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SHORT COMMUNICATION

Endogenous or Exogenous Origin of Vaginal Candidiasis in Polish Women?

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Abstract

Vaginal candidiasis is a common problem of clinical practice. Many studies have been conducted to explain its origin but only a few have included Polish women. The aim of the study was to determine the prevalence and similarity of oral, anal and vaginal *Candida albicans* strains isolated from Polish women with vaginal candidiasis. The study involved 20 from 37 recruited women. Swab samples were collected from their vagina, anus, and oral cavity at two-month intervals. All the women were treated with nystatin. Yeast were recovered and identified by the germ-tube test, API /Vitek system, typed by API ZYM and RAPD-PCR. Chi-square test was used to analyze the data. A total of 170 *Candida albicans* isolates were recovered from 180 samples collected 3 times from 3 sites of 20 women. Positive yeast vaginal cultures were found in all patients before administration of nystatin. Vaginal yeast recovery rate was decreased statistically significant in both follow-up visits ($p = 0.001$; $p = 0.003$). The same and different genotypes/biotypes were found concomitantly in a few body sites and/or repeatedly at time interval from the same body site. The results support the concept of dynamic exchange of yeast within one woman and endogenous or exogenous origin of vaginal candidiasis.

Key words: *Candida albicans*, candidiasis, molecular typing

Vaginal candidiasis (VC) is recognized in 20–50% of women attending primary care clinics because of vaginal complaints (Eschenbach, 2008; Ilkit and Guzel, 2011; Vazquez *et al*., 1994). Peak incidence of VC corresponds to a period of high sexual activity (20–40 years of age) and high-estrogen host status, uncommon before menarche and after menopause (Amouri *et al.,* 2010; Lanchares and Hernández, 2000). The most important factors predisposing to VC are: uncontrolled hyperglycemia, pregnancy, use of high-estrogen oral/intrauterine contraceptives, decreased immune response due to neoplastic diseases or immunosuppression, as well as abuse of broad-spectral antibiotics in combination with yeast vaginal colonization (Amouri *et al.*, 2010; Sobel, 2007). Some studies have in several cases failed to find predisposing factors to VC (Sobel, 1985), thus from the clinical point of view VC is characterized as an oestrogen – related hypersensitivity response to a commensal organism (Fischer, 2012). About 75% of women have at least one episode of VC during their adult life and are treated with antifungal drugs (Hurley and DeLouvois, 1979; Lanchares and Hernández, 2000; Maffei *et al.*, 1997; Sobel, 2007). Some of them can be culture-positive for yeast in the vagina, experience relapse (20–50%) or progress of symptoms within 1–3 months after clinically successful therapy (Amouri *et al.*, 2010; Lanchares and Hernández, 2000; Mercure *et al.*, 1993; Ringdahl, 2000; Sobel, 1996; Sobel, 1990; Vazquez *et al*., 1994) and approximately 5% have a recurrent infection (Sobel, 2007; Sobel, 1993).

Although infections due to non-*albicans Candida* species are increasing in prevalence (10–30%), multiple research studies permanently recognize *Candida* (*C.*) *albicans* as the most important infectious agent of VC with prevalence to 80–95% in various reports (Amouri *et al.*, 2010; Chong *et al.*, 2003; Darce Bello *et al*., 2002; Mahmoudi Rad *et al.*, 2011; Mendling *et al.*, 2000; Sobel, 2007; Sobel, 1993; Sobel, 1985). *C. albicans* belongs also to the most common fungal species isolated from vaginas of women without clinical manifestation (al-Rawi and Kavanagh, 1999; Chong *et al*., 2003; Darce Bello *et al*., 2002; Li *et al*., 2008; Mendling *et al.,* 2000; Sobel, 1985; Vazquez *et al*., 1994). Vaginal colonization is asymptomatic in 20% (range 10–80%) of women, higher in pregnancy (especially at week 28 or later) and in the women, who possess VC risk factors (Lanchares and Hernández, 2000; Mahmoudi Rad *et al.,* 2011; Sobel, 2007).

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Circulation of *Candida* strains between the vagina and other colonized body sites can be responsible for the difficulties in treatment and management of VC. The oral cavity and anus appear to be a site of *C. albicans* persistence and a source of endogenous re-infection (Lanchares and Hernández, 2000; Sobel, 2007). Molecular methods used for genetic typing of strains have revealed similarity as well as diversity between oral, anal and vaginal *C. albicans* isolates (Ge *et al.*, 2012; Schmid *et al.*, 1993; Xu *et al.*, 1999). Colonization of the vagina by isolates of the same type for a longer time period can explain why moderate symptoms of VC or relapse after treatment are observed in some women (Lanchares and Hernández, 2000; Sobel, 1985; Vazquez *et al.*, 1994). The diversity of molecular types among strains isolated from the site of infection and colonized areas may suggest re-infection, not necessarily caused by endogenous strain, but by vaginopathic isolate derived from exogenous sources (Ge *et al.*, 2012; Li *et al.*, 2008). Up to now, many studies regarding VC have been conducted to explain the microbiological origin and epidemiology of VC in the world. However, only a few have included Polish women.

The objective of this work was to determine the prevalence and similarity of oral, anal and vaginal *C. albicans* strains isolated from women with VC in a period of 4-months with using phenotyping and genotyping methods.

Patients with VC from West Pomeranian region of Poland were recruited to the study (n = 37) based on a written questionnaire. Women in pregnancy, with diabetes mellitus, immunodeficiency, neoplastic diseases, receiving oral contraceptives, immunosuppressed drugs or antibiotics were excluded from the study. All signed a written consent for participation and re-attendance at follow-up visits after VC treatment. Of the women recruited 20 completed the study. They were aged 21–44 years (average 33). No known sister-sister, mother-sister, or cohabitation relationships were identified among the subjects. All the women presented symptoms of vaginal yeast infection during the first visit and received nystatin. All of them were under the care of the same gynecologist, who collected samples from the vagina, anus, and oral cavity three times at two-month intervals: I – after diagnosis of VC and before topical treatment with nystatin, II – after 2 months, III – after 4 months. Swab specimens were cultured at 37°C for 48 h on SDA (Sabouraud Dextrose Agar) medium for yeast isolation and on chromogenic agar medium for estimation of yeast morphology. All morphologically distinct yeast colonies were recorded as "different" isolates and were identified by the germ tube test, API ID 32 C kit or by the Vitek Compact 2 system (BioMérieux SA, France). Subsequently, all strains were biotyped using API ZYM (BioMérieux SA, France) and genotyped using optimized RAPD-PCR (random amplified polymorphic DNA). Biotypes were determined based on activity of 5 hydrolases: esterase C4, valine arylamidase, naphtol-AS-BI-phosphohydrolase, α-glucosidase, N-acetyl-beta-glucosamidase (Kurnatowska and Kurnatowski, 1998; Wiliamson *et al.*, 1986). In optimized RAPD-PCR method was used the primers set: 1290 (5'-GTG GAT GCG A-3')/1247 (5'-AAG AGC CCG T-3') (Cobb and Clarkson, 1994; Cobb and Clarkson, 1992; Schmid *et al.*, 1990). The genetic similarity of the isolates was calculated using the Dice similarity coefficient (S_{AB}), clustered by the unweighted pair group method (UPGMA) and visualized by the dendrogram (Sobel, 2003; Soll *et al.*, 1991). The threshold for clusters of genetically related and different isolates was calculated respectively at a $S_{AB} \geq 0.80$ and at a $S_{AB} < 0.80$. The control organism used in the study was *C. albicans* ATCC 24433.

The occurrence of yeast-positive cultures in a single body site in the following visits was compared χ-square test with Yates correction factor and Fisher's exact probability test (two side-test). A comparison of yeast-positive cultures between two or three examined body sites within a single visit performed respectively by McNemar's test and Cochrane Q test was made. Distribution of the number of *C. albicans* isolates in a single body site between visits was analyzed using comparison of 2 counts. In all calculations assumed p-values of < 0.05 statistically significant.

A total of 139 yeast-positive cultures (77.2%) and 170 isolates of *C. albicans* were recovered from 180 swab samples collected three times from 3 body sites of 20 women. During the first sampling before treatment, positive vaginal cultures were found at all symptomatic patients. In the follow-up visits (II and III) yeast-positive samples from vagina were recovered respectively in 50% and 60% of cases.

Statistically significant differences were noted in the yeast recovery rate from vagina between visits I and II as well as between visits I and III, $p = 0.001$ and $p = 0.003$, respectively. Significantly less vaginal *C. albicans* strains isolated from visit II in comparison with the visit I ($p = 0.05$) (Table I). During one sampling *C. albicans* positive cultures were most commonly found in all three body sites at once – 46.7%. Differences in yeast-positive samples between three studied body sites within visits I and II were statistically significant ($p = 0.04$) (Table I). The colonization of two sites occurred with 38.4%, mostly in the combinations: anus – oral cavity and vagina – oral cavity. Single sites were colonized rarely, mostly oral cavity (Table II).

Mixed cultures as a combination of *C. albicans* and non-*albicans* species (*C. parapsilosis* or *C. dubliniensis*) were found at 2 women (K.I, F.U.) only but the non-*albicans* strains (n = 2) were excluded from further

Table I
The number and percentage of yeast-positive cultures and *C. albicans* strains isolated from 3 body sites of women during 3 consecutive visits

No of visits/ samples	Yeast – positive cultures; n (%)							*Candida albicans* isolates; (n)		
	vagina	oral cavity	anus	p-value (Cochrane Q test)	p-value McNemar's test			vagina	oral cavity	anus
					vagina-oral	vagina-anus	oral-anus			
I n=20/20/20	20 (100)	18 (90)	15 (75)	0.04	0.5	0.1	0.3	24	25	20
II n=20/20/20	10 (50)	17 (85)	16 (80)	0.04	0.05	0.1	1.0	10	19	18
III n=20/20/20	12 (60)	18 (90)	13 (65)	0.09	0.1	1.0	0.7	15	22	17
p-value – χ-square test with Yates correction factor or Fisher's exact probability test (two side-test)								p-value – comparison of 2 counts		
I vs II	0.001	1.0	1.0					0.05	0.3	0.75
I vs III	0.003	1.0	0.73					0.15	0.7	0.65
II vs III	0.75	1.0	0.48					0.3	0.7	0.9

Table II
The incidence of *C. albicans* in different body sites of women during one visit (60 samples)

Body site	Number of *C. albicans* samples (%)
oral cavity	6 (10.0)
anus	2 (3.3)
vagina	1 (1.6)
anus – oral cavity	10 (16.7)
vagina – oral cavity	9 (15.0)
vagina – anus	4 (6.7)
vagina – oral cavity – anus	28 (46.7)

study because of their single colonies in the culture in comparison with *C. albicans*. From 4 women 2 morphotypes of *C. albicans* in vaginal sample were isolated, whereas 3 (15%) women experienced recurrent VC (J.A, P.I, S.I.); the remaining ones were colonized without symptoms of vaginal infection in the 2 follow-up visits.

C. albicans strains were classified to 6 biotypes – Fig. 1. The most common was biotype C (58.8%) which was found in 14 (70.0%) of women, at the infection site (vagina I) and colonized body sites throughout the study time. *C. albicans* strains classified to biotype Z (described by the authors of the study) exhibited the activity three (esterase C4, valine arylamidase, N-acetyl-beta-glucosaminidase) of five enzymes used for biotyping. Those strains were isolated only from vagina of 3 different women (B.Ka, K.D, W.Z.) during follow-up visits after vaginal candidiasis treatment. Each strain of biotype "Z" presented a different genetic pattern, not observed in other body sites of these women.

Based on the average similarity coefficient (S_{AB}=0.80) calculated for all studied *C. albicans* strains 31 genotypes

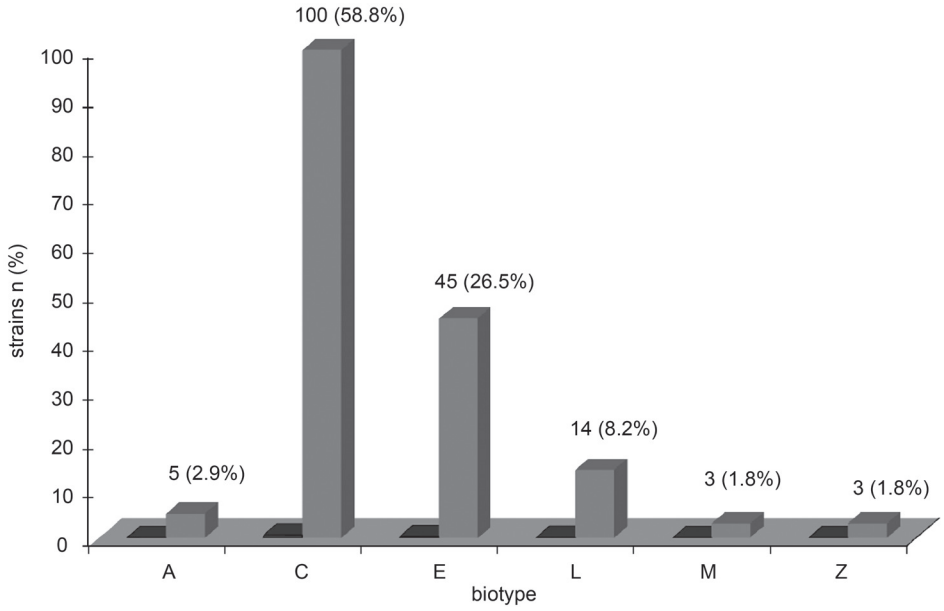

Fig. 1. Biotypes of *C. albicans* strains isolated from women with vaginal candidiasis

Table III
The genotypes and biotypes of *C. albicans* strains isolated from vagina, oral cavity and anus of women with vaginal candidiasis during three consecutive visits

No.	Patient	Age (years)	VISIT I (before treatment)			VISIT II (follow-up visit)			VISIT III (follow-up visit)		
			vagina	oral cavity	anus	vagina	oral cavity	anus	vagina	oral cavity	anus
1.	B.K.	36	27. E 27. E	27. E	27. E*			27. E		27. E	
2.	B.Ka.	23	u13. E			7. Z		22. E	22. E*		22. E
3.	B.M.	44	7. E	12. M	12. M		13. E	13. C		12. E	12. E
4.	F.A.	25	16. E	u20. L	14. C*		6. C		13. E	1. L	
5.	F.U.	36	17. C 17. E	17. C			17. C	14. C		7. C	
6.	G.D.	29	20. C	u8. E 21. C 23. E	18. C		21. E	18. C		u11. E 26. E	18. C 23. E
7.	J.A.[R]	32	17. C		u7. C	17. C	4. C*	4. C	17. C	4. C	4. C 4. C
8.	K.M.	36	1.C	9. C 10. L	10. L	10. E	9. A	11. C	9. C	9. C	9. C 10. C
9.	K.D.	38	u16. C	15. C			15. C		24. C u15. Z	25. C*	24. C
10.	K.I.	31	29. C u18. C	u9. C u19. C	29. E*	28. E	u17. C	28. L	28. E	30. C	
11.	M.A.	23	10. E	1. C 7. E	11. E		1. E	11. E		8. E	1. E
12.	M.K.	26	u3. C u5. A	u4. A	4. E		4. A	4. A 4. L		4. M	
13.	P.I.[R]	42	6. L	8. C 16. C		6. L	u2. C u6. L	15. L 16. C	6. L*	13.C 16. L	16. C
14.	R.A.	29	1. C	2. C	2. C		2. C u1. C	2. C		2. C	2. C
15.	R.Ag.	26	u12. C	17. C	17. C	17. C	17. C	17. C	17. C	17. C	
16.	S.I.[R]	32	31. E	31. C	31. E	31. E	31. C	31. E	31. E	31. C	31. E
17.	S.A.	36	30. C	23. C	30. C			30. C	22. C		3. C
18.	S.Z.	41	5. C	26. C	26. L	4. C	5. C			12. C	
19.	W.Z.	44	19. C	17. C*		19. C	17. E		20. Z	17. E	22. C
20.	Z.E.	21	u10. C	19. C	19. C*	19. C	19. C	19. C	3. C	3. C	19. C u14. C

[R] – women with recurrent vaginal candidiasis; * two *C. albicans* isolates with different morphotypes presented the same genotype
Numbers denote genotype, capital letters denote biotype, u – unrelated genetic patterns

were revealed that grouped strains being identical ($S_{AB} = 1$) and genetically related isolates ($S_{AB} = 0.80–0.99$). The 20 unrelated isolates ('u') with unique patterns ($S_{AB} < 0.80$) were found (Table III). *C. albicans* strains from each woman were assigned to 1– 5 genotypes. Usually a single genotype of *C. albicans* was found in oral cavity, anus and vagina, in 40%, 65% and 55% of patients, respectively. The occurrence of two genotypes of *C. albicans* at a single body site was observed in 30–35% of the women, while the three genotypes in 5–20%. There were no statistically significant differences in the occurrence of one, two or three genotypes of *C. albicans* at a single body site. Based on average similarity coefficient between pairs of *C. albicans* isolates revealed that pairs of vaginal and anal isolates were more similar ($S_{AB} = 0.80 \pm 0.05$) than other pairs of isolates. The similarity of vaginal/oral and anal/oral pairs of strains was $S_{AB} = 0.74 \pm 0.04$ and $S_{AB} = 0.75 \pm 0.01$, respectively.

C. albicans strains belonging to the same genotype and biotype were isolated concomitantly from 2

or 3 body sites, from 13 and 4 women, respectively as well as 2 or 3 times at time intervals from the same body site, from 12 and 7 women, respectively. The same genotypes were found in epidemiologically and geographically unrelated women; genotype 17 (18 isolates) was observed in the case of 4 (F.U, J.A, R.Ag, W.Z.) and genotype 4 (12 isolates) for 3 women (J.A, M.K, S.Z.). The same genotypes were found in the vagina (J.A, P.I.) and in oral/anal samples (S.I.) of women with recurrent VC as well as in vagina (W.Z.) or in oral/anal sites of women without symptoms of VC (B.K, F.U, S.A, S.Z.).

The *C. albicans* strains of different genotypes and biotypes were isolated concomitantly from different body sites and repeatedly at time intervals from the same body site (K.I, K.M, S.Z, F.A.). Some women had a unique genetic pattern of strains at the site of infection (vagina/visit I): u13 (B.Ka.); u16 (K.D.); u18 (K.I.); u3 i u5 (M.K.); u12 (R.Ag.); u10 (Z.E.) that were characteristic of the site of infection. Unique genetic patterns of strain also occurred at colonized body sites.

The results of the study show that *C. albicans* is still the most isolated yeast species regardless of body site and sampling time. Most of the strains of this species were eliminated from the vagina after antifungal therapy with nystatin but some of them persisted a few months in the vagina of women with and without VC symptoms. Similarly, the results of other authors suggest that topical antifungal agents resolve the symptoms of VC in the majority of women, but do not completely eliminate yeast from the vagina (Lanchares and Hernández, 2000; Mercure *et al.,* 1993; Sobel, 2007; Sobel, 2003; Sobel, 1996; Soll *et al.,* 1991; Vazquez *et al*., 1994). Unfortunately, some women are still colonized after VC treatment, yet others have a relapse of VC (Sobel, 1985). Yeast-positive cultures appeared 30 days or 3 months after the completion of therapy of VC, in 15–20% of women in Odds findings (1982) and in 20–25% of women according to Sobel (2007), respectively. In our study up to 50% of women had a yeast-positive vaginal culture 2 months after treatment and re-colonization increased after 4 months. Some authors intensify the research concerning the yeast colonization of the other body sites to help in the treatment of VC and to improve the satisfactory efficacy in the management of VC in clinical practice. The origin of VC is multifactorial and may be associated not only with incomplete elimination of yeasts from the vaginal epithelium but also with re-colonization from other host body sites or from the external environment. Although it was commonly believed that the proximity of the rectum colonized by yeast is a predisposing factor for VC, our report exhibited more frequent oral than anal colonization before and after antifungal therapy of VC. Similarly to our results, Soll *at al.* (1991) found a higher percentage of yeast in the oral cavity (56%) than in the anorectal region (24%) and the frequent relationship of carriage between pairs of oral-anal and oral-vaginal isolates. The mentioned above data together with Sobel's results (2007, 1985) suggested that the importance of rectal isolates is overestimated in the pathogenesis of VC. Based on typing systems we tried to answer the question of yeast source involved in VC. Biotyping poorly typed *C. albicans* isolates because up to 90% of women with VC were colonized by strains belonging to biotype C before and after antifungal therapy. Comparative analysis with other studies (Brajer *et al*., 2005; Kurnatowska and Kurnatowski, 1998) was difficult because of geographically different samples, not related patients or application of different kinds of classification. The results of molecular typing, by means of optimized RAPD technique, revealed greater diversity of *C. albicans* types in different body sites of the same patient in comparison with the results of biotyping.

The DNA-based typing methods of *C. albicans* strains revealed the highest genetic similarity between pairs of vaginal and anal strains in comparison with other pairs of isolates. The results of genotyping seem to be consistent with the results of earlier studies conducted by Mercure *et al.* (1993) and Mendling *et al.* (2000). The former authors suggested the anal isolates could be a reason for VC because their genetic similarity with vaginal isolates was found in 68% of episodes, the latter demonstrated that different body sites including oral cavity might be the endogenous source of yeast. The results of the present genotyping study do not exclude both points of view.

Colonization of one body site by the same biotype/genotype over an extended time can suggest endogenous re-infection or vaginal relapse. The hypotheses seem to be more acceptable for women with recurrent VC (J.A, P.I, S.I) and they are consistent with the results of Vazquez *et al.* (1994). The studies conducted by other authors (Lockhart *et al.* 1996; Maffei *et al.*, 1997) in women with recurrent VC also suggested the persistence of the single yeast genotype within vagina during consecutive episodes of VC. It can be associated with incomplete elimination of the specific genotype after topical treatment (El-Din *et al.*, 2001; Stein *et al.*, 1991). In the light of Fischer's (2012) data patients frequently do not have recurrent symptoms of VC but their disease is chronic as a result of oestrogen-related yeast persistence (Fischer, 2012).

The variability of biotypes/genotypes within one body site and within one woman over an extended time could be the result of topical antifungal therapy as well as may suggest the acquisition of new isolates by the host. The emergence of biotype Z after antifungal therapy in the control visits can be the result of selection of *C. albicans* phenotypes, while the occurrence of unique genetic patterns can suggest the acquisition of the

infecting isolates from an exogenous source. The occurrence of multiple genotypes and biotypes of *C. albicans* at the woman's body sites suggests a dynamic process of woman colonization by yeast, including the exchange of strains/genotypes within body sites. The findings are consistent with the results of Stein *et al.* (1991), who observed the exchange of strains in 4 of 7 women with VC. The study conducted by Samaranyake *at al.* (2003) on oral isolates also confirms the dynamic colonization of a body site by multiple genotypes.

No specific relationships were found between genotype/biotype/body site and clinical symptoms. The data are consistent with the results of Lian *et al.* (2004); Mercure *et al.* (1993) and Xu *et al.*(1999). Thus, these results rather suggest a circulation of the multiple genotypes in the population and the need to seek other factors responsible for the symptoms of infection. The factors can be associated with the host immune response and yeast adaptation to specific environmental conditions, *i.e.* with the adhesion to host tissues or with susceptibility to drugs (work in progress).

In summary, the results of our study indicate that VC can be both endogenous and exogenous in origin. The occurrence of an identical genotype and biotype at the site of infection and/or colonization over several months confirmed the endogenous relapse, transmission of the strains between body sites and the possibility of endogenous re-infection. The diversity of genotypes at the body sites of the same individual over a few months confirmed the periodic exchange of strains and their acquisition from an exogenous source. Do these processes appear occasionally or are they dependent on some other factors? This is a question for further research.

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