SUSCEPTIBILITY TO SOME ANTIFUNGAL DRUGS OF VAGINAL LACTOBACILLI ISOLATED FROM HEALTHY WOMEN

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SUMMARY

Samples were taken from the lateral vaginal wall of 19 healthy women. Lactobacilli were isolated from ten of these women. Fifty-eight *Lactobacillus* isolates were identified as *L. gasseri*, *L. vaginalis*, *L. acidophilus*, *L. delbrueckii* spp. *lactis*, *L. crispatus*, *L. plantarum*, *L. cellobiosus*, *L. jensenii*, *L. salivarius*, *L. curvatus*, *L. brevis* and *L. oris*. The inhibitory effect of isoconazole and oxiconazole in 0.2, 0.1 and 0.05 μg/ml concentrations on 58 *Lactobacillus* spp. and Candida albicans (AJD 180) were investigated, using an agar diffusion method. Although both antifungal drugs showed a large inhibitory effect on *C. albicans*, oxiconazole had a very low inhibitory effect on *Lactobacillus* isolates. The natural resistance of lactobacilli to antifungal drugs may enable the development of an antifungal drug(s)/ probiotic combination for antifungal activity and lactobacilli replacement therapy.

KEY WORDS

vaginal lactobacilli, antifungal, probiotic

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INTRODUCTION

Lactobacilli are the dominant bacteria of a healthy human vagina and their presence and number are influenced by estrogen production which undergoes age and menstrual cycle dependent changes /1/. It is generally assumed that vaginal lactobacilli are responsible for protection against pathogenic microorganisms /2/. There is growing interest in the use of lactobacilli of human origin as probiotics against urogenital tract infections /3-5/. It was reported that selected *Lactobacillus* strains can be used to treat vaginal infections as an alternative to antibiotics /4/. Several selection criteria have been used for novel probiotic strains, which can be summarized into three groups: safety, functional and technological aspects. For safety considerations it is important that new probiotic strains have a healthy human origin /6/.

Women worldwide have been plagued by vaginal infections caused by *Candida* spp., generally referred to as candidal vaginitis /2/. Candidal vaginitis is treated with intravaginal or oral antifungal drugs. Lactobacilli have been shown to reduce the adherence of *C. albicans* to vaginal epithelial cells /3/.

After completion of the course of antibiotic therapy, the urogenital flora is not immediately restored, but it can take some 4 weeks to normalize /7/. The main source of candidal vaginitis is disruption of the normal flora, such as with use of broad-spectrum antibiotics. Resistance to antibiotics and antifungal drugs is considered of primary importance for the selection of probiotic microorganisms /8/.

Thus, the present study was undertaken (i) to characterize the dominant species of lactobacilli colonizing the vagina of healthy women, and (ii) to determine some antifungal drugs to which lactobacilli are resistant using *in vitro* techniques. Furthermore, new strains with resistance to antifungal drugs can be selected for the use as a probiotic according to the results of this study. Finally, the use of stable antifungal-resistant strains of normal microflora may be favorable in addition to antifungal/probiotic combination therapies.

MATERIALS AND METHODS

Vaginal swabs were taken from the lateral vaginal wall of 19 healthy women, and were inoculated on lactic agar as a selective medium /13/. Colonies forming a yellow zone were isolated and identified by Gram characteristics, colonial appearance, and cell morphology and motility. The growth of bacteria was examined at 15°C, 45°C, and 50°C in MRS broth with 2%, 4% and 6.5% NaCl. The following identification tests were carried out: utilization of citrate, arginine, esculin and starch hydrolysis, production of acid from fructose, glucose, arabinose, xylose, mannitol, lactose, salicin, sucrose, galactose, raffinose, ribose, melezitose, mannose, sorbitol, trehalose, and maltose. Production of gas from glucose and production of catalase were also tested. The species designation of isolates was confirmed by substrate fermentation (API 50 CHL, Bio-merieux SA) /14/. According to the results of the identification tests, 58 strains were identified as *Lactobacillus* spp.

In preparation for experiments, *Lactobacillus* spp. isolates were cultured under anaerobic conditions for 24 h at 37°C in MRS broth (Oxoid). *C. albicans* (AJD 180) was isolated from human vagina. Candida isolate was identified with the API Candida system (Biomerieux) as *Candida albicans*. The *C. albicans* (AJD 180) was grown for 24 h at 35°C in YPD broth /15/. The determination of the inhibitory effect of isoconazole and oxiconazole in 0.2, 0.1 and 0.05 μg/ml concentrations on *Lactobacillus* isolates and *C. albicans* (AJD 180) was carried out according to an agar diffusion method /16,17/. Inhibition zone diameters (mm) were measured after anaerobic incubation at 37°C for 24 h (BBL GasPak System) using sliding calipers.

RESULTS

In this study, *Lactobacillus* spp. were isolated from 10 of the 19 healthy women tested. As a result of the identification tests, 58 *Lactobacillus* spp. isolates were identified as 12 *L. gasseri* (20%), nine *L. vaginalis* (16%), nine *L. acidophilus* (16%), eight *L. delbrueckii* spp. *lactis* (14%), eight *L. crispatus* (14%), three *L. plantarum* (5%), two *L. cellobiosus* (3%), two *L. jensenii* (3%), two *L. salivarius* (3%), one *L. curvatus* (2%), one *L. brevis* (2%), and one *L. oris* (2%). We found

that *L. gasseri* was the predominant vaginal *Lactobacillus*, followed by *L. acidophilus* and *L. vaginalis,* in healthy Turkish women.

The inhibitory concentrations of isoconazole and oxiconazole used as antifungal drugs were determined on *Candida albicans* (AJD 180) isolated from the vagina. *In vitro* assays demonstrated that both antifungal drugs showed an effect on *C. albicans* (AJD 180). *Lactobacillus* strains were significantly more inhibited by isoconazole than by oxiconazole (Table 1). Many *Lactobacillus* isolates were resistant to oxiconazole. In addition, it was also found that *L. gasseri* H14 and *L. delbrueckii* spp. *lactis* H13 were more resistant to different concentrations of isoconazole and oxiconazole than other strains.

DISCUSSION

That *Lactobacilli* in the vaginal microflora function as a microbiological barrier to infection by urogenital pathogens has suggested their use as a probiotic for treatment and prevention in recent years. The strains selected to be used as a probiotic have to be resistant to antibiotic and antifungal drugs. Some research on vaginal lactobacilli has been carried out in recent years, but there are no published reports concerning vaginal *Lactobacilli* isolated from Turkish women.

Lactobacilli are dominant in the vaginal microflora, at 10^7 or 10^8 cfu/g in the vaginal fluid of healthy premenopausal women /9/. In the present study, the predominant species found in the vagina of 10 healthy women included *L. gasseri*, *L. vaginalis* and *L. acidophilus*. Similar results have been reported by other authors. Benno *et al.* demonstrated that *L. gasseri* was the dominant species among *Lactobacilli* isolated from the intestinal tracts of Japanese people /10/. Song *et al.* reported that *L. crispatus* and *L. gasseri* were the predominant *Lactobacilli* in the vagina of Japanese women /11/. Among them, those belonging to the *L. acidophilus* group and *L. fermentum* were the most frequently isolated. Others species, such as *L. plantarum*, *L. brevis*, *L. jensenii*, *L. casei*, *L. delbruckii* and *L. salivarius* were also isolated /12/. Recently, a new species was found, *L. vaginalis*, resembling *L. fermentum* and *L. reuteri,* whereas phylogenetically it is most closely related to *L. oris* and *L. reuteri* /3/.

Candidal vaginitis is a superficial infection characterized by the presence of vulval and vaginal pruritus or pain and discharge. Intravaginal administration of clotrimazole for 1 week is the recommended

TABLE 1

The inhibitory effect of antifungal drugs on *Candida albicans* AJD 180 and *Lactobacillus* isolates

Strain	Antifungal drug (μg/ml)					
	Isoconazole			Oxiconazole		
	0.2	0.1	0.05	0.2	0.1	0.05
C. albicans (AJD 180)	++++	+++	+++	++++	++++	+++
L. gasseri H14	+	+	–	–	–	–
L. gasseri H15	+	+	+	–	–	–
L. gasseri H16	++	+	+	–	–	–
L. gasseri I2	++	+	+	–	–	–
L. gasseri F1	++	+	+	+	+	+
L. gasseri O2	+	+	+	–	–	–
L. gasseri R3	++	++	+	+	–	–
L. gasseri R4	+	+	+	–	–	–
L. gasseri R5	+	+	+	–	–	–
L. gasseri R7	++	++	+	–	–	–
L. gasseri R10	+	+	+	–	–	–
L. gasseri L1	+	+	+	–	–	–
L. acidophilus A1	++	+	-	–	–	–
L. acidophilus G6	+	+	+	–	–	–
L. acidophilus G7	+	+	+	+	+	+
L. acidophilus G8	+	++	+	–	–	–
L. acidophilus R1	+	+	+	+	–	–
L. acidophilus R6	+	+	+	–	–	–
L. acidophilus 14	++	+	+	–	–	–
L. acidophilus R9	+	+	+	+	+	+
L. acidophilus G11	++	++	+	+	+	–
L. crispatus G1	+++	++	++	–	–	–
L. crispatus G2	++	++	+	–	–	–
L. crispatus G3	++	++	++	–	–	–
L. crispatus G5	+++	++	++	–	–	–
L. crispatus G9	++	+	+	–	–	–
L. crispatus G10	++	+	+	+	+	+
L. crispatus G14	++	++	+	+	+	+
L. crispatus O3	++	++	+	–	–	–

Strain	Antifungal drug (μg/ml)					
	Isoconazole			Oxiconazole		
	0.2	0.1	0.05	0.2	0.1	0.05
***L. delbrueckii* spp. *lactis* G4**	++	++	++	–	–	–
***L. delbrueckii* spp. *lactis* G12**	++	++	++	+	+	+
***L. delbrueckii* spp. *lactis* G13**	+	+	+	+	–	–
***L. delbrueckii* spp. *lactis* H9**	+	+	+	–	–	–
***L. delbrueckii* spp. *lactis* H11**	+	+	+	+	+	–
***L. delbrueckii* spp. *lactis* H12**	++	+	+	–	–	–
***L. delbrueckii* spp. *lactis* H13**	+	+	–	–	–	–
***L. delbrueckii* spp. *lactis* R8**	+	+	+	+	+	+
***L. vaginalis* H1**	+++	++	++	–	–	–
***L. vaginalis* H2**	++	++	+	–	–	–
***L. vaginalis* H3**	++	++	++	–	–	–
***L. vaginalis* H4**	++	++	+	–	–	–
***L. vaginalis* H5**	++	++	+	–	–	–
***L. vaginalis* H6**	+	+	+	–	–	–
***L. vaginalis* H7**	++	++	+	++	++	–
***L. vaginalis* H8**	+	+	+	+	+	–
***L. vaginalis* S1**	++	+	–	–	–	–
***L. plantarum* H17**	+	+	+	–	–	–
***L. plantarum* T1**	++	++	++	–	–	–
***L. plantarum* H10**	+	++	–	–	–	–
***L. jensenii* R11**	++	+	+	–	–	–
***L. jensenii* R12**	++	+	+	–	–	–
***L. salivarius* 11**	++	+	+	+	–	–
***L. salivarius* S2**	++	++	+	–	–	–
***L. cellobiosus* L2**	++	+	+	+	+	+
***L. cellobiosus* 13**	+	+	+	–	–	–
***L. brevis* R2**	++	++	++	–	–	–
***L. curvatus* L3**	+	+	+	–	–	–
***L. oris* A2**	++	+	+	+	+	+

Diameter of inhibition zone:
++++ = 17 mm and over; +++ = 11-16 mm; ++ = 6-10 mm; + = 1-5 mm;
– = no inhibition.

therapy for vaginal candidiasis /3/. However, in the present study, both oxiconazole and isoconazole showed a great inhibitory effect on *C. albicans* (AJD 180).

Many isolates of *Lactobacillus* were found resistant to oxiconazole. This may be important when deciding which agent to use as treatment. The use of oxiconazole may preserve more of the natural vaginal microflora. In addition, it was also found that *L. gasseri* H14 and *L. delbrueckii* spp. *lactis* H13 were more resistant to different concentrations of isoconazole and oxiconazole than other strains.

CONCLUSION

New strains isolated from healthy women may be used as a probiotic for safety considerations. Using these strains as a probiotic together with an antifungal drug could be recommended for the treatment of candidal vaginitis.

ACKNOWLEDGEMENT

This study was supported by Gazi University BAP project no. FEF 05/2002-54 and TR Prime Ministry State Planning Organization project no. 2003 K 12470-06.

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