

Antibiotics sensitivity of *Candida* clinical and food-borne isolates*

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Candida is a yeast species recognized as the most frequent etiological agent of systemic and invasive thrush in humans. Invasions can affect all tissues, organs and systems of human in various stages of development. In the last 10 years *Candida* infections have increased 15 times. The purpose of our study was to determine the sensitivity of four antibiotics belonging to three different groups of antifungal agents against clinical and food-borne *Candida* strains. Our studies showed that of all tested strains, 7% was resistant to nystatin, 32% to fluconazole, 23% to voriconazole, and no strains grew in the presence of caspofungin. Despite the differences in biochemical profiles of clinical and food-borne isolates of *Candida*, a group of strains showing resistance to antibiotics include both types of isolates. At the same time circulating of antibiotic-resistant strains outside the hospital environment and the yeast infection *via* food is possible.

Key words: *Candida*, fluconazole, voriconazole, caspofungin, nystatin, clinical and food strains

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INTRODUCTION

Candida yeasts are natural inhabitants of the alimentary, genital and upper respiratory tracts of mammals (Szczepaniak *et al.*, 2004; Tunka *et al.*, 2006). Approximately 40–60% of people are carriers of *Candida*, however, it does not mean that the clinical candidiasis occurs (Ibanez-Nolla *et al.*, 2004). *Candida* is recognized as the most frequent etiological agent of systemic and invasive thrush in humans among which the most common is *Candida albicans* (80–90%). Invasions can affect all tissues, organs and systems of human in various stages of development. Probability of infections is growing with specific conditions of the organism and in particular during predisposing situations, like: diabetes, pregnancy, use of antibiotics, cortisones, contraceptives, estrogens, immunosuppressors, methronidazol, genetic factors, etc. (Tunka *et al.*, 2006; Torba *et al.*, 2012; Silva *et al.*, 2012). In the past ten years, *Candida* infections have increased 15 times (Kenedy & Wolz, 2007; Torba *et al.*, 2012).

The discovery and development of antibiotics for the treatment of microorganisms infections was one of the most significant medical achievements of the twentieth century. Nowadays, at least a few hundred of antibiotics are known, but this does not mean a sense of security. Having the vast number of drugs, which at the

time of entry into treatment were completely new agents, we used them fairly lightly. In last few years, among factors contributing to fungal colonization, use of antibiotics with broad spectrum of antibacterial activity is the most significant. According to statistics from the monitoring of antibiotic consumption of European Surveillance of Antibiotic Consumption, Poland is in the top ten among European countries in terms of quantitative antibiotic consumption per capita (Dzierżanowska, 2001; Kostecka, 2011). Unfortunately, many of existing antimicrobial agents are under threat from the widespread emergence of microorganisms resistance (Kuźma *et al.*, 2007; Deptuła & Gospodarek, 2010). This huge number of resistant species is mainly caused by abuse of all antibiotics in the treatment and also in the treatment of viral infections (Dzierżanowska, 2001, Lewis & Ausubel, 2006; Straus & Hancock, 2006; Kaufman, 2011). Antibiotic-resistant strains of *Candida* circulate in human environment and colonize diverse environment, they get also into the food. Highly-processed food is not generally contaminated with yeasts of the *Candida* genus. However, the food products, fermented products and products with a low degree of processing are not free from these yeasts. The presence of the yeast in the food is well documented (Tornai-Lehoczki *et al.*, 2003; Tyrer *et al.*, 2004; Bajpai *et al.*, 2011), but only few works concern the study of antibiotic resistance of food-borne isolates.

Within the presented study the sensitivity of both clinical and food-borne *Candida* strains against antibiotics belonging to three different groups was determined. The following antibiotics were used: nystatin (polyenes), fluconazole and voriconazole (triazoles I and II generation, respectively) and caspofungin (echinocandins).

MATERIALS AND METHOD

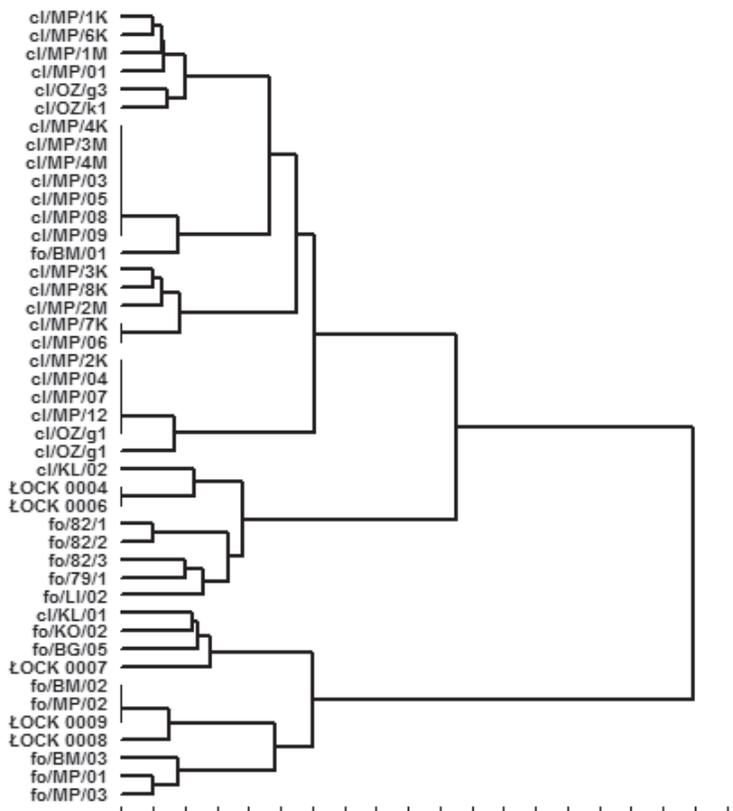
Yeast species. 44 strains of *Candida* were tested (Table 1). Clinical strains of *Candida albicans* (cl/MP/1K, cl/MP/2K, cl/MP/3K, cl/MP/4K, cl/MP/6K, cl/MP/7K, cl/MP/8K, cl/MP/1M, cl/MP/2M, cl/MP/3M, cl/MP/4M, cl/MP/01, cl/MP/03, cl/MP/04, cl/MP/05, cl/MP/06, cl/MP/07, cl/MP/08, cl/MP/09, cl/MP/12, cl/OZ/g1, cl/OZ/g2, cl/OZ/g3, cl/OZ/k1), *Candida glabrata* (cl/KL/01) and *Candida lusitanae* (cl/KL/02) were obtained from Department of Laboratory Diagnostics of Polish Mother's Memorial Hospital and Health

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Table 1. Number and origin of isolates tested.

Strains	No. of isolates	Origin
Clinical isolates		
<i>C. albicans</i>	15	faeces
	3	throat
	3	vagina
	1	blood
	1	skin
	1	stomach
<i>C. glabrata</i>	1	urogenital tract
<i>C. lusitaniae</i>	1	faeces
Food-borne isolates		
<i>C. lusitaniae</i>	2	fruit yoghurt
	2	feed
<i>C. krusei</i>	3	pickled cucumber
	1	bakery
<i>C. boidinii</i>	3	pickled cucumber
<i>C. famata</i>	1	fruit yoghurt
	1	salad
<i>C. colliculosa</i>	1	fruit yoghurt
<i>C. parapsilosis</i>	1	fruit yoghurt
<i>C. tropicalis</i>	1	pickled cucumber
<i>C. pelliculosa</i>	1	feed
<i>C. rugosa</i>	1	sauerkraut

Figure 1. Dendrogram of similarity of *Candida* strains biochemical properties estimated by API 20C AUX tests (bioMerieux).

Care Team of Lodz Polesie, while food-borne strains of *Candida lusitaniae* (fo/82/1, fo/79/1, ŁOCK 0004, ŁOCK 0006), *Candida famata* (fo/82/2, fo/LI/2), *Candida parapsilosis* (fo/82/3), *Candida colliculosa* (fo/KO/02), *Candida tropicalis* (fo/BM/01), *Candida krusei* (fo/BM/02, fo/MP/02, ŁOCK 0008, ŁOCK 0009), *Candida boidinii* (fo/BM/03, fo/MP/01, fo/MP/03), *Candida rugosa* (fo/BG/05) and *Candida pelliculosa* (ŁOCK 0007) were obtained from Institute of Fermentation Technology and Microbiology of Lodz University of Technology and various commercial companies.

Yeast maintenance and activation. Yeast strains were maintained on agar YPG medium (1% bacto yeast extract, 2% bacto peptone, 2% glucose, 2% bacto agar) slants. The strain activation was carried out by double passaging of 24 hours yeast culture in YPG liquid medium, in temperature 37°C.

Biochemical properties. API 20C AUX tests (bioMerieux, France) were used for biochemical characteristics of the yeast. All procedures were conducted according to manufacturer recommendation. The biochemical profiles of *Candida* isolates were estimated according to the yeast abilities to assimilate 19 substrates: glucose, glycerol, 2-keto-D-gluconate, L-arabinose, D-xylose, adonitol, xylitol, galactose, inositol, sorbitol, α -methyl-D-glucoside, N-acetyl-D-glucosamine, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, raffinose.

Antibiotics. The following antibiotics were used: caspofungin acetate (1-[(4R,5S)-5-[(2-aminoethyl)amino]-N2-[(10R,12S)-10,12-dimethyl-1-oxotetradecyl]-4-hydroxy-L-ornithine]-5-(threo-3-hydroxy-L-ornithine) pneumocandin B0 Diacetat at the concentration of 5 μ g/mL (Laboratories Merck Sharp & Dohme-Chibret, France); voriconazole ((2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluor-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol) at the concentration of 1 μ g/disc (Becton, Dickinson & Co., USA); fluconazole (2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol) at the concentration of 25 μ g/disc (Becton, Dickinson & Co., USA); nystatin (1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E,21E,25E,27E,29E,31E,33R,35S,36R,37S)-33-[(3-amino-3,6-dideoxy- β -D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo-nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid) at the concentration of 50 μ g/mL (PPH Galfarm Sp z o.o., Polska).

Antifungal activity assessment. Agar disc diffusion method was used for screening of antifungal activity of each antibiotic. Yeast inoculum (10^3 cfu/mL) in 0.85% NaCl solution was spread on the surface of YPG agar plate. Sterile filter paper discs (6mm in diameter) with 50 μ g of nystatin, 25 μ g of fluconazole, 1 μ g of voriconazole and with 10 μ l of caspofungin acetate in the concentration of 5 μ g/ml were placed on the inoculated plates. The positive control consisted of water instead of antibiotics, while uninoculated plates con-

Table 2. Interpretive chart of diameter zone of growth inhibition for nystatin, fluconazole, voriconazole and caspofungin according to manufacturers of antibiotics.

Antifungal agent	Zone Diameter Interpretive Standards [mm]		
	Resistant (R)	Intermediate (I)	Susceptible (S)
Caspofungin acetate	≤12	13–15	≥16
Voriconazole	≤13	14–16	≥17
Fluconazole	≤14	15–18	≥19
Nystatin	no zone	10–14	≥15

taining antibiotics served as a negative control. All plates were incubated for 48 h at 37°C. Size of inhibition zone diameters surrounding filter paper disc was measured and compared to the Zone Diameter Interpretive Standards (Table 2).

Statistical analysis. Hierarchical cluster analysis was used to estimate the similarity of *Candida* strains according to their biochemical profiles and susceptibility to antibiotics. Outcomes of biochemical properties were displayed as binary data, 0 or 1 when the feature was absent or present in the yeast profile, respectively. Whereas the results of yeasts antibiotic-sensitivity were presented as the average value of growth inhibition zones diameter for each examined antibiotic. The algorithm for hierarchical clustering was calculated by Ward's method (Statistica 6.0, Stat Soft Inc.) and cluster hierarchy was presented using a tree structure (dendrogram) with horizontal agglomeration distance.

Statistic development zones of inhibition. Diameter zone of growth inhibition is presented as average value of four measurements and standard deviations were calculated (Microsoft Excel 2010).

RESULTS AND DISCUSSION

Most of 26 clinical strains used in researches were isolated from the faeces (61%), vagina (11%) and throat (11%). *C. albicans* was predominated in this group and

only two isolates were identified as *C. glabrata* and *C. lusitanae*. Pickled cucumbers (39%) and fruit yoghurt (28%) were the main source of food-borne *Candida*. *C. lusitanae* and *C. krusei* represented the largest number of isolates. Neither *C. albicans* nor *C. glabrata* typically isolated from human were not found in food. The only genus isolated from these two sources simultaneously was *C. lusitanae*.

Hierarchical cluster analysis applied for similarity assessment of *Candida* isolates according to their biochemical profiles showed differentiation of the strains in the two clusters (Fig. 1). The first cluster includes 24 clinical strains of *C. albicans* and food-borne *C. tropicalis* (fo/BM/01) isolated from pickled cucumbers. The second cluster, except 17 strains originating from food matrices, includes two clinical strains *C. glabrata* (cl/KL/01) and *C. lusitanae* (cl/KL/02). At the same time, distinct biochemical heterogeneity was stated among the isolates of *C. albicans*. It is probably conditioned by an adaptation of the yeast to host organism.

Candida isolates were evaluated for sensitivity to four antibiotics. All isolates were found sensitive to caspofungin that was expressed by growth inhibition zones ranged from 18 to 49 mm. Caspofungin is a valuable fairly new antifungal agent with a novel mechanism of action belonging to the class echinocandins (McCormack & Perry, 2005; Wiczorek *et al.*, 2008). In the early 2000's, echinocandins were licensed for the treatment and prevention of fungal infections (Finkelman, 2010; Al-Badriyeh *et al.*, 2012; Lobo *et al.*, 2013). Spectrum of caspofungin activity is relatively broad including *Candida* and *Aspergillus* species, and mycelial forms of endemic fungi (Wiczorek *et al.*, 2008; Wiederhold & Herrera, 2012). The high effectiveness of this antibiotic is attributed to an inhibition of the glucan synthase complex in yeast cells, that is why it is an attractive tar-

Table 3. Antibiotics sensitivity of *Candida* strains classified as diameter of growth inhibition zones:

R — resistant, I — intermediate, S — susceptible, measured by the disc diffusion method.

Yeast*	Average diameter zone of antibiotics [mm]	Average diameter zone of antibiotics [mm]							
		Caspofungin	Voriconazole	Fluconazole	Nystatin				
<i>C. albicans</i> cl/MP/1M	46.75±5.38	S	0	R	0	R	20.00	S	
<i>C. albicans</i> cl/MP/01	18.50±0.58	S	0	R	0	R	0	R	
<i>C. albicans</i> cl/MP/03	20.50±0.58	S	20.50±1.73	S	15.75±2.50	I	20.00	S	
<i>C. albicans</i> cl/MP/04	19.50±1.29	S	0	R	0	R	19.00	S	
<i>C. albicans</i> cl/MP/05	23.50±0.58	S	0	R	0	R	25.50±0.58	S	
<i>C. albicans</i> cl/MP/08	23.00±1.41	S	0	R	0	R	19.25±0.96	S	
<i>C. albicans</i> cl/MP/09	23.50±0.58	S	13.25±1.26	I	10.50±0.58	R	20.50±0.58	S	
<i>C. glabrata</i> cl/KL/01	25.00	S	11.50±0.58	R	9.00	R	25.00	S	
<i>C. boidinii</i> fo/MP/01	27.50±1.29	S	30.25±1.26	S	16.00±1.63	I	30.50±0.58	S	
<i>C. boidinii</i> fo/MP/03	26.00±0.82	S	15.25±1.26	I	0	R	31.50±1.29	S	
<i>C. rugosa</i> fo/BG/05	23.25±0.50	S	14.50±0.58	I	7.50±0.58	R	29.25±0.96	S	
<i>C. tropicalis</i> fo/BM/01	23.50±0.58	S	0	R	0	R	0	R	
<i>C. krusei</i> fo/BM/02	28.75±1.50	S	23.75±2.06	S	14.50±1.29	I	34.50±5.20	S	
<i>C. boidinii</i> fo/BM/03	29.50±0.58	S	32.75±2.22	S	16.75±2.06	I	30.50±0.58	S	
<i>C. pelliculosa</i> ŁOCK 0007	30.75±0.96	S	35.00±0.82	S	33.50±1.29	S	0	R	

*All strains not included in the Table 3 were sensitive to all antibiotics tested.

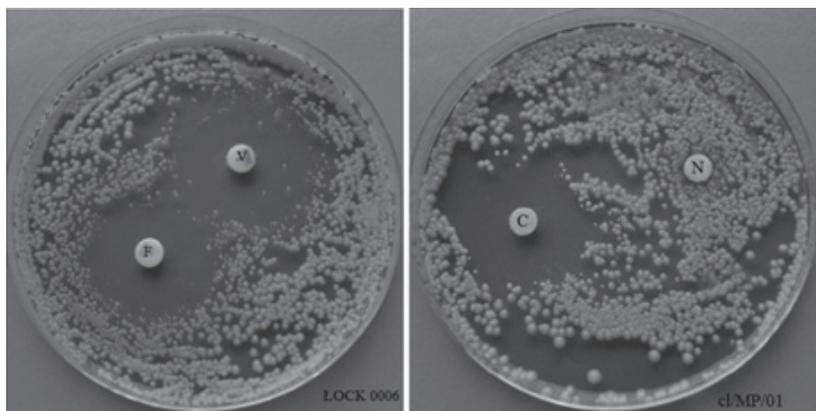


Figure 2. Examples of antibiotics susceptibility of food borne isolate *Candida lusitanae* LOCK 0006 (left) and clinical isolate *Candida albicans* cl/MP/01 (right) estimated by agar disc diffusion method.

Symbols: F — fluconazole, V — voriconazole, C — casprofungin, N — nystatin.

get due to the lack of homologous enzymes in human cells (Dominguez-Gil *et al.*, 2007; Marchettia *et al.*, 2007; Wiczorek *et al.*, 2008). Casprofungin is mainly used in hospital treatment, so it is possible that the yeast had not yet developed resistance systems to this antibiotic and are still sensitive.

Due to different sensitivity to nystatin, fluconazole and voriconazole 15 strains were grouped together (Table 3).

Triazoles, which did not totally inhibit growth of the clinical isolates and only against five food-borne strains

showed the lowest effectiveness. Azoles were introduced to the therapy in the 1980s. Mechanism of these drugs action is impaired synthesis of ergosterol in the fungal plasma membrane, with the participation of cytochrome P450. Voriconazole also inhibits the synthesis of chitin (Pfaller *et al.*, 2003, Cuenca-Estrella *et al.*, 2005; Szymankiewicz & Danciewicz, 2008). Similar mechanism of action can explain the similarity of activity against tested *Candida* strains. Triazoles, particularly fluconazole, are first-line drugs in the treatment of fungal infections. Spectrum of activity of fluconazole does not include infections caused by *Candida glabrata* and *Candida krusei* (Pfaller *et al.*, 2003; Cuenca-Estrella *et al.*, 2005; Spelberg *et al.*, 2006; Szymankiewicz & Danciewicz, 2008). However, in

our study, two food-borne strains of *C. krusei* showed a susceptibility to fluconazole, while the third one was intermediate. There were no resistant strains of *C. krusei* to fluconazole. Only one clinical isolate of *C. glabrata* was tested on the activity of antibiotics and it was not sensitive to fluconazole. Voriconazole is a triazole of the second generation. It is used for invasive *Candida* infections caused by strains resistant to fluconazole. This antibiotic shows a strong activity against *Candida* including *C. krusei* and *C. glabrata* (Pfaller *et al.*, 2003; Cuenca-Estrella *et al.*,

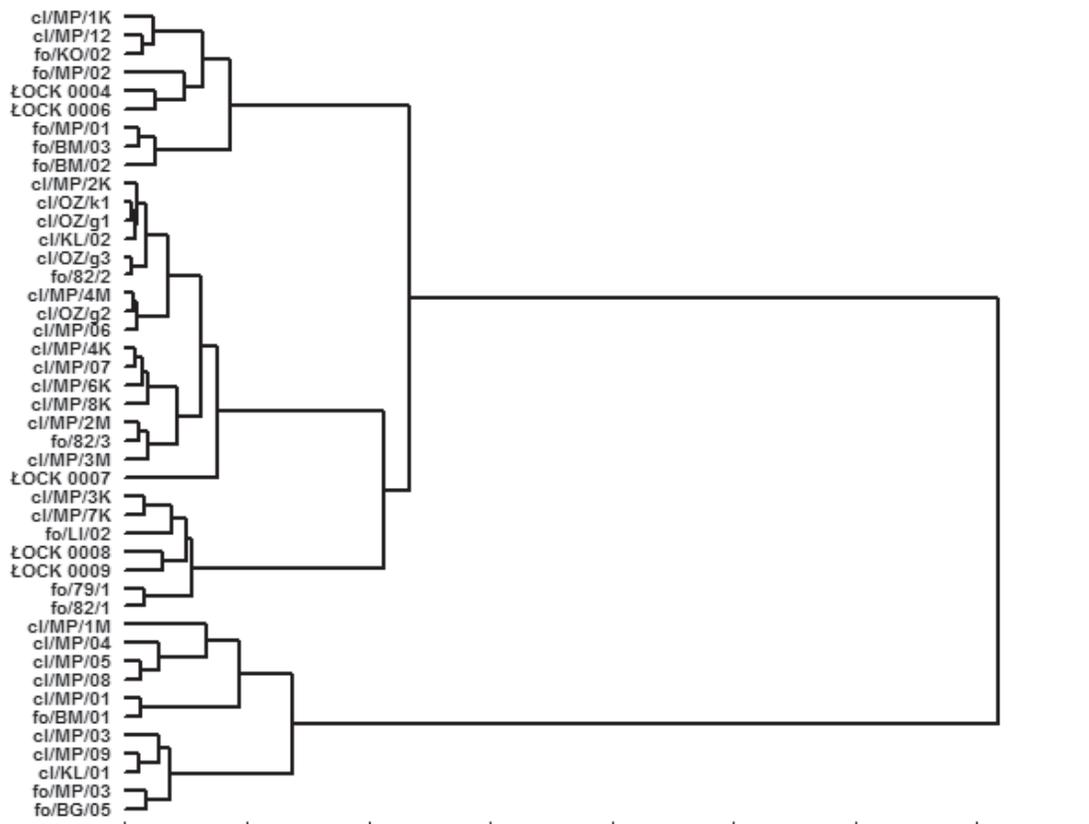


Figure 3. Dendrogram of similarity of *Candida* yeasts sensitivity to different antibiotics. The sensitivity was estimated by the agar disc diffusion method.

2005; Panackal *et al.*, 2006; Spelberg *et al.*, 2006; Szymankiewicz & Danciewicz, 2008). In our research, voriconazole effectively inhibited growth of *C. krusei*, however, *C. glabrata* was not sensitive neither to fluconazole nor to voriconazole.

Three isolates were resistant to nystatin: one *C. albicans* clinical strain isolated from faeces and two food-borne strains *C. tropicalis* and *C. pelliculosa* isolated from pickled cucumber and animal feed respectively. Nystatin inhibited growth of 93% of all the tested strains.

Figure 2 shows representative examples of plates illustrating action of antibiotics on *Candida* isolates.

According to Ng *et al.*, 2003 it has been proved to be the most effective agents in the treatment of various fungal infections. In the study of Hossain *et al.*, 2000 was shown that nystatin was effective against some fungal pathogens unresponsive to amphotericin B and azoles. Pharmacological activity of nystatin comes from the interaction with ergosterol found in the plasma membrane of fungal cells with subsequent functional disorder of the membrane, resulting in yeast death (Barão de Aguiar *et al.*, 2010). Unfortunately, the toxicities caused by polyene antibiotics limit their use (Ng *et al.*, 2003; Hossain *et al.*, 2000; Chandrasekar, 2011; Semis *et al.*, 2012). Nystatin is not absorbed through the skin or from the gastrointestinal track, and so topical and oral administration of nystatin is not associated with significant toxicities (Hossain *et al.*, 2000; Ng *et al.*, 2003; Chandrasekar, 2011; Semis *et al.*, 2012). That is why it was used as the first-choice drug for the treatment of oral candidiasis (Barão de Aguiar *et al.*, 2010). In Poland, this antibiotic was widely used during antibioticotherapy in bacterial infection as a prophylaxis of gastrointestinal candidiasis. Reduced nystatin consumption in the recent years, especially in the prophylactic treatment of fungal infection, could be the reason of sensitivity recovering fungi of recovered its resistance to this antibiotic. The only one resistant strain for voriconazole, fluconazole and nystatin was *C. albicans* (cl/MP/01) isolated from the faeces.

Similarity of *Candida* isolates according to their sensitivity to different antibiotics is shown in Fig. 3. Eleven of the 15 isolates showed a resistance or at least intermediate sensitivity to one antibiotic tested form cluster significantly different from the other strains. *C. albicans* are the majority (64%) in this cluster. As in the case of biochemical profiles, food-borne *C. tropicalis* strains (fo/BM/01) showed high homology with the clinical strains.

CONCLUSIONS

Among 44 *Candida* strains tested, neither *C. albicans* nor *C. glabrata* typically isolated from human, were not found in food. However, according to biochemical profiles *C. albicans* isolates were distinct from the food-borne ones, a substantial heterogeneity within this group was also noted. Simultaneously, the incidents of biochemical profiles conformity of some clinical and food-borne isolates may be a result of *Candida* transmission via food. The presented research shows that a group of strains expressing intermediate sensitivity or resistance to the tested antibiotics include both types of isolates. At the same time, the similarity of food-borne *C. tropicalis* to the clinical strains of *C. albicans* does not exclude the possibility of circulating of antibiotic-resistant strains outside the hospital environment and the yeast infection entered into the body with food. Local monitoring the resistance of environmental *Candida* strains will be help-

ful in making strategic decisions on the use of specific drugs in therapy.

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