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Correlation between hydrophobicity and resistance to nonoxynol-9 and vancomycin for urogenital isolates of lactobacilli

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1. SUMMARY

Seven clinical isolates of lactobacilli were found to be relatively hydrophobic with a mean water-contact angle of 66 ± 15 degrees, and to be susceptible to 1% nonoxynol-9 and vancomycin. However, seven other strains were relatively hydrophilic with a mean water-contact angle of 32 ± 13 degrees, and found to be resistant to 25% nonoxynol-9 and vancomycin. Thus, the surface properties of lactobacilli that influence susceptibility to antimicrobial agents may involve surface hydrophobicity. Possibly the penetration barrier posed by the cell surface towards these two non-ionic antimicrobials is lower for hydrophobic cells than for hydrophilic cells.

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2. INTRODUCTION

Urogenital lactobacilli are believed to play a protective role in maintaining host resistance to infection [1]. Although selected strains have been shown to prevent recurrent urinary tract infections in humans [2,3], much remains to be understood about the microbiol ecology of the urogenital tract. The adhesion of lactobacilli to the mucosa, their competitive exclusion of uropathogens and their production of inhibitory substances, possibly including hydrogen peroxide, are believed to be important in maintaining a dominant presence in the flora [1,4]. Although specific adhesins, possibly including lipoteichoic acid [5] probably aid lactobacillus adhesion to mucosal cells, hydrophobicity is also presumed to have an effect.

Previous studies in vitro [6,7] and in vivo [8] have shown that nonoxynol-9, the non-ionic detergent in many spermicides, can kill lactobacilli, while uropathogens and *Candida* thrive in its presence and induce an increased incidence of infection. Vancomycin is also a non-ionic antimicrobial agent that affects bacteria at the cell wall. Resistance to vancomycin of Gram-positive organisms such as coagulase-negative staphylococci and *Enterococcus faecalis* has particular clinical importance [9,10]. The presence of urogenital flora resistance to vancomycin may have ecologic implications. The aim of the present study was to measure the hydrophobicity of 14 strains of lactobacilli and to determine whether there was any correlation with the extent of resistance to nonoxynol-9 and vancomycin.

3. MATERIALS AND METHODS

3.1. Bacteria

Fourteen *Lactobacillus* strains were isolated from the urethra or vagina of adult females with no history of urogenital infection, except for three isolates which came from women with a history of yeast vaginitis (strains 36 and 62) and urinary tract infection (strain 65). The organisms were stored in skim milk at −70°C, and cultured on MRS agar and in MRS broth (Difco, Detroit) in 5% CO_2 for the assays. Strains 55, 60, 68, 75, RC-20, RC-15, and RC-28 produced hydrogen peroxide, while the others did not.

3.2. Contact angle measurements

A suspension of washed cells in water was sucked through a cellulose acetate filter by negative pressure until a multi-layer of bacterial cells was deposited. Subsequently, the drying time of these filters prior to the occurrence of so-called 'plateau contact angles' [11,12] was determined and found to be 2–3 h. Water-contact angles were then measured using the sessile-drop technique. Two filters were prepared out of one culture and three separate cultures were made in order to take account of possible biological variations.

3.3. Nonoxynol-9 susceptibility

Doubling dilutions of nonoxynol-9 were made in MRS broth ranging from 0.1–25%, and the testing of lactobacillus susceptibility was carried out over 18 h as reported previously [6]. The culture tubes were scored for growth and the minimum inhibitory concentration was recorded as the lowest concentration demonstrating no growth. Susceptibility and resistance of lactobacilli to nonoxynol-9 was assigned by us to strains inhibited by < 1.0% and those able to grow in all concentrations up to 25%.

3.4. Vancomycin resistance

Lactobacilli were cultured on Mueller-Hinton medium (Difco) supplemented with 5% sheep blood overnight at 37°C in 5% CO_2. Colonies were resuspended in phosphate buffered saline (PBS, pH 7.1) to achieve point 1 on the McFarland scale and then swabbed onto fresh plates. Vancomycin disks (BBL Sensi-Disc, Becton Dickinson, Cockeysville, MD) were put on the plates and the zones of inhibition were measured after a 24-h incubation.

4. RESULTS AND DISCUSSION

The water-contact angles presented in Table 1 range from very hydrophilic (19°) to very hydrophobic (87°). Clearly, these organisms have quite different surface properties, even within a single species, as witnessed here by comparing the hydrophilic *L. plantarum* RC-6 with the more hydrophobic *L. plantarum* RC-20. The fact that these lactobacilli differ in hydrophobicities may explain their differential adhesion capacities to epithelial cells. For example, hydrophilic *L. casei* GR-1 is much more adherent than hydrophobic *L. plantarum* RC-20 (mean of 64 bacteria per epithelial cell compared to 23 per cell) [13]. The fact that most of the hydrophilic strains tested were *L. casei* might indicate that this reflects a property of that particular species.

As shown in Table 1, two groups of strains emerged based upon their resistance or susceptibility to nonoxynol-9 and vancomycin. The susceptibility and resistance patterns for the 14 strains corresponded to a highly statistically significant degree ($P < 0.001$) to relative cell-surface hydrophobicity. It is possible that a hydrophobic

Table 1

Hydrophobicity of lactobacilli and relationship with susceptibility to vancomycin and nonoxynol-9

Strain	Contact angle (degrees)	Vancomycin	Nonoxynol-9
L. gasseri 60	67	S	S
L. casei 55	36	S	S
L. acidophilus 68	74	S	S
L. acidophilus 75	65	S	S
L. plantarum RC-20	79	S	S
L. casei RC-15	52	S	S
L. jensenii RC-28	87	S	S
	Mean 66 ± 15		
L. casei 8	30	R	R
L. casei 11	43	R	R
L. plantarum RC-6	25	R	R
L. casei 36	19	R	R
L. casei 62	19	R	R
L. casei 65	58	R	R
L. casei GR-1	33	R	R
	Mean 32 ± 13		

R = resistant, S = susceptible.
Mean of 66 is significantly greater than 32 (Chi-squared test, $P < 0.001$).

cell surface posed less of a barrier for the penetration of the non-ionic spermicide than did hydrophilic cell surfaces. While no other studies of this nature have been carried out with lactobacilli, experiments with single strains of four *Bacteroides* species showed that a hydrophobic cell surface allowed the penetration of hydrophobic antimicrobial agents [14]. This agrees with our findings. Only 2 of the strains (55 and 65) did not quite follow the trend of the other strains for hydrophobicity, but their antimicrobial patterns placed them firmly in the two groupings. The finding of exceptions is not unexpected, as there are likely some lactobacillus isolates which can resist antimicrobial agents through mechanisms other than the ones being proposed here.

The results showed that the hydrophobic strains were susceptible to nonoxynol-9, while the hydrophilic isolates were resistant to its action. Although not the focus of this paper, the results do have ecological implications. The advent of condom products containing high concentrations of spermicide (up to 18% in some products) to which the vagina is exposed, and the general increase in use of spermicides due to their effectiveness against sexually transmitted diseases, suggests that the normal vaginal flora of many women might become disrupted. If the persistant use of a non-ionic spermicide leads to a highly adherent and selected population of lactobacilli resistant to nonoxynol-9 and able to interfere with infectious agents (uropathogens, *Candida*), the patient may continue to be protected from an increase in urogenital infections. However, a patient could be at risk of urogenital infection after nonoxynol-9 use if the lactobacilli are susceptible to nonoxynol-9 or if surviving strains are poorly adherent and without protective properties.

The discovery of vancomycin resistant organisms in 7 of the 14 strains confirms recent results by Swenson and colleagues [15]. What has not been reported before was the finding that the more hydrophobic lactobacilli (mean contact angle of 66 degrees) were susceptible to the vancomycin. while the resistant organisms were relatively hydrophilic (mean 32 degrees). It is uncertain whether increased exposure of the vaginal and intestinal flora to vancomycin has altered their resistance patterns, hydrophobicity and adhesiveness. Such an ecological effect of antimicrobial therapy is feasible, as vancomycin is not absorbed from the gastrointestinal tract [16]. Since vancomycin is also a non-ionic antimicrobial agent, the influence of cell surface hydrophobicity upon resistance may be explained on a similar basis as nonoxynol-9. However, a preliminary testing of highly ionogenic tetracycline (unpublished data) has not shown a correlation between resistance and hydrophobicity, probably due to the additional involvement of electrostatic forces in the penetration of the antibiotic through the cell surface.

In conclusion, the cell surface hydrophobicity of lactobacilli may play an important role in the susceptibility and resistance to antimicrobial agents used in the urogenital tract of adult women.

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