Enzymatic activity and biotypes of Candida fungi isolated from the surfaces of mobile phones and hands

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ABSTRACT

Introduction: The secretion of hydrolytic enzymes is a factor facilitating pathogenic fungi invasion into the tissues.
Purpose: To assess hydrolytic activity and biotypes of Candida strains isolated from samples collected from the surfaces of mobile phones and the hands of their owners.
Materials and methods: The study included 175 mobile telephones and hands. The API ZYM test was used to assess enzymatic activity; biotyping was performed according to Williamson's classification.
Results: Among the strains isolated from hand surfaces, the highest activity was shown for *C. albicans* (acid phosphatase, esterase), *C. glabrata* (leucine arylamidase, acid phosphatase, esterase), and *C. krusei* (acid phosphatase). Of the strains isolated from phone surfaces, the highest activity was shown for *C. albicans* (leucine arylamidase, acid phosphatase), *C. glabrata* (esterase, leucine arylamidase, esterase lipase), and *C. krusei* (acid phosphatase). Biotypes G, B and F were dominant for all types of fungi, both for strains isolated from phones and hand surfaces. Additionally, biotype A was dominant for *C. krusei*.
Conclusions: *C. albicans*, *C. glabrata*, and *C. krusei* showed activity for all hydrolytic enzymes. The strongest correlation between the hydrolytic activity of fungi isolated from hand and phone surfaces was shown for *C. albicans*.
Keywords: *Candida*, hands, telephone, API Zym

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INTRODUCTION

According to the International Telecommunication Union, the number of mobile telephone users will come close to Earth's population and reach 7 billion by the end of 2014 [1]. The consistently increasing number of mobile phone users has led to the growth of interest in the impact of mobile phones on human health. Another issue associated with their use is their role in microbial transmission.

Akinyemi et al. [2] emphasized that a mobile phone is an essential item for both professional and social use, but is frequently used in environments inhabited by high numbers of bacteria. The authors [2] investigated 400 samples collected from mobile phones belonging to representatives of the following groups: group A – food producers (100 subjects); group B – professors and students (104 subjects); group C – civil servants (106 subjects); and group D – health care providers (90 subjects). They found a high percentage (62.0%) of bacterial contamination. The highest rate of mobile phone contamination was observed in group A (92.4%), followed by group B (73.6%), C (16.9%), and D (15.3%). Coagulase-negative Staphylococcus, which was isolated in the lowest percentage from phones in group D (26.3%), was the most common in group A (50.1%). Other identified microbes included, among others: *Staphylococcus aureus, Enterococcus faecalls, Pseudomonas aeruginosa, Escherichia coli and Klebsiella* spp. [2].

The secretion of hydrolytic enzymes is a well-known factor that facilitates the invasion of pathogenic fungi (dermatophytes, yeast-like fungi, mold) into the tissues. It is believed [3-7] that the activity and the nature of the secreted fungal enzymes may play an essential role in adaptation and reflect fungal virulence.

The aim of the study was to assess the hydrolytic activity and biotypes of *Candida* strains isolated from samples collected from the surfaces of mobile telephones and the hands of their owners.

MATERIALS AND METHODS

The Bioethics Committee of the Medical University of Bialystok approved the study, approval no. RI-002/489/2010. A total of 175 mobile phones and hands of students and professors of the Medical University of Bialystok and university hospital personnel were included in the mycological evaluation.

Biological monitoring of mobile phone and hand surface contamination was performed with Count-TactTM applicator using Count-Tact plates (bioMerieux) containing a medium complying with the requirements of the Draft European Standard CEN/TC 243/WG2. CandiSelect (Bio-Rad) was used to identify yeast-like fungi.

The enzymatic activity of fungi was determined using the API ZYM test by BioMerieux, containing substrates for the identification of 19 hydrolases.

Strains were biotyped according to Williamson's classification (1986) [6] distinguishing 8 biotypes (A to H) based on the analysis of five enzymes: esterase (II), valine arylamidase (VI), naphthol phosphohydrolase (XI), alpha-glucosidase (XV), and N-acetyl-beta-D-glucosidase (XVII). Additional biotypes (I to N) described by Kurnatowska and Kurnatowski [7] as well as biotypes described by Krajewska-Kułak et al. [9, 10], by Batura-Gabryel [11], and Bajer et al. [12] were also included in the assessment (Table 1).

The mycological procedures were in accordance with the manufacturer's instructions.

Selected numerical characteristics of the evaluated parameters such as: the arithmetic mean; median; the highest (maximum) and the lowest (minimum) values; standard deviation (s), which is a measure of "average" deviation from the mean value; 25th and 75th percentile, first and third quartiles; Spearman rank correlation coefficient; and Wilcoxon test were used for statistical analysis.

RESULTS

Candida glabrata dominated among the fungi identified in the collected samples; however, *C. albicans* and *C. krusei* were also common. These three species were found on over half of the respondents, both on their hands and phone surfaces. In contrast, *C. tropicalis* and the genus *Candida species* occurred sporadically (Table 2).

Enzymatic activity was determined using the API ZYM test. The tables below show a comparison of the distribution of hydrolytic activity of different enzymes, exhibited by only three fungal species. *C. tropicalis* and *C. species* were not included in the analysis due to an insufficient number of colonies for reliable analysis.

Table 3 shows the values of the selected descriptive statistics for the hydrolytic activity of *C. albicans* strains, along with the result of the Wilcoxon test, which was used to compare the activity of strains on hands and phones. The *C. albicans* strains isolated from the surface of hands and phones showed the activity of all hydrolytic enzymes. The strains isolated from hand surfaces had the highest activity of acid phosphatase (an average of 12.4 nmol) and esterase (an average of 12.1 nmol), and the lowest activity of beta-glucuronidase (an average of 0.1 nmol); while strains isolated from phone surfaces had the highest activity of leucine arylamidase (an average of 10.4 nmol) and acid phosphatase (an average of 12.1

nmol), and the lowest activity was beta-glucuronidase (an average of 0.2 nmol).

We found that the hydrolytic activity of *C. albicans* strains isolated from hand surfaces was higher compared with strains isolated from phone surfaces. We found statistically significant differences for the majority of enzymes. Valine arylamidase was the exception.

Table 1. List of biotypes based on the available literature [165-169]

BIOTYPES ENZYMATIC	**ENZYMES**				
	E 2 Esterase	**E 6** Valine arylamidase	**E 11** Naphtol-AS-BI-phosphodydrolase	**E 15** -glucosidase	**E 17** N-acetyl--glucosaminidase
according to Williamson (1986) [7]					
A	+	+	+	+	+
B	+	-	+	+	+
C	+	+	+	-	+
D	+	+	-	+	+
E	+	+	+	-	-
F	+	+	+	+	-
G	+	-	+	+	-
H	+	+	-	-	-
according to Kurnatowska and Kurnatowski (1998) [7]					
I	-	-	-	-	+
J	-	-	-	+	+
F	+	+	-	+	-
L	+	-	+	-	+
M	+	-	+	-	-
N	+	-	-	-	+
according to Krajewska-Kułak et al. (2000) [9]					
O	+	-	-	-	-
P	+	-	-	+	-
R	-	+	+	+	+
according to Krajewska-Kułak et al. (2001) [10] and Batura-Gabryel (2003) [11]					
S	+	+	-	-	+
T according to Krajewska-Kułak et al. (2001) and S according to Batura-Gabryel (2003)	+	-	-	+	+
acccording to Brajer et al. (2005) [12]					
T	-	+	+	-	-
U	-	+	+	-	+
In	-	+	-	+	-

Table 2. Species/genera of fungi isolated from the samples collected from hand and mobile phone surfaces

Species/genera of fungi	**The occurrence of fungal strains in samples taken from**			
	hand surface		**phone surface**	
	Number	**Percent**[1)]	**Number**	**Percent**[1)]
Candida glabrata	156	89.1%	131	74.9%
Candida albicans	146	83.4%	114	65.1%
Candida krusei	122	69.7%	95	54.3%
Candida tropicalis	9	5.1%	11	6.3%
Candida species	1	0.6%	0	0.0%
none	1	0.6%	10	5.7%

[1)] Sums do not have to add up to 100%, as any number of response options could be chosen.

Table 3. Hydrolytic activity of *Candida albicans* strains

Enzyme type	Activity of *Candida albicans* (nmol)												$p^{1)}$
	Hand (N = 146)						Mobile telephone (N =114)						
	$\overline{x}$	Me	c_{25}	c_{75}	min.	max.	$\overline{x}$	Me	c_{25}	c_{75}	min.	max.	
Phosphatase alcaline	10.6	10	5	10	1	30	8.8	10	5	10	5	30	0.0014**
Esterase (C4)	12.1	10	10	20	3	40	9.1	10	5	10	5	20	0.0000***
Esterase lipase (C8)	11.6	10	10	20	0	30	9.4	10	5	10	0	30	0.0005***
Lipase (C14)	6.3	5	5	10	0	30	6.9	5	5	10	0	20	0.0829
Leucine arylamidase	11.9	10	5	20	0	40	10.4	10	5	10	0	30	0.0499*
Valine arylamidase	1.4	0	0	0	0	10	1.6	0	0	0	0	20	0.0110*
Cystine arylamidase	7.0	5	5	10	0	30	7.5	5	5	10	0	20	0.1542
Tyrpsin	2.0	0	0	0	0	20	2.3	0	0	5	0	20	0.0745
Chymotripsin	2.3	0	0	5	0	20	2.6	0	0	5	0	20	0.2135
Phosphatase acid	12.7	10	10	20	2	40	10.2	10	5	10	5	30	0.0000***
Naphtol-AS-BI-phosphodydrolase	9.4	10	5	10	0	40	8.9	10	5	10	0	30	0.3614
α-galactosidase	7.0	5	5	10	0	20	6.9	5	5	10	0	20	0.6265
β-galactosidase	6.5	5	5	10	0	20	6.1	5	5	10	0	20	0.2934
β-glucuronidase	0.1	0	0	0	0	10	0.2	0	0	0	0	10	0.1797
α-glucosidase	6.7	5	5	10	0	20	6.5	5	5	10	0	20	0.9499
β-glucosidase	7.0	5	5	10	0	40	6.6	5	5	5	0	30	0.5940
N-acetyl-β-glucosaminidase	2.1	0	0	5	0	10	2.5	0	0	5	0	10	0.1075
α-mannosidase	5.5	5	5	5	0	10	5.5	5	5	5	0	10	1.0000
α-fucosidase	4.9	5	5	5	0	10	4.9	5	5	5	0	10	0.1797

p – value of statistical significance calculated using the Wilcoxon test; 1) Statistical significance was evaluated using simultaneous measurements of activity on the hands and phones (N = 109)

The strains isolated from hand surfaces had the highest activity ofleucine arylamidase (an average of 14.3 nmol), acid phosphatase (an average of 13.7 nmol), and esterase (an average of 13.3 nmol), whereas the lowest activity was beta-glucuronidase (an average of 0.1 nmol); the isolates from mobile phone surfaces had the highest activity of esterase (an average of 15.0 nmol), leucine arylamidase (an average of 13.7 nmol), and esterase lipase (an average of 11.0 nmol), whereas the lowest activity was beta-glucuronidase (an average of 0.7 nmol). We found that the activity of *C. glabrata* strains isolated from hand surfaces was higher compared with strains isolated from phone surfaces, for which statistically significant differences were shown, for lipase and lower for esterase, valine arylamidase, cystine arylamidase, beta-glucuronidase, and alpha-mannosidase (Table 4).

The *C. krusei* (Table 5) strains isolated from the surface of hands and phones showed the activity of all hydrolytic enzymes. The strains isolated from hand surfaces had the highest activity of acid phosphatase (an average of 21.2 nmol), and the lowest activity of beta-glucuronidase (an average of 0.1 nmol), while the isolates from phone surfaces had the highest activity of acid phosphatase (an average of 18.6 nmol) and the lowest activity of beta-glucuronidase (an average of 0.2 nmol). We found that the activity of *Candida krusei* isolated from hand surfaces was higher compared with strains isolated from phone surfaces, for which statistically significant differences were shown for enzymes such as alkaline phosphatase, cystine arylamidase and acid phosphatase, and lower activity for naphthol phosphohydrolase and N-acetyl-beta-D-glucosidase.

We also determined fungal activity for each strain isolated from hand and phone surfaces by averaging the value of 20 measurements (Table 6). The distribution of the calculated mean values in the study population was described using a set of descriptive statistics. The mean level of activity shown by a different genera/species of fungi seems to be similar; however, the activity of fungi isolated from mobile phone surfaces was slightly lower compared with those from hand surfaces. More advanced statistical analyses were performed to determine whether this difference was statistically significant.

Table 4. Hydrolytic activity of *Candida glabrata* strains

Enzyme type	**Activity of *Candida glabrata* (nmol)**												***p*** [1)]
	Hand (*N* = 156)						**Phone (*N* =131)**						
	$\overline{x}$	Me	c_{25}	c_{75}	min.	max.	$\overline{x}$	Me	c_{25}	c_{75}	min.	max.	
Phosphatase alcaline	11.5	10	5	20	0	30	10.2	10	5	10	0	30	0.0738
Esterase (C4)	13.3	10	10	20	5	40	15.0	20	10	20	0	40	0.0033**
Esterase lipase (C8)	11.8	10	10	10	0	30	11.0	10	5	10	0	40	0.6054
Lipase (C14)	7.2	5	5	10	0	30	5.8	5	5	10	0	20	0.0278*
Leucine arylamidase	14.3	10	5	20	0	40	13.7	10	0	20	0	40	0.6350
Valine arylamidase	1.1	0	0	0	0	20	1.6	0	0	0	0	20	0.0249*
Cystine arylamidase	5.8	5	5	5	0	20	6.7	5	5	10	0	20	0.0364*
Trypisn	1.2	0	0	0	0	10	1.3	0	0	0	0	20	0.5316
Chymotripsin	1.7	0	0	5	0	20	1.7	0	0	5	0	10	0.5614
Phosphatase acid	13.7	10	5	20	0	40	12.5	10	5	20	0	40	0.8245
Naphtol-AS-BI-phosphodydrolase	9.3	10	5	10	0	40	8.7	10	5	10	0	40	0.1807
α-galactosidase	6.3	5	5	10	0	20	6.4	5	5	10	0	20	0.7318
β-galactosidase	5.8	5	5	5	0	20	5.8	5	5	5	0	20	0.8448
β-glucuronidase	0.1	0	0	0	0	5	0.7	0	0	0	0	50	0.0180*
α-glucosidase	6.6	5	5	10	0	20	6.4	5	5	10	0	20	0.9831
β-glucosidase	5.9	5	5	5	0	40	5.9	5	5	5	0	40	0.1444
N-acetyl-β-glucosaminidase	1.9	0	0	5	0	10	2.2	0	0	5	0	10	0.2112
α-mannosidase	5.5	5	5	5	0	10	6.1	5	5	10	0	20	0.0077**
α-fucosidase	4.7	5	5	5	0	10	5.0	5	5	5	0	10	0.0526

p – value of statistical significance calculated using the Wilcoxon test; [1)] Statistical significance was evaluated using simultaneous measurements of activity on the hands and phones (*N* = 122)

An analysis of correlation was performed in order to assess the relationship between the activity of fungi isolated from hand and phone surfaces. Only subjects that tested positive for the presence of fungi on both hand and phone surfaces were included in this analysis. Therefore, a reliable analysis could be performed only for *C. albicans*, *C. glabrata*, and *C. krusei*. The strongest correlation between the activity of fungi isolated from hand and cell phone surfaces was shown for *C. albicans* (R=0.72; p=0.0000***); the correlation was lower for *C. glabrata* (R=0.55; p=0.0000***), and the lowest for *C. krusei* (R=0.41; p=0.0002***). All correlations were statistically significant, though their strength varied considerably.

Comparison of the activity levels in the fungi isolated from hand and phone surfaces was another issue to investigate. Correlation analysis itself does not enable answering this question, as the nature of correlation between the two features does not change, for example, after deducting any number from one of them. Therefore, the Wilcoxon test was used to compare the level of two features measured for the same units.

A statistically significant difference was shown for the hydrolytic activity of *C.albicans* isolated from hand and phone surfaces, with lower activity shown by fungi isolated from phone surfaces (mean difference of approx. 0.4). We also found that fungi isolated from phone surfaces of nearly 60% of subjects who also had *C. albicans* on hand surfaces showed lower hydrolytic activity. On the other hand, no statistically significant differences were shown for the hydrolytic activity of *C. glabrata* isolated from phone and hand surfaces (the mean difference was 0.1; however, the correlation was statistically insignificant, p=0.131). We found no statistically significant differences between the activity distribution for *C. krusei* isolated from phone and hand surfaces, p=0.272. Details are provided in Table 6.

The activity distributions of different enzymes for three fungal species are compared in tables 7, 8, and 9. *C. tropicalis* and *C. species* could not be included in the analysis due to an insufficient number of measurements. The assessment of significance of differences between hand and phone measurements was performed using the Wilcoxon test, which involves a simultaneous measurement of both compared values. Therefore, only individuals who had a certain strain isolated from phone and hand surfaces were considered. In the case of *C. tropicalis* and *C. species*, the number of such cases excluded performing a reliable analysis.

Table 5. Hydrolytic activity of *Candida krusei* strains

Enzyme type	**Activity of *Candida krusei* (nmol)**												***p*** [1)]
	Hand (*N* = 122)						**Phone (*N* =95)**						
	$\overline{x}$	Me	c_{25}	c_{75}	min.	max.	$\overline{x}$	Me	c_{25}	c_{75}	min.	max.	
Phosphatase alcaline	12.6	10	10	20	0	40	10.3	10	5	10	0	20	0.0129*
Esterase (C4)	14.0	10	10	20	5	40	13.7	10	10	20	5	40	0.7431
Esterase lipase (C8)	10.2	10	5	10	0	40	10.7	10	5	10	0	30	0.5955
Lipase (C14)	5.8	5	5	10	0	20	5.7	5	0	10	0	20	0.3014
Leucine arylamidase	16.1	10	10	20	0	40	15.1	10	10	20	0	40	0.1615
Valine arylamidase	3.8	5	0	5	0	20	4.2	5	0	5	0	10	1.0000
Cystine arylamidase	5.1	5	0	10	0	20	4.2	5	0	5	0	20	0.0127*
Trypsin	2.1	0	0	5	0	20	1.6	0	0	5	0	10	0.4080
Chymotripsin	1.7	0	0	5	0	20	1.6	0	0	5	0	10	0.5012
Phosphatase acid	21.2	20	10	30	0	40	18.6	20	10	30	0	40	0.0283*
Naphtol-AS-BI-phosphodydrolase	9.6	10	5	10	0	20	10.9	10	10	10	5	30	0.0432*
α-galactosidase	5.9	5	5	10	0	10	6.2	5	5	10	0	20	0.7022
β-galactosidase	5.4	5	5	5	0	40	5.1	5	5	5	0	10	0.3570
β-glucuronidase	0.1	0	0	0	0	5	0.2	0	0	0	0	5	0.1088
α-glucosidase	6.3	5	5	10	0	40	6.1	5	5	10	0	10	0.9909
β-glucosidase	4.7	5	5	5	0	10	5.3	5	5	5	0	10	0.1075
N-acetyl-β-glucosaminidase	1.3	0	0	2.5	0	5	2.0	0	0	5	0	10	0.0136*
α-mannosidase	4.8	5	5	5	0	10	5.4	5	5	5	0	20	0.1235
α-fucosidase	4.5	5	5	5	0	10	4.7	5	5	5	0	10	0.0910

p – value of statistical significance calculated using the Wilcoxon test;[1)] Statistical significance was evaluated using simultaneous measurements of activity on the hands and phones (*N* = 81)

Table 6. Fungal activity for all strains isolated from hand and phone surfaces with averaged value for 20 measurements

Activity	*N*	$\overline{x}$	Me	*s*	c_{25}	c_{75}	min.	max.
Hand								
Candida albicans	146	6.4	6.3	1.7	5.0	7.3	2.3	12.3
Candida glabrata	154	6.4	6.5	2.0	5.0	7.5	1.3	12.3
Candida krusei	124	6.8	6.8	1.9	5.8	8.3	1.3	13.3
Candida tropicalis	10	7.2	7.0	1.3	6.3	8.3	5.5	9.5
Candida species	1	7.3	7.3	0	7.3	7.3	7.3	7.3
Phone								
Candida albicans	113	5.8	5.8	1.4	5.0	6.5	2.5	9.8
Candida glabrata	133	6.3	6.5	1.8	5.3	7.5	1.0	12.3
Candida krusei	95	6.6	6.5	1.9	5.5	8.0	1.3	10.5
Candida tropicalis	9	7.4	8.3	1.2	6.8	8.3	4.8	8.3

In the case of *C. albicans*, strains isolated from hand surfaces showed statistically significantly higher hydrolytic activity of alkaline, acid phosphatase, esterase, esterase lipase, and leucine arylamidase compared with strains from phone surfaces; whereas, the activity of valine arylamidase was higher in phone strains compared with those isolated from hands. Details are presented in Table 8.

Table 7. Correlations between the hydrolytic activity of Candida albicans strains depending on sampling site

Candida albicans								
Hydrolytic activity	*N*	$\overline{x}$	Me	*s*	c_{25}	c_{75}	min.	max.
hands	146	6.4	6.3	1.7	5.0	7.3	2.3	12.3
telephone	113	5.8	5.8	1.4	5.0	6.5	2.5	9.8
phone – hand (*p* = 0.0005*)**	**109**	**-0.4**	**-0.3**	**1.2**	**-1.3**	**0.3**	**-2.8**	**3.3**

	Number	**Percent**
smaller on the phone	65	59.6%
the same	11	10.1%
greater on the phone	33	30.3%

Candida glabrata								
Hydrolytic activity	*N*	$\overline{x}$	Me	*s*	c_{25}	c_{75}	min.	max.
hand	154	6.4	6.5	2.0	5.0	7.5	1.3	12.3
phone	133	6.3	6.5	1.8	5.3	7.5	1.0	12.3
phone – hand (*p* = 0.1313)	122	0.1	0.1	1.7	-0.6	1.3	-7.3	4.0

	Number	**Percent**
smaller on the phone	47	38.5%
the same	12	9.8%
greater on the phone	63	51.6%

Candida krusei								
hydrolytic activity	*N*	$\overline{x}$	Me	*s*	c_{25}	c_{75}	min.	max.
hand	124	6.8	6.8	1.9	5.8	8.3	1.3	13.3
phone	95	6.6	6.5	1.9	5.5	8.0	1.3	10.5
phone – hand (*p* = 0.2726)	81	-0.3	0.0	1.9	-1.3	1.0	-7.3	4.3

	Number	**Percent**
smaller on the phone	40	49.4%
the same	9	11.1%
greater on the phone	32	39.5%

Table 8. Distribution of the hydrolytic activity in *Candida albicans* strains depending on sampling site

Enzyme type	**Activity of *Candida albicans* (nmol)**												***p*** [1)]
	Hand (*N* = 146)						**Phone (*N* =114)**						
		Me	c_{25}	c_{75}	min.	max.		Me	c_{25}	c_{75}	min.	max.	
Phosphatase alcaline	10.6	10	5	10	1	30	8.8	10	5	10	5	30	0.0014**
Esterase (C4)	12.1	10	10	20	3	40	9.1	10	5	10	5	20	0.0000***
Esterase lipase (C8)	11.6	10	10	20	0	30	9.4	10	5	10	0	30	0.0005***
Lipase (C14)	6.3	5	5	10	0	30	6.9	5	5	10	0	20	0.0829
Leucine arylamidase	11.9	10	5	20	0	40	10.4	10	5	10	0	30	0.0499*
Valine arylamidase	1.4	0	0	0	0	10	1.6	0	0	0	0	20	0.0110*
Cystine arylamidase	7.0	5	5	10	0	30	7.5	5	5	10	0	20	0.1542
Trypsin	2.0	0	0	0	0	20	2.3	0	0	5	0	20	0.0745
Chymotripsin	2.3	0	0	5	0	20	2.6	0	0	5	0	20	0.2135
Phosphatase acid	12.7	10	10	20	2	40	10.2	10	5	10	5	30	0.0000***
Naphtol-AS-BI-phosphodydrolase	9.4	10	5	10	0	40	8.9	10	5	10	0	30	0.3614
α-galactosidase	7.0	5	5	10	0	20	6.9	5	5	10	0	20	0.6265
β-galactosidase	6.5	5	5	10	0	20	6.1	5	5	10	0	20	0.2934
β-glucuronidase	0.1	0	0	0	0	10	0.2	0	0	0	0	10	0.1797
α-glucosidase	6.7	5	5	10	0	20	6.5	5	5	10	0	20	0.9499
β-glucosidase	7.0	5	5	10	0	40	6.6	5	5	5	0	30	0.5940
N-acetyl-β-glucosaminidase	2.1	0	0	5	0	10	2.5	0	0	5	0	10	0.1075
α-mannosidase	5.5	5	5	5	0	10	5.5	5	5	5	0	10	1.0000
α-fucosidase	4.9	5	5	5	0	10	4.9	5	5	5	0	10	0.1797

p – value of statistical significance calculated using the Wilcoxon test, [1)] Statistical significance was evaluated using simultaneous measurements of activity on the hands and phones (*N* = 109)

Table 9. Distribution of the hydrolytic activity in *Candida glabrata* strains depending on sampling site

Enzyme type	Activity (nmol)												$p^{1)}$
	Hand (N = 156)						Phone (N =131)						
		Me	c_{25}	c_{75}	min.	max.		Me	c_{25}	c_{75}	min.	max.	
Candida glabrata													
Phosphatase alcaline	11.5	10	5	20	0	30	10.2	10	5	10	0	30	0.0738
Esterase (C4)	13.3	10	10	20	5	40	15.0	20	10	20	0	40	0.0033**
Esterase lipase (C8)	11.8	10	10	10	0	30	11.0	10	5	10	0	40	0.6054
Lipase (C14)	7.2	5	5	10	0	30	5.8	5	5	10	0	20	0.0278*
Leucine arylamidase	14.3	10	5	20	0	40	13.7	10	0	20	0	40	0.6350
Valine arylamidase	1.1	0	0	0	0	20	1.6	0	0	0	0	20	0.0249*
Cystine arylamidase	5.8	5	5	5	0	20	6.7	5	5	10	0	20	0.0364*
Trypsin	1.2	0	0	0	0	10	1.3	0	0	0	0	20	0.5316
Chymotripsin	1.7	0	0	5	0	20	1.7	0	0	5	0	10	0.5614
Phosphatase acid	13.7	10	5	20	0	40	12.5	10	5	20	0	40	0.8245
Naphtol-AS-BI-phosphodydrolase	9.3	10	5	10	0	40	8.7	10	5	10	0	40	0.1807
α-galactosidase	6.3	5	5	10	0	20	6.4	5	5	10	0	20	0.7318
β-galactosidase	5.8	5	5	5	0	20	5.8	5	5	5	0	20	0.8448
β-glucuronidase	0.1	0	0	0	0	5	0.7	0	0	0	0	50	0.0180*
α-glucosidase	6.6	5	5	10	0	20	6.4	5	5	10	0	20	0.9831
β-glucosidase	5.9	5	5	5	0	40	5.9	5	5	5	0	40	0.1444
N-acetyl-β-glucosaminidase	1.9	0	0	5	0	10	2.2	0	0	5	0	10	0.2112
α-mannosidase	5.5	5	5	5	0	10	6.1	5	5	10	0	20	0.0077**
α-fucosidase	4.7	5	5	5	0	10	5.0	5	5	5	0	10	0.0526
Candida krusei													
	Hand (N = 122)						Phone (N =95)						
Phosphatase alcaline	12.6	10	10	20	0	40	10.3	10	5	10	0	20	0.0129*
Esterase (C4)	14.0	10	10	20	5	40	13.7	10	10	20	5	40	0.7431
Esterase lipase (C8)	10.2	10	5	10	0	40	10.7	10	5	10	0	30	0.5955
Lipase (C14)	5.8	5	5	10	0	20	5.7	5	0	10	0	20	0.3014
Leucine arylamidase	16.1	10	10	20	0	40	15.1	10	10	20	0	40	0.1615
Valine arylamidase	3.8	5	0	5	0	20	4.2	5	0	5	0	10	1.0000
Cystine arylamidase	5.1	5	0	10	0	20	4.2	5	0	5	0	20	0.0127*
Trypsin	2.1	0	0	5	0	20	1.6	0	0	5	0	10	0.4080
Chymotripsin	1.7	0	0	5	0	20	1.6	0	0	5	0	10	0.5012
Phosphatase acid	21.2	20	10	30	0	40	18.6	20	10	30	0	40	0.0283*
Naphtol-AS-BI-phosphodydrolase	9.6	10	5	10	0	20	10.9	10	10	10	5	30	0.0432*
α-galactosidase	5.9	5	5	10	0	10	6.2	5	5	10	0	20	0.7022
β-galactosidase	5.4	5	5	5	0	40	5.1	5	5	5	0	10	0.3570
β-glucuronidase	0.1	0	0	0	0	5	0.2	0	0	0	0	5	0.1088
α-glucosidase	6.3	5	5	10	0	40	6.1	5	5	10	0	10	0.9909
β-glucosidase	4.7	5	5	5	0	10	5.3	5	5	5	0	10	0.1075
N-acetyl-β-glucosaminidase	1.3	0	0	2.5	0	5	2.0	0	0	5	0	10	0.0136*
α-mannosidase	4.8	5	5	5	0	10	5.4	5	5	5	0	20	0.1235
α-fucosidase	4.5	5	5	5	0	10	4.7	5	5	5	0	10	0.0910

p – value of statistical significance calculated using the Wilcoxon test, [1)] Statistical significance was evaluated using simultaneous measurements of activity on the hands and phones (N = 122 - *Candida glabarata*; N = 81 - *Candida krusei*)

Table 10. Biotype distribution for the different genera/species of fungi isolated from hand surfaces

Biotype hand surface	**Fungi type**									
	C. albicans n=146		*C. glabrata* n=156		*C. krusei* n=122		*C. tropicalis* n=9		*C. species* n=1	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%
according to Williamson (1986)										
A	8	5.5%	5	3.2%	21	16.9%	0	0.0%	0	0.0%
B	37	25.3%	48	31.2%	7	5.6%	2	22.2%	0	0.0%
C	0	0.0%	3	1.9%	2	1.6%	0	0.0%	0	0.0%
E	0	0.0%	1	0.6%	1	0.8%	0	0.0%	0	0.0%
F	22	15.1%	10	6.5%	46	37.1%	2	22.2%	0	0.0%
G	69	47.3%	76	49.4%	34	27.4%	5	55.6%	1	100.0%
Total according to Williamson (1986)	136	93.2%	143	92.8%	111	89.4%	9	100%	1	100%
according to Kurnatowska and Kurnatowski (1998)										
L	0	0.0%	0	0.0%	1	0.8%	0	0.0%	0	0.0%
M	6	4.1%	6	3.9%	8	6.5%	0	0.0%	0	0.0%
Total according to Kurnatowska and Kurnatowski (1998)	**6**	**4.1%**	**6**	**3.9%**	**9**	**7.3%**	**0**	**0.0%**	**0**	**0.0%**
according to Krajewska-Kułak et al. (2000)										
R	0	0.0%	1	0.6%	2	1.6%	0	0.0%	0	0.0%
P	3	2.1%	4	2.6%	2	1.6%	0	0.0%	0	0.0%
Total according to Krajewska-Kułak et al. (2000)	**3**	**2.1%**	**5**	**3.2%**	**4**	**3.2%**	**0**	**0.0%**	**0**	**0.0%**
acccording to Brajer et al. (2005)										
T	1	0.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Total acccording to Brajer et al. (2005)	**1**	**0.7%**	**0**	**0.0%**	**0**	**0.0%**	**0**	**0.0%**	**0**	**0.0%**
Number of biotypes total	**7**		**9**		**10**		**3**		**1**	

Table 11. Biotype distribution for the different species of fungi isolated from phone surfaces

Biotype phone surface	**Fungi type**									
	C. albicans n=131		*C. glabrata - n=114*		*C. krusei - n=95*		*C. tropicalis - n=11*		*C species - n=10*	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%
according to Williamson (1986)										
A	10	8.8%	18	13.5%	24	25.8%	0	0.0%	0	0.0%
B	38	33.6%	31	23.3%	11	11.8%	1	11.1%	0	0.0%
C	0	0.0%	0	0.0%	2	2.2%	0	0.0%	0	0.0%
E	2	1.8%	1	0.8%	0	0.0%	0	0.0%	0	0.0%
F	14	12.4%	11	8.3%	37	39.8%	1	11.1%	0	0.0%
G	44	38.9%	62	46.6%	14	15.1%	7	77.8%	0	0.0%
H	0	0.0%	1	0.8%	0	0.0%	0	0.0%	0	0.0%
Total	**108**	**95.5%**	**124**	**93.3%**	**88**	**94.7%**	**9**	**100%**	**0**	**0.0%**
according to Anna Kurnatowska and Piotr Kurnatowski (1998)										
L	1	0.9%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
M	1	0.9%	7	5.3%	5	5.3%	0	0.0%	0	0.0%
Total	**2**	**1.8%**	**7**	**5.3%**	**5**	**5.3%**	**0**	**0.0%**	**0**	**0.0%**
according to Krajewska-Kułak et al. (2000)										
R	0	0.0%	2	1.4%	0	0.0%	0	0.0%	0	0.0%
P	3	2.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Total	**3**	**2.7%**	**2**	**1.4%**	**0**	**0.0%**	**0**	**0.0%**	**0**	**0.0%**
Number of biotypes total	**8**		**8**		**6**		**3**		**0**	

Table. 12. Biotype classification of the isolated strains

Biotype classification	Fungi type									
	C. albicans		*C. glabrata*		*C. krusei*		*C. tropicalis*		*C. species*	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%
according to Williamson (1986)										
hand	136	93.2%	143	92.8%	111	89.4%	10	100%	1	100%
phone	108	95.5%	124	93.3%	88	94.7%	9	100%	0	0.0%
according to Kurnatowska and Kurnatowski (1998)										
hand	6	4.1%	6	3.9%	9	7.3%	0	0.0%	0	0.0%
phone	2	1.8%	7	5.3%	5	5.3%	0	0.0%	0	0.0%
according to Krajewska-Kułak et al. (2000)										
hand	3	2.1%	5	3.2%	4	3.2%	0	0.0%	0	0.0%
phone	3	2.7%	2	1.4%	0	0.0%	0	0.0%	0	0.0%
acccording to Brajer et al. (2005)										
hand	1	0.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
phone	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

We found differences in biotype distribution for the different genera/species of fungi isolated from hand surfaces (Tables 10-12). The number of biotypes was dependent on the fungal species; biotypes classified according to Williamson were dominant for strains isolated from both mobile phone and hand surfaces.

DISCUSSION

A study conducted at the Manchester Metropolitan University [as cited in 13] showed that approximately 4 thousand microbes were present on one square centimeter of a mobile phone; therefore, the device is classified as the largest concentration of bacteria in the human environment. For comparison, it is estimated that a landline phone receiver is inhabited by an average of about 25 thousand bacteria [13].

According to Bardy et al. [14-16], 9-25% of mobile phones are inhabited by bacteria. The authors [14-16] also believe that bacterial infections of communication devices should be considered an important issue that should be taken into account when implementing effective infection control measures to reduce cross-infections.

Over the past few years, there has been an alarming increase in fungal infections, which are a serious epidemiological, clinical, diagnostic, and therapeutic problem, as well as the increased role of fungi in the spread of nosocomial infections. As already mentioned, it seems impossible to disregard the role of mobile phones in this aspect.

Jeske et al. [17] conducted an experiment, in which 40 anesthesiologists working in operating rooms were asked to use their in-hospital mobile phones following hand disinfection. Bacterial contamination of hands following the use of a mobile phone was shown in 38 out of 40 physicians. The experiment was repeated for land line phones, and bacterial contamination of hands was found in 33 out of 40 physicians. According to the authors [17], this confirms that the use of mobile phones may play a significant role in the spread of nosocomial infections since mobile phones, in contrast with land line phones, are commonly used in a patient's environment.

Since mobile phones are regarded as potential vectors of infections, it seems appropriate to investigate certain aspects related to the pathogenicity of fungi isolated from phone surfaces.

Secretion of hydrolytic enzymes by pathogenic fungi (dermatophytes, yeast-like fungi, and mold) is a known factor facilitating tissue invasion, while the activity and the nature of the secreted enzymes may play an essential role in adaptation and reflect fungal virulence.

Hydrolytic enzymes are responsible for catalyzing, among other things, the hydrolytic cleavage of C-O, C-N, C-C bonds, and the targets for this enzymatic attack are the host's cell membranes comprised of lipids and proteins [18].

According to the *Nomenclature Committee of The International Union of Biochemistry and Molecular Biology*: *Enzyme nomenclature* (1992), hydrolytic enzymes include [18]: esterases (carboxylic ester hydrolases - lipase and phosphor-lipase A2, phosphoric monoester hydrolases - alkaline and acid phosphatase, and sulphuric ester hydrolases - sulfatase), glycosidases (alphaglu-cosidase, beta-glucosidase, alpha-mannosidase, N-acetyl-beta-D-glucosidase), peptidases (amino-peptidases, arylamidases, proteinases, elastases, collagenases, keratinases), and ureases.

In the present study, we used the API ZYM to assess enzymatic activity. We found that all strains of fungi isolated from hand and mobile phone surfaces showed activity of all hydrolytic

enzymes. The mean level of hydrolytic activity in the individual fungal genera/species were comparable; however, fungi isolated from phone surfaces showed slightly lower activity compared with those from hand surfaces. The strongest correlation between the hydrolytic activity of fungi isolated from hand and phone surfaces was for *C. albicans*; whereas, the lowest was for *C. krusei*.

A number of techniques for strain differentiation among *C. albicans* species have been described in the available literature. The available typing methods include: serotyping, morphotyping, biotyping, auxotyping, enzyme-typing, an assessment of resistance to antifungal agents, the ability of adherence, and killer-yeast typing [18-28]; electrophoretic methods include immunoblotting, evaluation of isoenzymes or DNA, as well as karyotyping [22-24].

Otero *et al.* [29] compared seven biotyping methods for *C. albicans* (auxotyping; enzyme-typing; Phongpaichit's morphotyping; Hunter and Odds' morphotyping; assessment of drug resistance; and Abbott's biotyping, 1980 and 1983 versions) with a population comprising 94 strains. The authors obtained a high correlation between Hunter's morphotyping and biotyping according to Odds and Abbott [29]. Bernhardt *et al.* believe that the API ZYM system is the most effective biotyping method for *Candida albicans* [30]. Other authors also confirm this opinion [31-34].

Kurnatowska and Kurnatowski [8] found 12 biotypes characteristic of *C. albicans* based on the ability to secrete hydrolytic enzymes. These were mainly F biotypes (28.7%), followed by A (23.7%), E (18.9%), and C (10.2%). Only a few strains were I, J, K, or L biotypes [8]. None of the isolated strains showed features of biotypes B, D, or H described by Williamson [6].

Krajewska-Kułak et al. [9] included 62 strains of *C. albicans* isolated from material collected from oral cavity ontocenosis in oncological patients as well as 29 *C. albicans* strains isolated from healthy individuals. Enzymatic activity was assessed using the API ZYM test by BioMerieux. Drug resistance was assessed using the FUNGITEST® by Sanofi Pasteur. Strain biotyping was performed according to Williamson's or Kurnatowska's and Kurnatowski's classifications [9]. Isolates from healthy individuals did not show the activity of 8 hydrolases, and the highest enzymatic activity was reported for leucine arylamidase, esterase lipase, and esterase. It was also found that biotype F was dominant (30.6%) among the isolates from patients, and 6.5% of cultured strains showed activity of additional biotypes, including biotype O (4.8%) and biotype R (1.6%) [9]. Biotype E was identified in 24.1% of strains isolated from healthy individuals. Biotype O was additionally identified in 3.4%, and biotype P in 37.95% of strains [9].

These results indicate a variation of enzymatic biotypes of fungi dependent on the species as well as the site of isolation. The aim of this study was also to investigate the biotypes of fungi belonging to the genus *Candida* isolated from the surfaces of mobile phones and the hands of their owners.

The present study showed that, generally, biotypes G, B, and F were dominant for each fungal species isolated from hand surfaces. Additionally, biotype A was also dominant in *C. krusei* strains. Biotypes B, F, and G also dominated in the group of isolates from respondents' mobile phones, and biotype A was also dominant in *C. glabrata* and *C. krusei* strains. Biotypes classified into Williamson's groups A-H were dominant for strains isolated from both mobile phone and hand surfaces (95.5% and 93.2%, respectively).

CONCLUSIONS

- *Candida albicans*, *Candida glabrata*, and *Candida krusei* showed activity of all hydrolytic enzymes, with higher activity shown by strains isolated from hand surfaces compared with mobile phones.
- The strongest correlation between the hydrolytic activity of fungi isolated from hand and phone surfaces was shown for *C. albicans*; whereas, the lowest correlation was shown for *C. krusei*.
- Generally, the increased resistance of *Candida* fungi isolated from hand surfaces was associated with elevated activity of twelve hydrolytic enzymes; whereas, in the case of mobile phones, ten enzymes, with six identical enzymes for both groups.
- Although the number of biotypes was dependent on fungal species, biotypes classified according to Williamson were dominant for strains isolated from both mobile phone and hand surfaces.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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