INTRODUCTION

The vagina is a complex habitat for micro-organisms, and vaginal infections are considered to be among the main causes of morbidity around the world. Studies of the microbial flora of the human vagina reveal that microorganisms normally present in this habitat play an important role in preventing colonization by exogenous bacterial species (Donders et al., 2000; van De Wijgert et al., 2002), normally characterized by a high number of Lactobacillus species. Lactobacillus crispatus and Lactobacillus jensenii are of great importance in maintaining the vaginal pH at approximately 4.7 (or less), which causes growth inhibition of other micro-organisms (Marrazzo, 2003).

Three major types of infections that affect the vaginal habitat are vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis. The first two have been extensively characterized in the literature (Marrazzo, 2003). Bacterial vaginosis is the most common cause of vaginal discharge (Klebanoff et al., 2004) and is described as a disequilibrium in the vaginal microflora in which the normally predominant Lactobacillus species are overgrown by anaerobic and facultative species (Ness et al., 2005). This condition is not associated with vaginal inflammation as occurs in vaginitis. Vaginal infections, but principally bacterial vaginosis, have been associated with many complications, including second trimester miscarriage, pelvic inflammatory disease, preterm birth, preterm premature rupture of the membranes, chorioamnionitis, post-partum endometritis, post-operative infection after gynaecological surgery and easier acquisition of HIV (Beigi et al., 2004; Ness et al., 2004). The aetiological agents of vaginal infection are Candida albicans in vulvoginal candidiasis, and Trichomonas vaginalis in trichomoniasis. The predominant microorganisms that cause bacterial vaginosis are Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum and others such as Prevotella spp., Mobiluncus spp. and Bacteroides spp. (Klebanoff et al., 2004).

Escherichia coli is a commensal inhabitant of the intestinal tract of healthy humans and different animals (Clermont et al., 2000). It is accepted that pathogenic E. coli strains are derived from commensal strains by acquisition of chromosomal or extrachromosomal virulence operons, and although extraintestinal pathogenic E. coli strains are mostly opportunistic, they can cause a diverse spectrum of serious diseases (Escobar-Páramo et al., 2004; Sasakawa & Hacker, 2006). The presence of vaginal E. coli is
controversial with regard to its potential pathogenic role. Some studies report its presence as an opportunistic microorganism (Watt et al., 2003). Others concluded that this species has not been detected on healthy vaginal epithelium (Hyman et al., 2005), and also it has been isolated from aerobic vaginosis. This infection is a condition of abnormal vaginal flora that differs from bacterial vaginosis. Genital complaints are those of a real vaginitis. Group B streptococci, E. coli, Staphylococcus aureus and T. vaginalis are frequently cultured (Donders et al., 2002). Considering the serious obstetric and neonatal complications, and that about 30% of women with vaginosis complaints remain undiagnosed despite extensive testing (Anderson et al., 2004), it is of great importance to determine whether E. coli strains isolated as the sole micro-organism from vaginal infections have particular characteristics. Thus, the aims of this study were to identify E. coli strains isolated as the sole micro-organism from women of reproductive age and presenting vaginal infections, to analyse their phenotypic characteristics by means of biochemical tests, and to determine potential genotypic association among them. E. coli strains isolated in association with other known aetiological agents of vaginal infections were not considered in the present study.

METHODS

Study population. The study population was composed of 425 women of fertile age (18 to 41 years) who attended Talca City Hospital with clinical diagnosis of vaginal infection; they were non-pregnant, non-smokers, had no history of sexually transmitted infections, and had had one sexual partner during the previous 6 months. Also, they had had no antimicrobial treatment within the previous 6 months as revealed by a written questionnaire. In addition, they showed absence of menstruation and had no diarrhoea or urinary tract infections (UTI). Diagnosis of vaginosis was based on detection of three or more Amsel criteria (Amsel et al., 1983) and the microscopic record of bacterial morphology to detect bacterial vaginosis (Nugent et al., 1991). The study protocol was accepted by the Universidad de Talca Bioethical Committee and included an informed consent from the patients, before collecting clinical samples. As control samples, vaginal epithelium from 100 healthy women was inoculated in MacConkey agar (Difco) and Trypticase Soy agar (Difco) supplemented with sheep blood (5%) and incubated in aerobic conditions at 37 °C for 24 h. The same swab was streaked onto Thayer Martin agar (Difco) and incubated in the presence of CO2 (5%) at 37 °C for 48 h. Finally, the swabs were used to inoculate a Sabouraud agar plate (Merck) and on Trichomonas medium (Oxoid), for Trichomonas vaginalis growth. The third swab was used with the Mycoplasma IST-2 kit (bioMérieux) to detect the growth of Mycoplasma hominis and Ureaplasma urealyticum. Finally the same swab was used to detect Chlamydia trachomatis with the TestPack Chlamydia kit (Abbot). Identification of Enterobacteriaceae family members was done with the API 20-E test (bioMérieux) and anaerobic bacteria were identified by phenotypic methods (Joussines-Somer & Sumanen, 1999). Candida albicans was identified by the ID 32C kit (bioMérieux).

In order to compare phenotypic and genotypic characteristics of vaginal E. coli strains with other E. coli isolates from other origins, 15 E. coli isolates from non-pregnant women with UTI, and four E. coli isolates from blood samples of patients with septicaemia and one from a patient with otitis were used. These isolates, obtained with informed consent from patients (as described above), were identified by the same procedures as used with isolates from vaginal infections.

Determination of DNA polymorphism in E. coli strains isolated from vaginal infections by RAPD-PCR. Phylogenetic relationships among the E. coli strains isolated from vaginal infections and those used to compare phenotypic and genotypic characteristics were studied by means of random amplified polymorphic DNA (RAPD). This method has become a highly valuable molecular tool for intra-species characterization of E. coli (Cave et al., 1994; Wang et al., 1993). Genomic DNA was extracted from a single colony using the Aqua Pure Genomic DNA Isolation kit (Bio-Rad) and the DNA concentration was spectrophotometrically determined (A260). PCRs were conducted in 25 µl reaction mixture containing 25 ng DNA template, 1 U Taq DNA polymerase (Invitrogen) in 1× buffer, 300 µM of each dNTP and 0.8 nM of each primer. The primers used were 2H (5'-AAGCTTGCAGCTGT-3') and 3H (5'-AAGCTTGA-TGGCC-3'), which were randomly selected among four arbitrary primers. Amplification was done in a DNA Engine (Bio-Rad) thermal cycler programmed to one cycle of 5 min at 94 °C, 10 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C followed by 20 cycles of 1 min at 94 °C, 1 min at 50 °C and 2 min at 72 °C to allow the completion of DNA extension (Adam et al., 2004). A negative control, consisting of the same mixture but using water instead of DNA, was included in each run. This procedure was repeated twice per strain. Only fragments present in both amplifications were used to define the RAPD patterns. The amplification products were analysed by electrophoresis in 1% agarose gel, stained with ethidium bromide and photographed under UV light. A 1 kb DNA ladder (New England Biolabs) was included as molecular size marker. The RAPD patterns obtained from E. coli strains isolated from vaginal infections were compared to those obtained from E. coli strains isolated from UTI, septicaemia and otitis. The numerical analysis was carried out using Taxotron software (Taxolab) (Chatellier et al., 1997). For each strain, the RAPD type was defined as the combination of the band pattern obtained with both primers. The relationships among the RAPD types of the strains were calculated by the unlabeled pair-group method averages method and represented as a dendrogram.

Statistical analysis. Significance of differences between variables was tested by means of χ² test and Fisher’s exact test.

RESULTS

Bacteriological analysis of vaginal fluids

Table 1 shows that Gardnerella vaginalis was the most frequent micro-organism isolated (33.2%) followed by E. coli (23.0%) in the 425 women with clinical diagnosis of vaginal infection. Other micro-organisms frequently
isolated were Candida albicans and Trichomonas vaginalis, 15.5 % and 9.1 %, respectively. Gram-negative strictly anaerobic bacilli were isolated in only 2.2 % of the cases. No growth of Neisseria gonorrhoeae or Ureaplasma urealyticum was observed. Moreover, in 42 of the samples studied no bacterial growth was detected. Also, 26 vaginal samples from healthy women yielded G. vaginalis strains and a low growth of E. coli, Mobiluncus spp., Enterococcus faecalis, Streptococcus agalactiae and Gram-negative strictly anaerobic bacilli (Bacteroides fragilis, Porphyromonas gingivalis, Prevotella intermedia and Fusobacterium nucleatum).

Table 2 shows micro-organisms recovered from women with vaginal infection.

The molecular masses of the fragments obtained with the 3H primer ranged between 500 and 4500 bp and the fragments produced by the 2H primer ranged between 500 and 6000 bp (data not shown). A total of 46 RAPD profiles were observed among vaginal isolates and 17 among isolates from other sources. The dendrogram presented in Fig. 1 shows three main clusters, labelled A, B and C. Cluster A included a single isolate from otitis. Cluster B encompassed the 46 E. coli strains of vaginal origin plus four strains from septicaemia, and cluster C included the

### Table 2. Number of samples from women with vaginal infection that yielded growth of associated and individual micro-organisms.

<table>
<thead>
<tr>
<th>Micro-organism(s)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. vaginalis + E. coli</td>
<td>29</td>
</tr>
<tr>
<td>C. albicans + E. coli</td>
<td>12</td>
</tr>
<tr>
<td>T. vaginalis + E. coli</td>
<td>8</td>
</tr>
<tr>
<td>G. vaginalis + Mobiluncus spp.</td>
<td>6</td>
</tr>
<tr>
<td>G. vaginalis + C. albicans</td>
<td>5</td>
</tr>
<tr>
<td>G. vaginalis + GNAB*</td>
<td>4</td>
</tr>
<tr>
<td>G. vaginalis</td>
<td>97</td>
</tr>
<tr>
<td>C. albicans</td>
<td>49</td>
</tr>
<tr>
<td>E. coli</td>
<td>46</td>
</tr>
<tr>
<td>T. vaginalis</td>
<td>31</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>10</td>
</tr>
<tr>
<td>Mobiluncus spp.</td>
<td>6</td>
</tr>
<tr>
<td>M. hominis</td>
<td>5</td>
</tr>
<tr>
<td>GNAB*</td>
<td>5</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>3</td>
</tr>
<tr>
<td>C. trachomatis</td>
<td>2</td>
</tr>
</tbody>
</table>

*GNAB, Gram-negative strictly anerobic bacilli (B. fragilis, P. gingivalis, P. intermedia, F. nucleatum).
isolates from UTI. The B cluster was subdivided into two genogroups (B1, 23 strains, and B2, 20 strains), both with E. coli strains of vaginal origin. Genogroups B1 and B2 represented 93.5% of all E. coli strains obtained from a vaginal source.

### DISCUSSION

Vaginal infections are still a major cause of morbidity, leading to millions of dollars spent worldwide annually in physician services and medications. Vaginal infections are not reportable diseases, and therefore accurate estimates of incidence are unavailable (Schwebke, 2000). Infections usually respond to appropriate treatments; however, misdiagnosis and pharmacological failure may occur. Therefore, it is important to diagnose the problem accurately and prescribe the appropriate treatment (Owen & Clenney, 2004). The high prevalence of vaginal infections, along with controversies with regard to aetiological agents, may be related to unidentified bacteria as agents of such infections. The present study establishes differences among E. coli strains isolated from different human habitats, including the vagina, and identifies an association between strains isolated as the sole micro-organism from women with vaginal infections with regard to both phenotypic and genotypic properties. This finding supports a potential role of particular subgroups of E. coli in the pathogenesis of vaginal infections.

The aetiological role of E. coli in vaginal infections has been controversial. In general, their isolation from vaginal fluids is considered as normal, and women are not treated. Our study of vaginal specimens from 425 women with clinical diagnosis of vaginal infection showed 62.4% of bacterial vaginosis cases in comparison to 37.5% of vaginitis, in agreement with previous reports (Klebanoff et al., 2004; Beigi et al., 2004). Moreover, it is important to observe that T. vaginalis and C. albicans were restricted to vaginitis infection. E. coli was present in both bacterial vaginosis and vaginitis (23% of total bacterial isolates), which made it interesting to determine if E. coli strains can be associated with other aetiological agents. In this study E. coli was isolated as the sole micro-organism in 46 cases. The absence of other recognized aetiological agents of vaginal infection made these bacterial strains an interesting research topic.

When the strains from vaginal, UTI, septicaemia and otitis sources were analysed for ornithine decarboxylase, sucrose fermentation and rhamnose fermentation, only those isolated as the sole micro-organism from vaginal infections showed significant particular activities. Thus, only 63% of these E. coli strains showed positive activity for ornithine decarboxylase, and 95% and 52% showed positive activity on rhamnose and sucrose, respectively (versus 100%, 75% and 100%, respectively, for the strains from other sources). It is important to note that phenotypic and chemotaxonomic characteristics are indirect markers of genomic changes (Coenye et al., 2005).

Results obtained by means of RAPD showed different profiles of genomic DNA. These molecular profiles were highly reproducible, allowing the analysis of different genotypic profiles by means of a dendrogram. The results showed that the E. coli strains grouped in three clusters. The strains of vaginal origin were clustered together in a distinct cluster, subdivided into two genogroups, which constituted 93.5% of the total vaginal isolates. This suggests that these strains, isolated as the sole micro-organism from vaginal infections, constitute a separate subpopulation within the species. Our study further demonstrated that E. coli strains isolated from UTI are closely related to the strains of vaginal origin. Other studies have suggested that E. coli vaginal residents present a common phylogeny to uropathogenic E. coli strains (Obata-Yasuoka et al., 2002) and also that uropathogenic E. coli are better adapted than other E. coli to the urethra, periurethra and vagina (Foxman et al., 2002). It is possible that in the referenced studies E. coli was not isolated as the sole micro-organism from vaginal infections and was not causally associated with the vaginal infection.

According to our results, it is plausible to postulate that the E. coli strains isolated as the sole micro-organism from the vagina may belong to a particular phylogenetic group. This could be based on the high genomic plasticity of E. coli and its capacity to acquire or eliminate genes. Since micro-organisms are defined by their environment, it would be unlikely that micro-organisms capable of causing human diseases evolve in such an environment where there are no humans or other animals (Wilson & Salyers, 2003). This is
Fig. 1. Genetic relationships among the *E. coli* strains studied and the respective biochemical tests used for vaginal *E. coli* (0, negative biochemical test; 1, positive biochemical test). ADH, arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; INO, *myo*-inositol fermentation; RHA, L-rhamnose fermentation; SAC, sucrose fermentation; MEL, melibiose fermentation.
likely to apply with respect to mutations that lead to increasing virulence, considering that the amplification of those mutations requires a selective pressure, presumably provided by the host environment, the vagina habitat in this case.

Many genital infections occur as a result of new sexual practices, involving significant microbiological aspects. This could explain the presence of new aetiological agents of vaginal infections or the adaptation of micro-organisms such as Escherichia coli. Finally, the potential role of E. coli in vaginal infections deserves further molecular studies to learn more about the particular subset of this species that appears to be aetiologically involved.

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