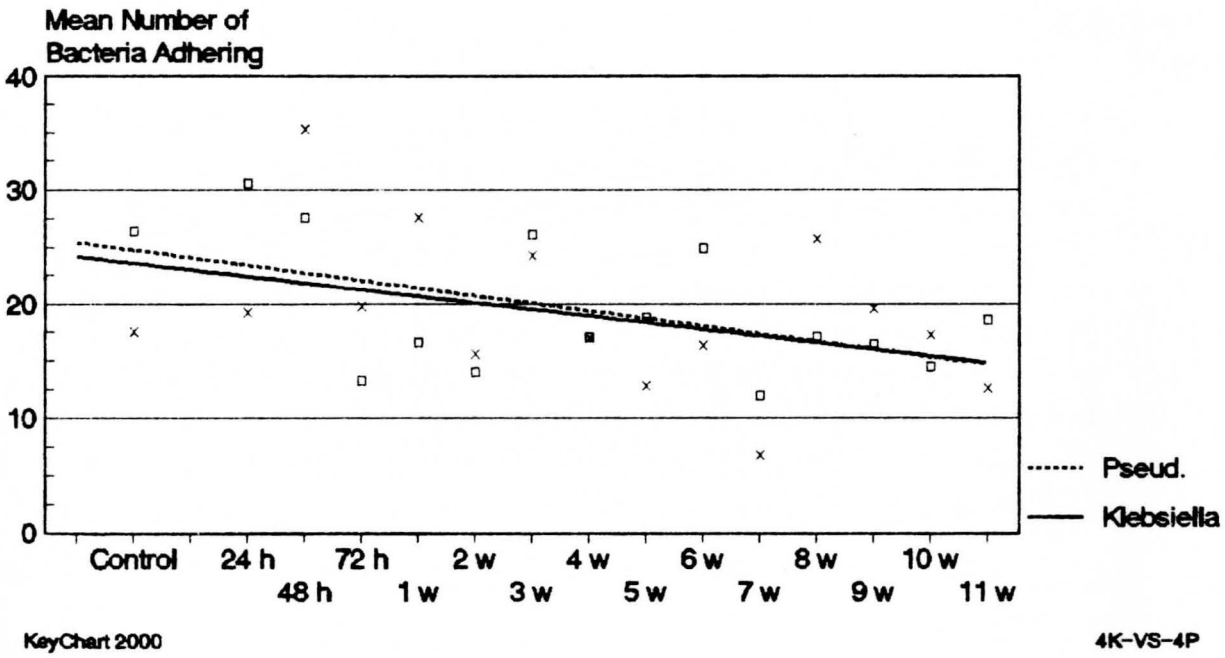
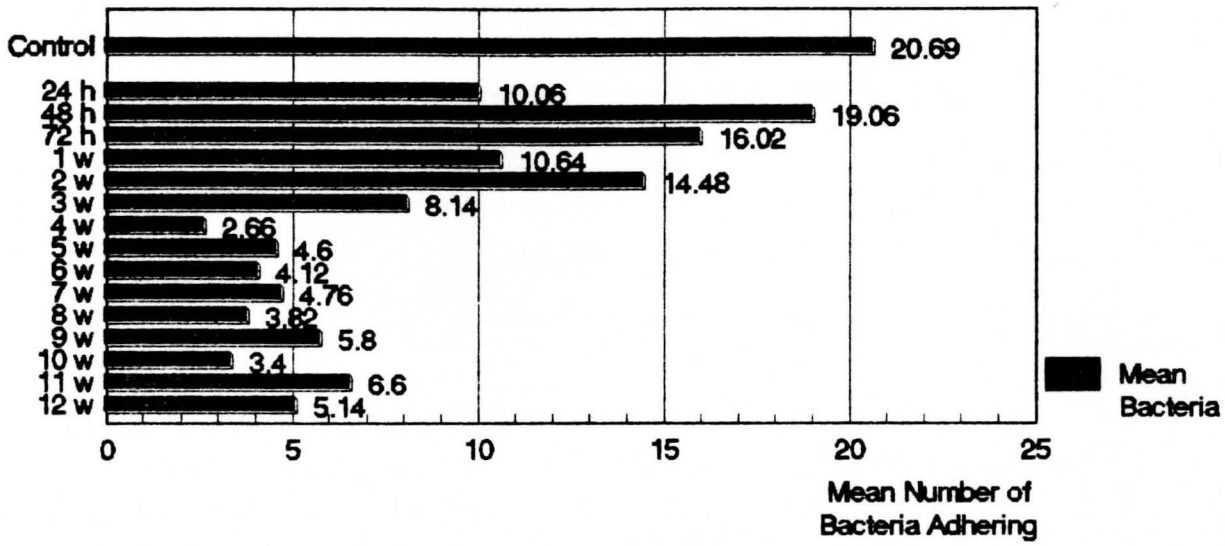


Figure 7: Bacterial adherence data for Patient 5. Bacterial isolate: *Pseudomonas aeruginosa.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

A) Patient 5: Mean Bacterial Adherences to Uroepithelia



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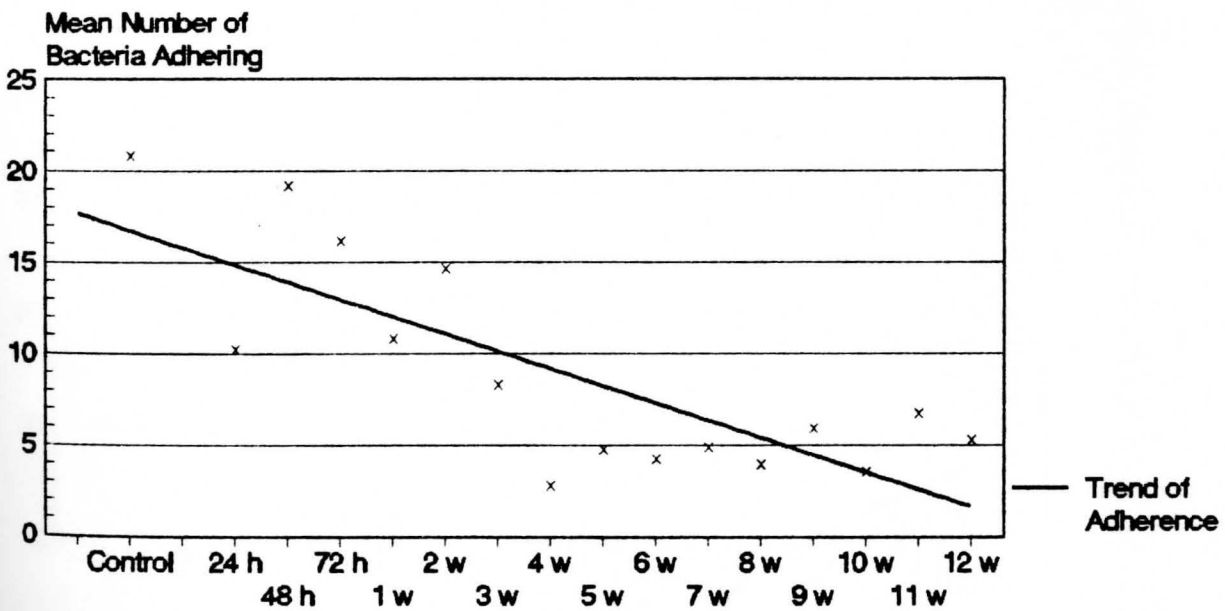
PATNT-5

B) Patient 5: Trend of Bacterial Adherence to Uroepithelia

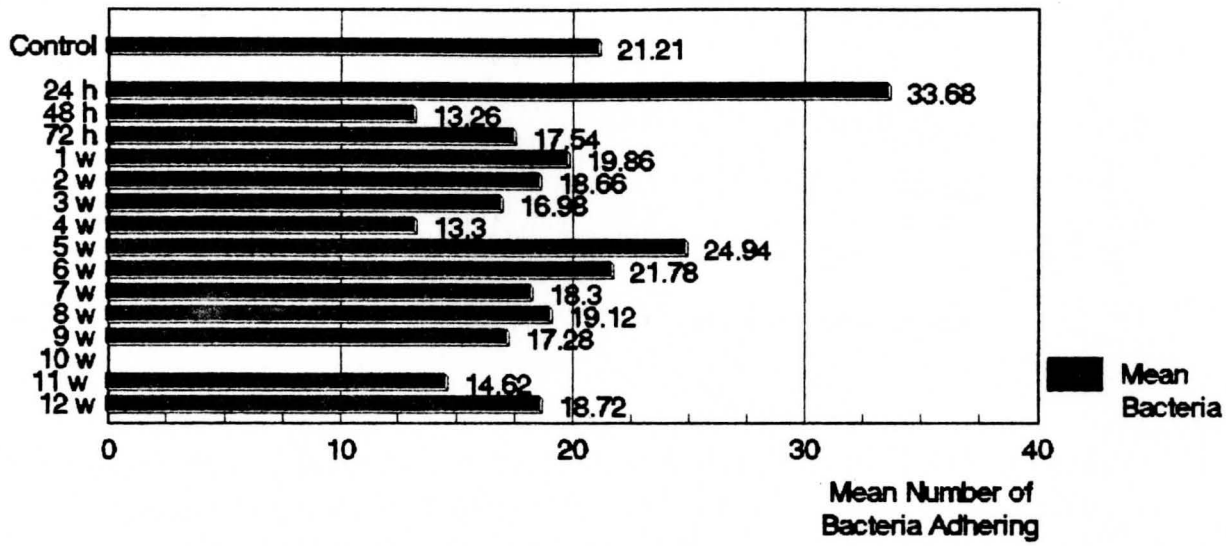


KeyChart 2000

5-TREND

Figure 8: Bacterial adherence data for Patient 6. Bacterial isolate: *Klebsiella pneumoniae.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected the indicated amount of time following the initiation of CJC ingestion.

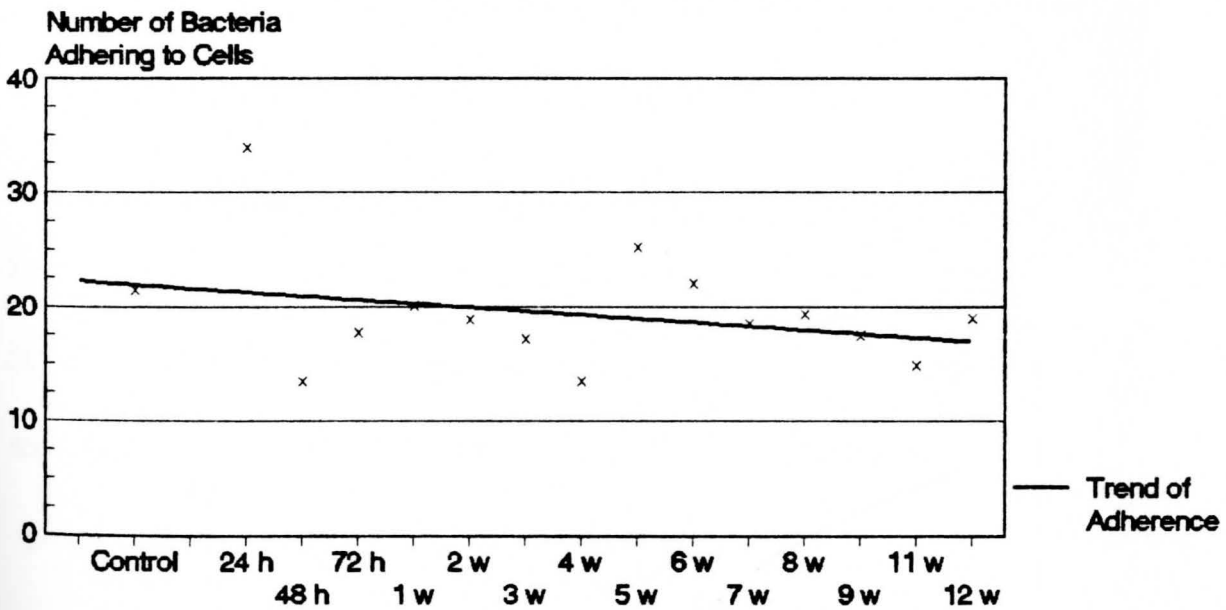
A) Patient 6: Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

PATNT-6

B) Patient 6: Trend of Bacterial Adherence to Uroepithelia

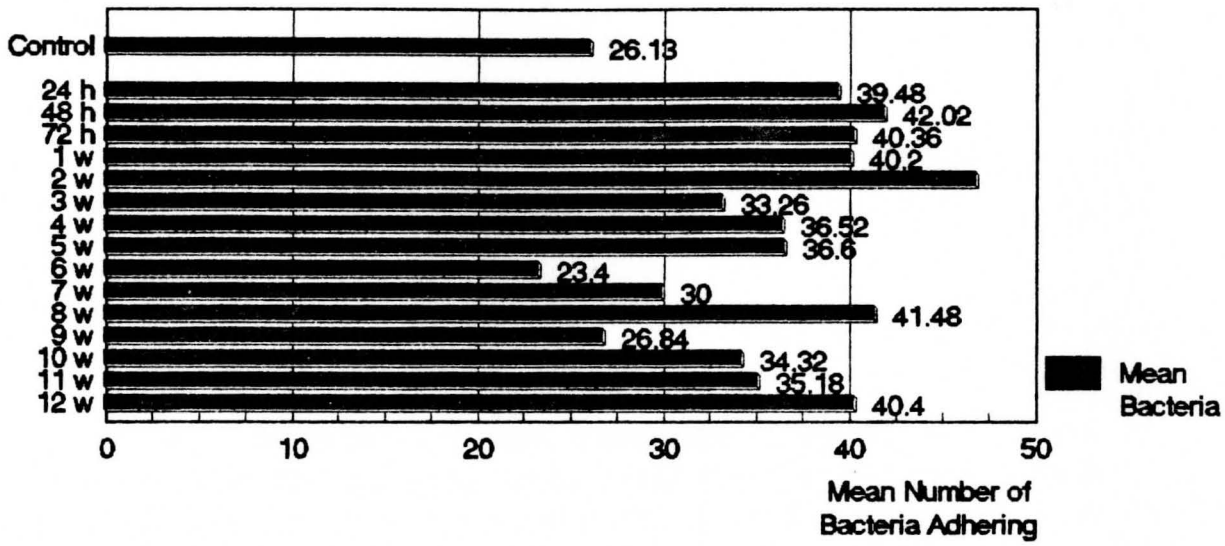


KeyChart 2000

6-TREND

Figure 9: Bacterial adherence data for Patient 7. Bacterial isolate: *Pseudomonas aeruginosa.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected the indicated amount of time following the initiation of CJC ingestion.

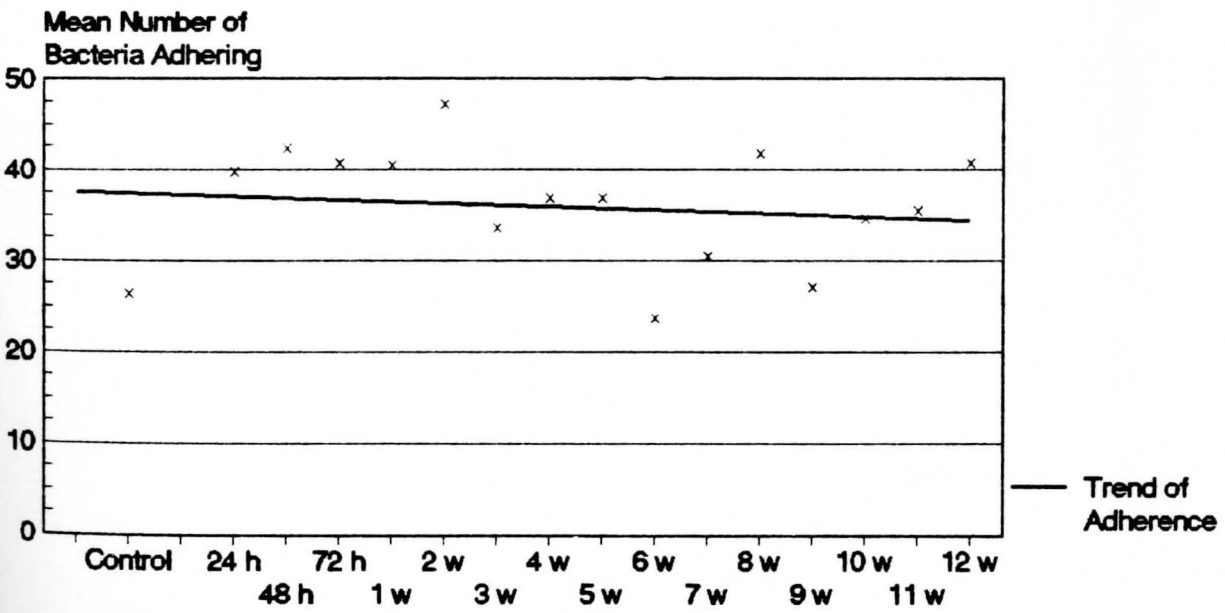
A) Patient 7: Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

PATNT-7

B) Patient 7: Trend of Bacterial Adherence to Uroepithelia

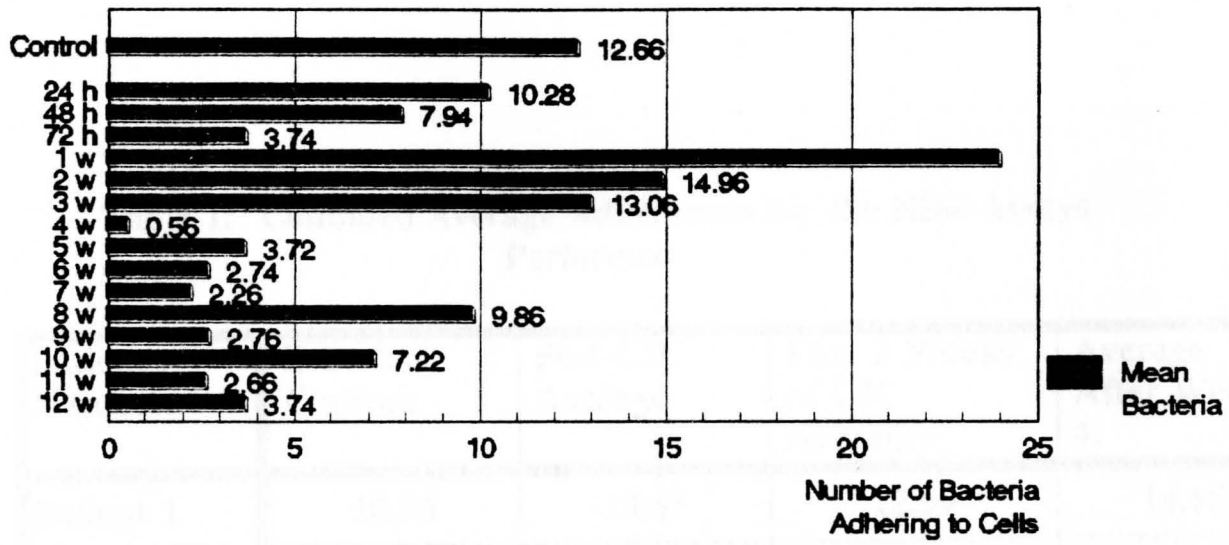


KeyChart 2000

7-TREND

Figure 10: Bacterial adherence data for Patient 8. Bacterial isolate: *Escherichia coli.* A) Bar graph of mean number of bacteria adhering to 50 uroepithelia cells with the indicated urine specimens as the reaction medium. The base-line control (control) represents the mean bacterial adherence to 150 uroepithelia cells including 50 cells assayed in the presence of urine of each of the specimens collected 3, 5, and 7 days prior to the initiation of CJC ingestion. B) Plot of the trend of bacterial adherence throughout the study. Control: Base-line control, all other specimens were collected at the indicated amount of time following the initiation of CJC ingestion.

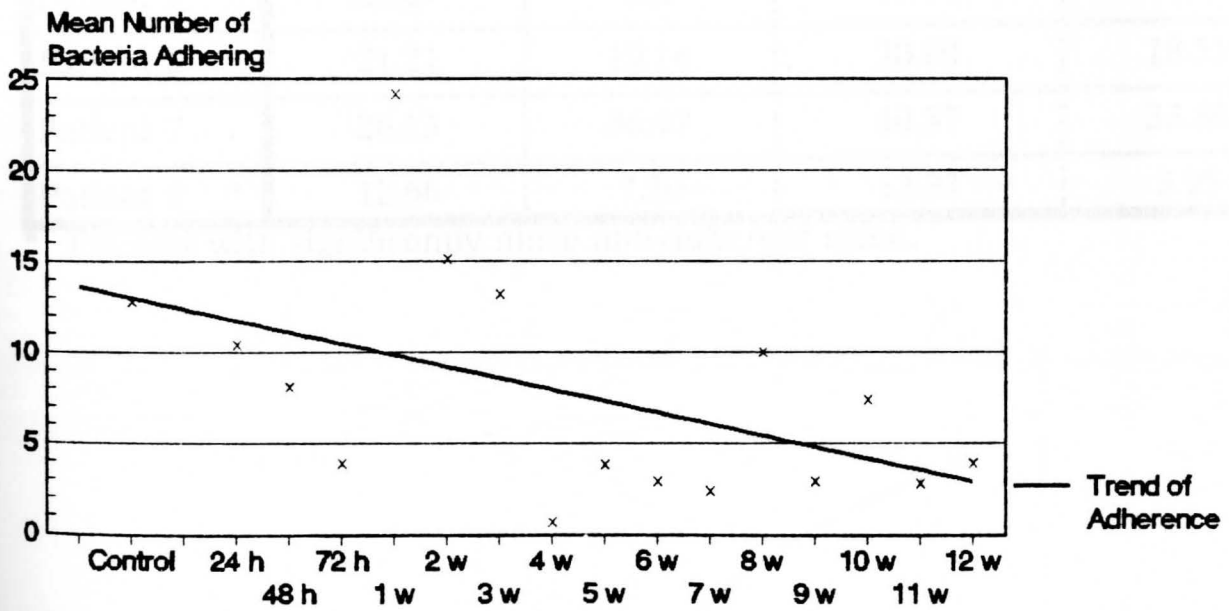
A) Patient 8: Mean Bacterial Adherences to Uroepithelia



KeyChart 2000

PATNT-8

B) Patient 8: Trend of Bacterial Adherence to Uroepithelia



KeyChart 2000

8-TREND

Table 1: Compiled Average Adherences for the Nine Assays Performed:

Patient Number:	Base-line Control:	Post-CJC Average:	First 3 Weeks of CJC Average:	Average After Week 4:
Patient 1	10.56	13.85	12.59	14.68
Patient 2	11.02	15.03	11.68	17.28
Patient 3 *	13.11	7.08	10.87	4.24
Patient 4K	17.39	19.06	23.40	15.80
Patient 4P *	26.19	18.90	21.16	17.22
Patient 5 *	20.69	7.95	13.06	4.54
Patient 6	21.21	19.14	20.00	18.51
Patient 7	26.13	36.47	40.37	33.86
Patient 8 *	12.66	7.30	12.33	3.95

* Patients with significantly more anti-adherent urine.

Table 2: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine from Patient Number One (and statistical data)

Bacteria Isolate: *Proteus miribalis*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	0.77	10.56	
24 Hour Post-CJC	0.80	28.78	0.0001
48 Hours Post-CJC	0.66	12.68	0.0394
72 Hours Post-CJC	1.24	**8.48**	0.0433
1 Week Post-CJC	1.26	**6.28**	0.0001
2 Weeks Post-CJC	0.78	**7.48**	0.0028
3 Weeks Post-CJC	0.74	11.92	0.3056
4 Weeks Post-CJC	2.30	32.02	0.0001
5 Weeks Post-CJC	0.34	**2.94**	0.0001
6 Weeks Post-CJC	0.66	**4.10**	0.0001
7 Weeks Post-CJC	10.94	25.34	0.0001
8 Weeks Post-CJC	2.82	25.84	0.0001
9 Weeks Post-CJC	1.64	12.30	0.1317
10 Weeks Post-CJC	0.44	9.30	0.2749
11 Weeks Post-CJC	0.96	10.64	0.9447
12 Weeks Post-CJC	1.84	9.86	0.5440

BOLD: Significantly lower than control.
UNDERLINED: Significantly higher than control.

Table 3: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine From Patient Number Two (and statistical data)

Bacteria Isolate: *Pseudomonus aeruginosa*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level ($\alpha = 0.05$)
Base-line Control (3, 5, and 7 days prior to CJC)	1.15	11.02	
24 Hour Post-CJC	0.48	**6.40**	0.0001
48 Hours Post-CJC	0.60	10.60	0.7242
72 Hours Post-CJC	0.74	21.46	0.0001
1 Week Post-CJC	0.82	13.78	0.0206
2 Weeks Post-CJC	0.98	10.76	0.8271
3 Weeks Post-CJC	0.48	**7.06**	0.0003
4 Weeks Post-CJC	1.10	31.68	0.0001
5 Weeks Post-CJC	1.26	11.24	0.8383
6 Weeks Post-CJC	1.26	11.10	0.9409
7 Weeks Post-CJC	1.08	14.38	0.0019
8 Weeks Post-CJC	0.46	15.08	0.0012
9 Weeks Post-CJC	0.40	26.48	0.0001
10 Weeks Post-CJC	0.66	15.32	0.0006
11 Weeks Post-CJC	0.68	23.16	0.0001
12 Weeks Post-CJC	0.86	**7.06**	0.0016

BOLD: Significantly lower than control.
UNDERLINED: Significantly higher than control.

Table 4: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine from Patient Number Three (and statistical data)

Bacteria Isolate: *Escherichia coli*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level ($\alpha = 0.05$)
Base-line Control (3, 5, and 7 days prior to CJC)	1.09	13.11	
24 Hour Post-CJC	0.87	11.18	0.1277
48 Hours Post-CJC	0.84	12.52	0.6399
72 Hours Post-CJC	1.11	**9.52**	0.0047
1 Week Post-CJC	1.95	**9.76**	0.0083
2 Weeks Post-CJC	0.84	12.08	0.4152
3 Weeks Post-CJC	1.33	**10.18**	0.0210
4 Weeks Post-CJC	0.80	**3.1**	0.0001
5 Weeks Post-CJC	0.92	**4.18**	0.0001
6 Weeks Post-CJC	0.72	**4.02**	0.0001
7 Weeks Post-CJC	0.81	**4.56**	0.0001
8 Weeks Post-CJC	0.68	**6.04**	0.0001
9 Weeks Post-CJC	1.20	**7.5**	0.0001
10 Weeks Post-CJC	No Specimen	No Specimen	No Specimen
11 Weeks Post-CJC	0.44	**2.9**	0.0001
12 Weeks Post-CJC	1.63	**1.58**	0.0001

BOLD: Significantly lower than control.
UNDERLINED: Significantly higher than control.

Table 5: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine from Patient Number Four (and statistical data)

Bacteria Isolate: *Klebsiella pneumoniae*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	0.87	17.39	
24 Hour Post-CJC	1.84	18.98	0.3223
48 Hours Post-CJC	0.28	35.08	0.0001
72 Hours Post-CJC	0.48	19.58	0.1727
1 Week Post-CJC	0.72	27.38	0.0001
2 Weeks Post-CJC	1.40	15.36	0.2048
3 Weeks Post-CJC	0.32	24.04	0.0001
4 Weeks Post-CJC	0.48	16.82	0.6957
5 Weeks Post-CJC	0.58	**12.58**	0.0011
6 Weeks Post-CJC	0.42	16.10	0.3777
7 Weeks Post-CJC	0.56	**6.56**	0.0001
8 Weeks Post-CJC	0.98	25.56	0.0001
9 Weeks Post-CJC	0.58	19.36	0.1454
10 Weeks Post-CJC	0.82	17.04	0.7935
11 Weeks Post-CJC	0.96	**12.40**	0.0002
12 Weeks Post-CJC	No Specimen	No Specimen	No Specimen

BOLD: Significantly lower than control.
UNDERLINED: Significantly higher than control.

Table 6: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine from Patient Number Four (and statistical data)

Bacteria Isolate: *Pseudomonas aeruginosa*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level ($\alpha = 0.05$)
Base-line Control (3, 5, and 7 days prior to CJC)	1.09	26.19	
24 Hour Post-CJC	0.68	<u>30.34</u>	0.0030
48 Hours Post-CJC	0.58	27.40	0.3871
72 Hours Post-CJC	1.22	**13.10**	0.0001
1 Week Post-CJC	0.66	**16.40**	0.0001
2 Weeks Post-CJC	1.18	**13.78**	0.0001
3 Weeks Post-CJC	1.08	25.92	0.8408
4 Weeks Post-CJC	1.08	**16.92**	0.0001
5 Weeks Post-CJC	1.02	**18.60**	0.0001
6 Weeks Post-CJC	0.72	24.72	0.2792
7 Weeks Post-CJC	2.38	**11.76**	0.0001
8 Weeks Post-CJC	0.92	**16.86**	0.0001
9 Weeks Post-CJC	1.36	**16.26**	0.0001
10 Weeks Post-CJC	1.54	**14.26**	0.0001
11 Weeks Post-CJC	1.62	**18.34**	0.0001
12 Weeks Post-CJC	No Sample	No Sample	No Sample

BOLD: Significantly lower than control.
<u>UNDERLINED</u>: Significantly higher than control.

Table 7: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine from Patient Number Five (and statistical data)

Bacteria Isolate: *Pseudomonas aeruginosa*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level ($\alpha = 0.05$)
Base-line Control (3, 5, and 7 days prior to CJC)	2.36	20.69	
24 Hour Post-CJC	2.48	**10.06**	0.0001
48 Hours Post-CJC	2.40	19.06	0.3334
72 Hours Post-CJC	1.02	**16.02**	0.0057
1 Week Post-CJC	1.18	**10.64**	0.0001
2 Weeks Post-CJC	2.48	**14.48**	0.0002
3 Weeks Post-CJC	1.18	**8.14**	0.0001
4 Weeks Post-CJC	1.58	**2.66**	0.0001
5 Weeks Post-CJC	1.98	**4.60**	0.0001
6 Weeks Post-CJC	1.38	**4.12**	0.0001
7 Weeks Post-CJC	1.42	**4.76**	0.0001
8 Weeks Post-CJC	1.34	**3.82**	0.0001
9 Weeks Post-CJC	1.90	**5.80**	0.0001
10 Weeks Post-CJC	1.44	**3.40**	0.0001
11 Weeks Post-CJC	0.94	**6.60**	0.0001
12 Weeks Post-CJC	1.36	**5.14**	0.0001

BOLD: Significantly lower than control.
UNDERLINED: Significantly higher than control.

Table 8: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Urine Specimen from Patient Number Six (and statistical data)

Bacteria Isolate: *Klebsiella pneumoniae*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	1.50	21.21	
24 Hour Post-CJC	1.28	<u>33.68</u>	0.0001
48 Hours Post-CJC	1.52	**13.14**	0.0001
72 Hours Post-CJC	1.32	17.54	0.0669
1 Week Post-CJC	0.72	19.86	0.5006
2 Weeks Post-CJC	0.84	18.66	0.2029
3 Weeks Post-CJC	1.12	**16.98**	0.0246
4 Weeks Post-CJC	0.88	**13.30**	0.0001
5 Weeks Post-CJC	1.88	<u>24.94</u>	0.0471
6 Weeks Post-CJC	2.22	21.78	0.7602
7 Weeks Post-CJC	1.12	18.30	0.1220
8 Weeks Post-CJC	1.72	19.12	0.5236
9 Weeks Post-CJC	2.78	26.34	0.1169
10 Weeks Post-CJC	No Sample	No Sample	No Sample
11 Weeks Post-CJC	0.66	**14.62**	0.0443
12 Weeks Post-CJC	1.94	18.72	0.4473

BOLD: Significantly lower than control.
<u>UNDERLINED</u>: Significantly higher than control.

Table 9: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine from Patient Number Seven (and statistical data)

Bacteria Isolate: *Pseudomonas aeruginosa*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	0.67	26.13	
24 Hour Post-CJC	0.60	39.48	0.0001
48 Hours Post-CJC	2.76	42.02	0.0001
72 Hours Post-CJC	1.16	40.36	0.0001
1 Week Post-CJC	0.48	40.20	0.0001
2 Weeks Post-CJC	0.54	46.90	0.0001
3 Weeks Post-CJC	1.02	33.26	0.0001
4 Weeks Post-CJC	0.84	36.52	0.0001
5 Weeks Post-CJC	0.76	36.60	0.0001
6 Weeks Post-CJC	0.80	23.40	0.1081
7 Weeks Post-CJC	0.80	30.16	0.0180
8 Weeks Post-CJC	1.10	41.48	0.0001
9 Weeks Post-CJC	0.72	26.84	0.6749
10 Weeks Post-CJC	0.82	34.32	0.0001
11 Weeks Post-CJC	0.82	35.18	0.0001
12 Weeks Post-CJC	1.24	40.40	0.0001

BOLD: Significantly lower than control.
UNDERLINED: Significantly higher than control.

Table 10: Mean Bacterial Adherence to 50 Uroepithelia Cells for Each Specimen of Urine from Patient Number Eight (and statistical data)

Bacteria Isolate: *Escherichia coli*

Sample:	Negative Control (no bacteria added)	Mean Bacterial Adherence	Probability Level (α = 0.05)
Base-line Control (3, 5, and 7 days prior to CJC)	0.12	12.66	
24 Hour Post-CJC	0.16	10.28	0.0741
48 Hours Post-CJC	0.20	**7.94**	0.0004
72 Hours Post-CJC	0.64	**3.74**	0.0001
1 Week Post-CJC	0.16	24.02	0.0001
2 Weeks Post-CJC	0.38	15.16	0.0607
3 Weeks Post-CJC	0.01	13.06	0.7104
4 Weeks Post-CJC	0.06	**0.56**	0.0001
5 Weeks Post-CJC	0.08	**3.72**	0.0001
6 Weeks Post-CJC	0.04	**2.74**	0.0001
7 Weeks Post-CJC	0.36	**2.26**	0.0001
8 Weeks Post-CJC	0.28	**9.86**	0.0121
9 Weeks Post-CJC	0.82	**2.76**	0.0001
10 Weeks Post-CJC	0.06	**7.3**	0.0001
11 Weeks Post-CJC	0.08	**2.66**	0.0001
12 Weeks Post-CJC	0.02	**3.74**	0.0001

BOLD: Significantly lower than control.
UNDERLINED: Significantly higher than control.

IV. Discussion:

The clinical value of cranberry juice in the reduction of urinary tract infection was first investigated in 1968 by Prodomas et al.[45] with favorable results, but this activity was unexplainable at the time. The recent work of Avorn et al.[2] is very convincing in terms of the effects of CJC on the incidence of bacteriuria and its effects on the clinical progression of UTI in a large population. It was first demonstrated in this laboratory that cranberry juice cocktail acts as a very strong anti-adherence agent both *in vitro* and *in vivo* and in this role may be potentially useful for the treatment of UTI.[52,58,59,72] Rather than looking for altered tendencies in the incidence and progression of UTI after cranberry juice consumption our study looks directly at the capacity of CJC to induce anti-adherent activity in the urine of eight compromised patients in a clinical setting. The population we chose to study is the population which stands to gain the most from advances in non-antibiotic reduction of urinary tract infection. This population has compound risk factors for UTI including age, gender, indwelling urethral catheterization, residency in a nursing home, plus various degrees of decreased functional capacity. With these risks it is nearly inevitable that these people will

suffer from chronic UTI for the remainder of their lives, increasing morbidity and mortality and decreasing their quality of life. Longitudinal studies have indicated that the prevalence of UTI over a five year period increases from 33% in an elderly female population to 57% when only the portion of these women living in nursing homes was considered[3] and studies of UTI in catheterized individuals has indicated a 5% to 10% risk per day of UTI, causing nearly all patients with catheters for any extended period of time to eventually become bacteriuric.[71] They will need to be treated repeatedly with antibiotics only to give temporary hiatus from the current infection which will most likely be replaced by infection with more virulent antibiotic-resistant strains of bacteria. The risk of infection with resistant bacteria in this population is greatly increased due to the high prevalence of resistant strains in the nursing home setting and the urethral catheterization of these patients. The work of Nickel et al.[35] suggested that antibiotics poorly penetrated the exopolysacharide biofilm of bacteria colonizing an indwelling catheter system, conferring a degree of antibiotic resistance to these microbes which could lead to the need for increased dosages of antibiotic for relief. The use of antibiotics in this population can be directly damaging

because they are likely to have impaired metabolism and clearance of these substances leading to toxic concentrations.[50] Interaction of the antimicrobials with other medications is also a risk factor. Nickel[32] suggested that the best strategy of attack on catheter-associated UTI is at the level of adherence of the bacteria to the uroepithelia. Antibiotic therapy has been shown to inhibit bacterial adherence to the bladder wall in experimental models[41] but the various dangers of antibiotic treatment make the investigation of other proven anti-adherent agents such as cranberry juice cocktail, which presents no known dangers to the recipients, a very attractive prospect.

The long-term goals of this and other studies in this lab are to bring about heightened interest and understanding of the specific activity of cranberry juice in the reduction of UTI. We would like to learn how valuable CJC is as a prophylactic against UTI and investigate its value as an adjuvant therapy in the treatment of UTI. The anti-adherent capacity of CJC has been demonstrated in several *in vivo* assays. Sobota[59] demonstrated that cranberry juice ingestion in 15 of 22 individuals tested produced significant anti-adherence activity when compared to urine collected prior to ingestion of the CJC, and he demonstrated a similar

activity in mice. Schmidt[52] demonstrated an anti-adherent effect in urine beginning one to three hours following ingestion of the cocktail. Wollet[72] had observed a significant difference in the capacity of urine from six female nursing home patients who ingested CJC daily to block bacterial adherence to uroepithelia cells as compared to 19 individuals from the same population but not on CJC. In the present study we look at the effects of CJC ingestion on the anti-adherence activity of the urine over time in a high risk population. We collected urine specimens over a thirteen week period including the initial week in which 3 base-line control samples were taken prior to giving 6 ounces of CJC per day for the next twelve weeks. Specimens were collected 24, 48 and 72 hours after the initiation of CJC ingestion, and then weekly for the remainder of the study. The urine specimens were used as the reaction medium for adherence assays in which all of the specimens from a particular patient were tested simultaneously using the same culture of bacteria and exfoliated uroepithelia cells isolated from a single specimen of female donor urine so that the only variable would be the patient urine (Bacteria used were isolated from each patients' base-line control specimens and used for the adherence assays of that patients urine). Urine from four

of the eight patients assayed exhibited significantly increased anti-adherence activity with reductions of 28%, 42%, 46%, and 62% in average bacterial adherence to uroepithelia cells in the presence of urine collected during the administration of CJC as compared to the base-line controls. These are convincing values not only in terms of the presence of anti-adherence activity afforded by the juice but also the level of activity observed is substantial. Further investigations are needed into the clinical value of CJC as a blocking agent against bacterial adherence to the uroepithelia. The effects of the juice were varied for the different bacterial isolates. The effects of cranberry juice on the adherence of *E. coli* has been well established by various investigators and anti-adherence activity against both *E. coli* isolates in this study was evident. The juice's effect on the *Pseudomonas* isolates were variable with activity seen against two of the four isolates assayed. Both strains of *Klebsiella* isolated exhibited decreased trends of adherence but not to significant levels. Urine from patient 4 which was significantly anti-adherent for a *Pseudomonas* isolate was not significantly anti-adherent for one of the *Klebsiella* isolates, but the overall trends of adherence throughout the study of these two isolates were similar. The anti-adherent urine may

have affected both isolates due to similar surface adhesins on the two strains but the effect on the *Klebsiella* isolate may have been decreased due to a blocking effect from its large capsule.

The mechanism of action of the cranberry juice in blocking bacterial adherence is of considerable interest. The isolation of a factor from the juice which is directly active or which is converted to or induces the production of an agent active in the urinary tract would be of significant pharmaceutical value. The traditional theory on the mode of action of cranberry juice in reduction of UTI was that it acidified the urine by increasing the secretion of organic acids. Investigators have since shown that there is no significant change in urinary pH after consumption of cranberry juice.[4,59] More recently investigators attributed the activity induced by CJC to components of the juice which interact with bacteria blocking their adherence to the uroepithelia. Sobota[59] demonstrated the anti-adherence activity of CJC in a series of *in vitro* and *in vivo* assays in 1984. He showed that preincubation of *E. coli* with cranberry juice, but not preincubation of uroepithelia cells with the juice, induced an anti-adherent effect and that this effect could be removed by washing, indicating the effect of the juice is most likely on

the bacterial surface molecules in a stable but not permanent manner. This effect was later confirmed by Zafriri et al.[73] in yeast agglutination experiments. Previously, it was shown *in vitro* that fructose effectively blocks the adherence of *E. coli* to uroepithelia cells.[49] Schaeffer et al.[49] suggested that D-fructose blocks the fimbrial adhesins of bacteria in a manner similar to the blocking activity of D-mannose observed in type 1 fimbriated hemagglutination studies. This hypothesis was based on structural similarities between the two carbohydrates and because anti-adherent activity was not seen from other sugars assayed. Fructose is a component of both natural cranberry juice and additional fructose is used as a sweetener in the CJC so a possible role for fructose in the activity of CJC has been suggested.[59,73] The *in vitro* anti-adherent activity of fructose was later confirmed by other investigators in relation to CJC[59,72] and it was also shown that the monosaccharide blocks the agglutination of yeast by *E. coli.*[73] Although fructose in the reaction media of adherence assays blocks bacterial adherence, it is questionable if fructose can pass in any substantial amount, or at all, in the urine following ingestion. Monosaccharides such as fructose are converted to glucose soon after absorption in normal metabolism and are not regularly

secreted in the urine. Although not necessarily relevant for an elderly population, in an HPLC assay of the urine of five young adults drinking 5%, 10%, and 15% solutions of fructose we were unable to detect any significant fructose accumulation in the urine (see addendum). Based on what is known about fructose metabolism and on the lack of fructose clearance in the urine it is unlikely that it is the active component of the juice. Zafriri et al.[73] found a non-dialyzable component of cranberry juice which they described as a polymeric compound of unknown nature that inhibited mannose resistant adhesins of pathogenic urinary isolates of *E. coli*. This group later found this anti-adherent polymer in the juice of blueberries as well which, along with cranberries, is from the Vaccinium genus. They partially purified the polymer by gel-filtration chromatography and found that it is heat stable and resistant to digestion by trypsin.[39] *In vitro* assays by Wollet[72] indicated that the non-dialyzable component of CJC also inhibits the adhesion of mannose sensitive, type 1 fimbriated *E. coli*.

An important clue in deciphering the mechanism of anti-adherent activity from cranberry juice would be to learn where the effect of the juice is occurring anatomically. Ofec et al.[39] suggested that the anti-

adhesive agent might act in the gut, the bladder, or both. In the clinical study by Avorn et al.[2] decreased incidence of bacteriuria and pyuria was not seen until after 4 to 8 weeks of cranberry juice use. As discussed in the results section a similar tendency was seen in this study in which it took four weeks for a significant anti-adherent effect to occur in three of the four patients for which activity was observed. The Avorn group theorized that the delayed activity might be do to time required for an alteration of the gut flora. Alternatively, the bacteria may be altered rapidly in the gut and the delay in activity due to the time required for carriage of the altered microbes from the GI tract and colonization of the periurethral area in a competitive manner with the more adherent flora already present. The suggestion of juice activity in the gut is supported by the numerous *in vitro* assays showing a direct effect of CJC when it contacts the bacteria[52,59,72,73] as it would in the gut. However, these *in vitro* assays also suggest the possibility that some active fraction of the juice is transferred to the blood and then directly or indirectly secreted into the urine. This latter suggestion is supported by various *in vivo* assays including the information presented in this study in which it has been shown that the anti-adherent activity is observed in the urine

after consumption of the juice. Bacteria not previously exposed to CJC have been shown repeatedly to lose their adherence capacity after exposure to urine produced following CJC consumption.[52,59,72,73] Also, Wollet[72] demonstrated that the significantly greater anti-adherent activity of urine from subjects that regularly drank CJC as compared to other groups that did not drink CJC could not be increased by addition of the nondialyzable component of the juice. However, urine from individuals not ingesting CJC became significantly more anti-adherent with the addition of the non-dialyzable component. Wollet suggests that in individuals ingesting CJC the urine is saturated with the active substance, indicating that a component of the juice is released into the urinary tract. This current study and the study of Avorn et al.[2] looked at an elderly population of women. The delayed effect in the activity of CJC in these clinical trials was not evident in previous *in vivo* assays of young adults in which anti-adherent effects could be demonstrated within hours of consumption. This could be due to altered metabolism of the juice in the populations studied or some other factor associated with the age difference in the two populations. It may have taken several weeks of CJC consumption for the metabolic pathway producing the active agent

to become active or for saturation of a renal clearance mechanism prior to secretion of the active substance. Orskov et al.[42] suggested that the function of Tamm-Horsfall protein (THP), a mucoid-like glycoprotein abundant in urine, is to trap urinary pathogens for clearance upon micturition after observing the trapping of type 1 fimbriated *E. coli* by the protein. Sobota and Apicella[58] demonstrated that THP is active in blocking the adherence of *E. coli* to uroepithelia. Preliminary work in this lab has suggested an increased anti-adherent activity of the protein when isolated from urine after consumption of CJC as compared to THP isolated from urine collected prior to ingesting the cocktail. This increased activity might result from the complexing or trapping of the active agent from the juice with the THP enhancing its effect, or the activity of the juice may be to cause a physiological change in the protein qualitatively or quantitatively. Another explanation could be simply an additive effect of the cranberry juice component and the THP due to coisolation of the juice component along with the THP in the technique used to isolate the THP. Further work is being done by other members of this lab to look more closely at the role of THP in the CJC anti-adherence mechanism.

Cranberry juice can be incorporated into the diet of high risk individuals easily and harmlessly and the accumulated evidence suggests that it could be highly beneficial in the reduction of UTI in this population. The isolation of the active factor responsible for the activity of the juice in the urinary tract would be especially beneficial for the population of patients examined in this study. Larger scale studies investigating the amount and type of protection afforded and studies of the site and type of action of cranberry juice are needed.

Addendum:

High Performance Liquid Chromatography to Detect the Passage of Ingested Fructose to the Urine:

A base-line control urine specimen was collected from five young adults. These subjects then drank 5%, 10%, and 15% solutions of D-fructose and collected urine specimens after four hours over a three day period with each concentration of fructose being ingested on a separate day. Standard solutions of 5%, 1%, 0.1%, and 0.01% D-fructose were prepared in urine and in deionized water. The collected urine specimens were compared to the standard fructose solutions by HPLC.

All HPLC work was performed by Marsha Malmer in the Youngstown State University Biology Departement Analytical Laboratory. The HPLC system consisted of an Isco model 2300 pump operated in the isocratic mode, a Waters 712 WISP autosampler, and a Hewlett-Packard 1073A refractive index detector. The column was a reversed phase 300mm x 4.1mm I.D. 10 micron carbohydrate column from Alltech Associates, Deerfield, IL. The temperature was maintained at 21°C. The mobile phase was prepared by mixing 925 ml acetonitrile with 75 ml of HPLC grade water. The acetonitrile used was OPTIMA

grade from Fischer Scientific, Pittsburgh, PA. The mobile phase was prepared fresh daily and filtered through a 0.45μ nylon filter. The flow rate was 3.0 ml/min. The mobile phase was degassed with helium during operation.

There was no detectable fructose accumulation in the urine of five individuals four hours after the consumption of 5%, 10%, or 15% fructose solutions when these specimens were compared to 5%, 1%, 0.1%, and 0.01% standard solutions of D-fructose in both control urine and deionized water by HPLC.

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