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VAGINAL MICROECOLOGY AND THE PATHOGENESIS OF URINARY TRACT INFECTIONS

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SUMMARY

Vaginal colonisation with *E. coli* appears to be an important prerequisite for the development of UTI. Whereas colonisation with P fimbriated strains has been clearly shown to predispose to pyelonephritis, virulence factors which predispose to cystitis have not been so clearly identified. Several host genetic and behavioural factors have been found to be associated with increased colonisation with *E. coli* and other uropathogens and subsequent UTI. Such factors include having the blood group antigen nonsecretor phenotype which is associated with increased *E. coli* vaginal colonisation, using spermicidal contraceptive products which increase *E. coli* vaginal colonisation probably through adverse effects on lactobacilli, exposure to certain antimicrobials which facilitate coliform colonisation through adverse effects on the anaerobic flora, and use of oestrogen products which appear to enhance *E. coli* adherence to vaginal and uroepithelial cells.

Increased knowledge about these and other factors which influence the vaginal microecology is important if we are to develop safe and effective strategies to prevent UTI. For example, we can expect a reduction in the risk of vaginal colonisation with uropathogens and subsequent UTI by a decrease in the use of spermicide-containing products and use when appropriate of antimicrobials that have less impact on the anaerobic rectal and vaginal flora, such as trimethoprim, trimethoprim-sulfamethoxazole, nitrofurantoin, or fluoroquinolones. In postmenopausal women, topical oestrogens clearly help normalise the flora and reduce the risk of recurrent UTIs. Further research is needed to evaluate the feasibility and effectiveness of re-establishing vaginal colonisation with lactobacilli, especially H_2O_2-producing strains, and whether recolonization can reduce the risk of UTI. In addition, a better understanding of the vaginal microecology and immunity is necessary in order develop a safe and effective vaccine to prevent UTI.

EPIDEMIOLOGY

Acute uncomplicated urinary tract infections are among the most common conditions causing individuals to seek medical care. Population based studies in Sweden have demonstrated that during the first year of life, approximately 1% of boys and girls have symptomatic UTI, and that by the age of seven al-

most 8% of girls and 2% of boys have had a culture-documented symptomatic UTI (*Hansson* et al., 1997). Symptomatic infections in the first year of life are relatively more likely to be pyelonephritis whereas infections in older children are more likely to be cystitis. In the United States, it is estimated from surveys of office practices, hospital-based clinics and emergency departments that there are over eight million episodes of urinary tract infection and over 350,000 episodes of acute pyelonephritis annually (S.M. Schappert, personal communication). In a recent large prospective study of young sexually active women, the incidence of cystitis was approximately 0.5 per person-year, suggesting that the incidence of UTI may be much higher than these national estimates (*Hooton* et al., 1996a). Recurrent UTI occurs in 27% to 44% of healthy women even though they generally have anatomically normal urinary tracts (*Hooton* and *Stamm*, 1997). Urinary tract infections in young healthy men are very uncommon. Urinary tract infections in healthy postmenopausal women are probably less common than in premenopausal women, but incidence data are lacking.

PATHOGENESIS

Urinary tract infections (UTIs) in women develop when uropathogens, almost always from the faecal flora, colonise the vagina, ascend into the bladder and, in some cases the kidney. Vaginal acquisition of uropathogens from a woman's male sex partner has been reported but probably only rarely predisposes to a UTI. Most uncomplicated UTIs in women cannot be explained by underlying functional or anatomic abnormalities of the urinary tract. The initial pathogenic event in the urinary tract occurs when the bacteria attach to the mucosa by interactions between bacterial surface adhesins and complementary epithelial cell receptors, and stimulate cytokine release resulting in an inflammatory response and symptoms (*Hooton* and *Stamm*, 1996). Vaginal colonisation is a prerequisite to bladder infection, and factors which increase the risk of UTI generally do so at least in part by facilitating vaginal colonisation. Vaginal colonisation and infection are facilitated by host behavioural factors such as spermicide use and sexual intercourse (*Hooton* et al., 1996a) and genetic factors such as blood group antigen nonsecretor status (*Hooton* and *Stamm*, 1996) which are discussed below.

Certain bacterial virulence factors provide a selective advantage to those strains possessing them with regard to colonisation and infection (*Johnson*, 1991). Whether vaginal colonisation and subsequent UTI occur is the result of a dynamic interaction between host characteristics and uropathogen virulence determinants. Colonisation with P-fimbriated strains of *E. coli* is a strong risk factor for acute uncomplicated pyelonephritis. The pathogenesis of cystitis is less well understood compared with that of pyelonephritis, and there are no bacterial properties that identify "cystitogenic" *E. coli* clones or distinguish them from strains that cause acute pyelonephritis, although haemolysin, type 1 fimbriae and the prsGJ96 type of P fimbriae may occur more often in acute cystitis strains than in other *E. coli* strains (*Svanborg* and *Godaly*, 1997). The relative importance of bacterial virulence factors and host factors in the pathogenesis of most episodes of acute uncomplicated UTI is not known.

VAGINAL MICROECOLOGY

The microflora of the healthy vagina includes a large number of aerobic, facultative anaerobic, and obligate anaerobic species (*Hooton* and *Stamm*, 1996). Facultative members of the genus *Lactobacillus* are the most prevalent organisms isolated and are found in 50 to 90% of women in mean quantities of $10^{7.2}$ to $10^{8.7}$ colony forming units per gram. Obligate anaerobic lactobacilli are found in up to 60% of women in similar quantities. Aerobic Gram-positive cocci, including *Staphylococcus* species, *Streptococcus* species, and *Enterococcus* species are found in approximately 30 to 50% of women but in lower quantities compared with lactobacilli. Anaerobes outnumber aerobes overall by 10:1 (*Hooton* and *Stamm*, 1996). Alterations in vaginal microflora are thought to play a critical role in facilitating vaginal colonisation with coliforms and, thus, UTI.

It has been hypothesised but not directly demonstrated that lactobacilli protect the vagina by competitive exclusion of pathogenic bacteria (*Redondo-Lopez* et al., 1990). Hydrogen peroxide (H_2O_2), produced by some vaginal lactobacilli strains in almost all normal women, may be important in colonisation resistance (*Eschenbach* et al., 1989). For example, the presence of H_2O_2-positive lactobacilli in the vagina of pregnant women is inversely correlated with infections such as bacterial vaginosis and symptomatic candidiasis or vaginal colonisation by some genital pathogens such as *Gardnerella vaginalis*, *Bacteroides*, *Peptostreptococcus*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, viridans streptococci, and *Enterococcus* (*Hillier* et al., 1992). In addition, *Hawes* et al. (1996) demonstrated that women with H_2O_2-producing lactobacilli colonising the vagina are at less risk of acquiring bacterial vaginosis compared with women who have an absence of H_2O_2-positive lactobacilli. Vaginal lactobacilli, especially H_2O_2-producing strains, may also have a role in protecting the vagina from colonisation with uropathogens as discussed below.

E. coli frequently colonises the vagina, even in women without frequent UTI recurrences (*Hooton* and *Stamm*, 1996). However, women with a history of recurrent UTI are more likely to be vaginally colonised with uropathogens compared with those without such a history (*Hooton* and *Stamm*, 1996). Approximately 32% of prepubertal girls, 16% of pregnant women, and 41% of post-menopausal women have been found to harbour vaginal *E. coli* in prevalence surveys. An even higher proportion of women are found to be colonised when serial vaginal cultures are done. Generally, the factors that predispose to vaginal colonisation also predispose to bladder colonisation and infection. Vaginal colonisation with uropathogens, however, does not inevitably lead to UTI, and it remains to be determined why vaginal colonisation progresses to UTI in some women and not in others. It is likely that vaginal colonisation is usually a necessary predeterminant to UTI, but that other events, such as sexual intercourse, generally must occur to allow infection to occur. Published literature suggests that vaginally colonising uropathogens can enter the bladder and cause infection whether they are transiently or persistently colonising the vagina.

SELECTED HOST FACTORS WHICH INFLUENCE VAGINAL MICROECOLOGY

Nonsecretor phenotype

Women with a history of recurrent UTIs are 2 to 4 times more likely to be nonsecretors of histo-blood group antigens than are women without such a history (*Kinane*, 1982; *Sheinfeld* et al., 1989). Children with febrile UTIs caused by *E. coli* also have a significantly higher prevalence of the nonsecretor phenotype than control subjects (*Jantausch* et al., 1994). Further, uro-epithelial cells from women who are nonsecretors show enhanced adherence of uropathogenic *E. coli* compared with cells from secretors (*Lomberg* et al., 1986). It has not been determined whether nonsecretors are at greater risk for pyelonephritis. Recent data suggests that the biochemical explanation for the increased adherence of *E. coli* to nonsecretors' uro-epithelial cells and for their propensity to develop recurrent UTI may be the presence of unique globoseries glycolipid receptors that bind *E. coli* expressing the P and F adhesins. Through extraction of glycosphingolipids from vaginal epithelial cells collected from nonsecretors and secretors, it has been demonstrated that two extended globoseries glycosphingolipids were selectively expressed by epithelial cells of nonsecretors, but not secretors, presumably as a result of sialylation of the gal-globoside precursor glycolipid, which in secretors is fucosylated and processed to ABH antigens (*Stapleton* et al., 1992).

Exposure to spermicides

Nonoxynol-9 is a nonionic surfactant which is the active ingredient in most spermicidal compounds marketed in the United States. It has been found to have *in vitro* antibacterial activity against several sexually transmitted bacteria, viruses and protozoans (*Bolch* and *Warren*, 1973; *Singh* and *Cutler*, 1982; *Asculai* et al., 1978; *Hicks* et al., 1985). *In vitro* studies have also shown that nonoxynol-9 is markedly less active against uropathogenic bacterial and yeast strains (MIC90 ≥25%) than against *Gardnerella vaginalis* strains (MIC90 ≤0.015%) and the *Lactobacillus* strains (MIC90 8%) tested (*McGroarty* et al., 1990; *Hooton* et al., 1991a). Hydrogen peroxide-producing strains of *Lactobacillus* appear to be more susceptible to nonoxynol-9 (MIC90 4%) than non-producers (MIC90 16%) (*Hooton* et al., 1991a). *Escherichia coli* strains which express type 1 fimbriae and vaginal strains of lactobacilli appear to adhere in significantly higher numbers to vaginal epithelial cells pre-incubated with nonoxynol-9 than to control cells (*Hooton* et al., 1991a).

These *in vitro* findings have generally but not always been confirmed in clinical studies. For example, a recent clinical study did not demonstrate nonoxynol-9 to be protective against human immunodeficiency virus, gonorrhoea, or *Chlamydia* (*Roddy* et al., 1998). We evaluated the effects of contraceptive method on the occurrence of bacteriuria and vaginal colonisation with *E. coli* in a study of 104 women who were seen prior to having sexual intercourse, the morning after intercourse, and 24 hours later (*Hooton* et al., 1991b). After intercourse, the prevalence of *E. coli* bacteriuria increased slightly in oral contraceptive users but dramatically in both spermicidal foam and condom users and diaphragm-spermicide users. Twenty-four hours later, the prevalence of bacteriuria remained significantly elevated only in the latter two groups. Similarly, vaginal colonisation with *E. coli* increased, compared with baseline, in all three groups but was more dramatic in the diaphragm-spermicide users (26% at

baseline vs. 61% after diaphragm-spermicide use; p=0.0002) than in foam and condom users (9% vs. 41%; p=0.001) and oral contraceptive users (15% vs. 35%; p=0.03). The effect on vaginal flora was more persistent in users of diaphragm-spermicide and foam and condoms than users of oral contraceptives. Vaginal colonisation with lactobacilli did not change significantly after intercourse in any of the groups.

In another study, sexually-active women were evaluated prospectively over a 6-month period to determine the effects of sexual intercourse and diaphragm-spermicide use on the vaginal microflora (*Hooton* et al., 1994). Two groups of young women, 20 with a history of recurrent UTI (3 or more UTIs in the past year) and 20 without such a history, were selected to determine if sexual and contraceptive practices had different effects in women at different risk for UTI. At visits at which sexual intercourse with diaphragm-spermicide use was reported in the three preceding days, there were marked alterations in vaginal flora as compared with visits preceded by no sex. Thus, the prevalence of *E. coli* colonisation increased from 13% to 59% (p<0.0001); other aerobic Gram-negative uropathogens increased from 4% to 20% (p=0.0045); Group D streptococci increased from 17% to 33% (p=0.014); Group B streptococci increased from 7% to 27% (p=0.0015); and *Candida* species increased from 7% to 40% (p<0.0001). In contrast to the effect of diaphragm-spermicide use on uropathogens and yeast, the prevalence of lactobacilli decreased to 68% in association with recent diaphragm-spermicide use from 87% at visits preceded by no sex (p<0.0001). Fewer visits were preceded by spermicide use without a diaphragm, but the effect on vaginal colonisation with *E. coli* was the same (66% of women were colonised at visits after spermicide use).

Given the differential effects of nonoxynol-9 on lactobacilli and uropathogens described above, it has been suggested that the adverse effects of spermicide on vaginal flora reported in studies such as those described may be in part due to a decrease in lactobacilli, especially protective H_2O_2-positive lactobacilli, after spermicide use. In a recent study of women with (cases) and without (controls) recurrent UTI, it was found that vaginal *E. coli* colonisation was significantly more frequent in cases than controls (35% vs. 11%; p<0.001) and in women without H_2O_2-positive lactobacilli than in women with H_2O_2-positive lactobacilli (odds ratio [OR], 4.0; p=0.01) (*Gupta* et al., 1998). Spermicide use was associated with greater risk of vaginal *E. coli* colonisation (OR, 12.5; p<0.001) and with absence of H_2O_2-positive lactobacilli (OR, 2.9; p=0.04). The inverse association between H_2O_2-positive lactobacilli and vaginal *E. coli* colonisation remained in case patients after controlling for spermicide use (OR, 6.5; p=0.02). Another recent study showed an increase in vaginal coliforms and decrease in vaginal lactobacilli after nonoxynol-9 instillation in the absence of sexual activity or diaphragm use (*Rosenstein* et al., 1998). Women with reduced lactobacilli were less likely to regain normal flora than were those whose lactobacilli were unaffected. However, coliform colonisation occurred whether lactobacilli produced H_2O_2 or not. *Hillier* et al. (1992) also did not find an association between vaginal colonisation with *E. coli* and absence of H_2O_2-producing lactobacilli in their study of pregnant women. Moreover, in a recent study among women using 3.5% nonoxynol-9 gel daily for two weeks, loss of H_2O_2-positive lactobacilli was not observed. However, only 23% of the study women had H_2O_2-positive lactobacilli at baseline suggesting that the study group

had a high rate of abnormal flora at baseline (*Richardson* et al., 1998).

Based on these *in vitro* and clinical studies, it seems likely that the differential antimicrobial activity of spermicides may alter the vaginal ecosystem, provide an environment conducive to the growth of uropathogens and, thus, predispose women who use these products to UTI. Spermicides may provide this selective advantage in colonising the vagina with nonoxynol-9-resistant uropathogens via a reduction in vaginal lactobacilli. Even the relatively small amounts of nonoxynol-9 on coated condoms increase the risk of UTI, presumably by altering flora and facilitating uropathogen colonisation (*Fihn* et al., 1996). Uropathogens from the faecal reservoir that come into contact with the vaginal introitus during insertion of a spermicide, especially if a diaphragm is used concomitantly, may be more likely to persist in the introitus in the presence of nonoxynol-9 because of a reduction in colonisation resistance attributable to a reduction in vaginal lactobacilli (especially H_2O_2-producing strains) and possibly an increased adherence to epithelial cells. Some studies as noted above, however, have not demonstrated that nonoxynol-9 significantly affects H_2O_2-producing lactobacilli. Thus, the mechanism whereby spermicide alters vaginal flora warrants further investigation.

Exposure to antimicrobials

Certain antimicrobials, particularly beta-lactams, can facilitate vaginal colonisation with uropathogens in animals. In a study of adult female cynomolgus monkeys, who carry the Gal-Gal receptor for P-fimbriae, persistent colonisation with P-fimbriated *E. coli* could be obtained in only 4 (17%) of 24 experiments in which the vagina was washed with a suspension of the strain (*Herthelius* et al., 1988). However, a persistent and heavy colonisation of the vagina occurred in 5 of 5 attempts when amoxicillin was administered intravaginally at the same time. Likewise, previous exposure to intravaginal cephadroxil was also shown to promote vaginal colonisation with cephadroxil-susceptible P-fimbriated *E. coli* (*Winberg* et al., 1993). There was a marked decrease in the total number of indigenous vaginal anaerobic bacteria following cephadroxil exposure. Data from these studies and other amoxicillin studies (*Herthelius* et al., 1989a; *Herthelius-Elman* et al., 1992a) suggest that facilitation of *E. coli* colonisation by these antimicrobials may be due to alterations in the indigenous anaerobic flora of the vagina and, thus, altered colonisation resistance. The natural colonisation resistance could not clearly be correlated with the presence of lactobacilli, which were only transiently reduced by amoxicillin. The colonisation resistance against *E. coli* could only partly be restored by vaginal instillation of lactobacilli, but was fully restored by flushing of the whole vaginal flora from a healthy monkey (*Herthelius* et al., 1989b). Trimethoprim and nitrofurantoin, which have much less effect on the periurethral anaerobic flora than does amoxicillin (*Lidefelt* et al., 1990), did not result in enhanced vaginal colonisation with *E. coli* in similar monkey experiments (*Herthelius-Elman* et al., 1992b).

Human data also suggest that certain antimicrobials facilitate susceptibility to UTI. Ampicillin given to adult women with acute cystitis induced a profound reduction in the indigenous genital flora and a concomitant increase in genital *E. coli* colonisation (*Reid* et al., 1990). Amoxicillin given to girls with respiratory tract infections resulted in a dramatic decrease in the peri-urethral anaerobic flora and a concomitant increase in the aerobic gram negative peri-urethral flora which normalised three weeks after therapy (*Lidefelt* et al., 1991). In contrast, ten girls given

trimethoprim-sulfamethoxazole had no major changes in their anaerobic or aerobic Gram-negative microflora during or after therapy. We have found that women with *E. coli* cystitis who are treated with amoxicillin or cefadroxil are more likely to have persistent vaginal and urethral colonisation with *E. coli* and more frequent recurrences of cystitis than women treated with trimethoprim-sulfamethoxazole or fluoroquinolones (*Hooton* et al., 1995). The superior efficacy of trimethoprim-sulfamethoxazole and fluoroquinolones in treating cystitis as determined by a 4 to 6 week follow-up suggests that drugs which eradicate introital *E. coli* while maintaining anaerobic flora are associated with the best outcome.

In a recent prospective study of premenopausal women starting a new contraceptive method, we found that 326 women in a university cohort and 425 women in a health-maintenance organisation cohort were at increased risk for UTI (OR 2.57 and 5.83, respectively) if antimicrobials had been taken during the previous 15 to 28 days but not during the previous 3, 7, or 14 days (*Smith* et al., 1997). The increased risks were noted both for women whose antimicrobial use was for treatment of a previous UTI and for women who received antimicrobials for other illnesses. These results are further convincing evidence that recent antimicrobial use increases a woman's risk of UTI, perhaps by altering the indigenous urogenital flora and predisposing to vaginal colonisation wit uropathogens.

Exposure to oestrogen

In vitro experiments suggest that oestrogens are more likely than progesterones to increase *E. coli* adherence. HeLa cells incubated with increasing concentrations of oestrogens had progressively enhanced attachment of *E. coli*, staphylococci and other bacteria whereas other hormones, including progesterone, had no such effect (*Sugarman* and *Epps*, 1982). *In vitro* studies have also demonstrated that adherence of *E. coli* and other uropathogens to human vaginal or uro-epithelial cells is highest for cells collected during the phase of the menstrual cycle when oestrogen peaks (*Hooton* and *Stamm*, 1996). Animal studies have also shown a peak in adherence of bacteria to vaginal epithelial cells in the pro-oestrus and oestrus of rats, which appeared to be related to oestrogen levels (*Hooton* and *Stamm*, 1996). *Sobel* and *Kaye* (1986) demonstrated significantly increased attachment of *E. coli* to both vaginal and bladder epithelial cells in oestrogenised rats compared with non-oestrogenised rats. Moreover, several studies have shown that oestrogen treatment facilitates experimental UTI in animals (*Hooton* and *Stamm*, 1996). Of note, some studies have not demonstrated variation of adherence to vaginal epithelial cells with uropathogens during the menstrual cycle (*Svanborg-Eden* et al., 1980). Conflicting study results may be due to strain variability in oestrogen-mediated alterations in adherence or to technical differences in the assays.

Human studies have shown that vaginal colonisation with *E. coli* is most likely during and just after the menses (*Hooton* and *Stamm*, 1996). *Sharma* et al. (1987) showed that women administered oral contraceptives had increased adherence of *E. coli* to their uro-epithelial cells compared with adherence to their cells before hormone administration. However, the cyclical variation in adherence, with the peak level just before the midcycle, was maintained after hormone administration, although the difference in the peak and trough adherence level was less after hormone administration. Contraceptives possibly increased the risk of UTI in a general population of post-menopausal women (*Orlander* et al., 1992), although these

results were not controlled for possible increases in sexual activity in pill users. In premenopausal women, oral contraceptive use appears not to be associated with an increased risk of UTI (*Strom* et al., 1987).

To evaluate a possible association between UTI and the menstrual cycle in women, we studied 577 women enrolled in antimicrobial treatment trials for acute cystitis (*Hooton* et al., 1996b). Patients were administered a standardised questionnaire which asked for the date of onset of the last menstrual cycle (LMP). Women were significantly more likely to present with UTI 8 to 15 days after the onset of their LMP, which is generally the time of peak oestrogen secretion, than at any other time of the cycle (41% presented during this interval) ($p<0.001$). This association was true for women with UTI caused by *E. coli* (41% presented 8 to 15 days after onset of their cycle; $p<0.001$) and for those with UTI caused by *S. saprophyticus* (47% presented 8 to 15 days after onset of their cycle; $p<0.001$). These data demonstrate a strong association between the time at which women present with acute cystitis and the time from the onset of their last menstrual period. We were not able to determine whether this association was due to a hormonal mechanism or to changes in sexual behaviour in relation to the menstrual cycle (James, 1971; *Spitz* et al., 1975; *Hedricks* et al., 1987).

Recent studies in postmenopausal women further support an association between hormonal status, vaginal flora and UTI. *Raz* and *Stamm* (1993) studied 93 postmenopausal women with a history of recurrent UTI in a randomised, double-blind, placebo-controlled trial of a topically applied intravaginal oestriol cream and evaluated patients serially monthly for 8 months. The incidence of UTI in the group given oestriol was significantly reduced compared with that in the placebo group (0.5 vs. 5.9 episodes per patient-year, $p<0.001$). Lactobacilli were absent in all vaginal cultures before treatment and reappeared after one month in 61% of the 36 oestriol-treated women compared with none of 24 placebo recipients ($p<0.001$). The prevalence of *Enterobacteriaceae* fell from 67% to 31% in oestriol recipients but was virtually unchanged in the placebo recipients ($p<0.005$). There appeared to be a relation between vaginal colonisation with lactobacillus and UTI in that 3 of 23 oestriol-treated women who were colonised with lactobacillus after therapy developed UTI compared with 7 of 13 who were not colonised. Although these findings appear to contradict the findings noted above in premenopausal women, any adverse effects of oestrogen in postmenopausal women may be overshadowed by oestrogen's effect on restoration of indigenous lactobacilli to the vaginal environment and reduction of colonisation and infection with uropathogens, perhaps by lowering pH, production of bactericidal substances, or competitive exclusion of uropathogens from uro-epithelial cells.

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