Drug susceptibility and enzymatic activity of Candida isolated from mobile phone and hand surfaces

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ABSTRACT

Introduction: Evaluation of susceptibility of isolated fungi as well as the type and activity of enzymes they release allows to determine their pathogenicity.
Purpose; To assess potential correlations between drug susceptibility and enzymatic activity of strains isolated from mobile phone and hand surfaces
Materials and methods: The mycological evaluation included 175 mobile phones and 175 hands of the phone owners. Drug susceptibility was assessed using the FUNGITEST; enzymatic activity was evaluated using the API ZYM test.
Results: We found significant correlations between an increased resistance to 5-fluorocytosine, ketoconazole, fluconazole and higher activity of six selected enzymes for *Candida glabrata* strains isolated from hand surfaces. We found also significant correlations between an increased resistance to 5-fluorocytosine, ketoconazole, miconazole, itraconazole and higher activity of six selected enzymes for *Candida albicans* strains isolated from hand surfaces. We found significant correlations between an increased resistance to 5-fluorocytosine, ketoconazole, itraconazole, fluconazole and higher activity of six selected enzymes for *Candida krusei* strains isolated from hand surfaces as well as an increased resistance to 5-fluorocytosine, ketoconazole, itraconazole, fluconazole and higher activity of five selected enzymes for strains isolated from phone surfaces.
Conclusions: We found varying correlations between enzymatic activity and drug resistance depending on the site of isolation and the species/genus of fungi. The drugs to which the evaluated strains showed resistance were the same for hand and mobile phone isolates.
Keywords: *Candida*, Fungitest, API ZYM, hands, mobile telephones

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INTRODUCTION

The latest Ericsson Mobility Report [1] indicates that the total number of mobile telephone users will exceed the global population in 2015. For the first time, the report revealed that by the year 2019 there will be a 3- up to 4-fold increase in the number of active mobile M2M devices, which was 200 million at the end of 2013 [1].

The number of mobile phone users was 56.7 million at the end of March 2014, which was 3.4% higher than the year before [2].

Ulger et al. [3] investigated the surfaces of 200 mobile phones belonging to health care personnel and isolated different bacterial species from 94.5% of the samples. They found [3] that approx. 35% of mobile phones were inhabited by two types of bacteria, and 11% by three or more strains.

Research [4] showed that one square centimeter of a mobile phone surface was covered by approximately four thousand microbes, including fungi, while a landline phone handset was covered by an average of approx. 25 thousand bacteria, which makes telephones the greatest concentrations of bacteria in the human environment.

There are no reports on enzymatic activity and drug susceptibility of fungal strains isolated from mobile phone surfaces in the world literature.

The activity and nature of enzymes released by fungi may play an essential role in adaptation and reflect fungal virulence. Secretion of hydrolytic enzymes is a known factor that facilitates pathogenic fungi to invade tissues. Hydrolytic enzymes are responsible for catalyzing, among other things, the hydrolysis of C-O, C-N, C-C bonds [5].

By exerting chemical and physical effects on the environment, these enzymes ensure the survival of fungi in the tissues and directly participate in host protein digestion. The released hydrolases [5] cause keratolysis of the stratum corneum, which is used as a source of nitrogen and carbon.

According to the *Nomenclature Committee of The International Union of Biochemistry and Molecular Biology*: *Enzyme nomenclature* (1992), hydrolytic enzymes include [6]:

- esterases (carboxylic ester hydrolases – lipase and phospholipase A2, phosphoric monoester hydrolases – alkaline and acid phosphatase and sulphuric ester hydrolases – sulfatase),
- glycosidases (alpha-glucosidase, beta-glucosidase, alpha-mannosidase, N-acetyl-beta-D-glucosidase),
- peptidases (aminopeptidases, arylamidases, proteinases, elastases, collagenases, keratinases),
- ureases.

Host's cell membranes, comprised of lipids and proteins, are a target for the enzymatic attack of microbes, which facilitate invasion of mammalian tissues.

In recent years, there has been a growth in fungal resistance to antifungal drugs.

The aim of this study was to assess the potential correlations between drug susceptibility and the enzymatic activity of strains isolated 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 175 hands of students and professors of the Medical University of Bialystok and the personnel of university hospitals were included in the mycological evaluation.

Biological monitoring of mobile phone and hand surface contamination was performed with Count-Tact^{TM} 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 tests by BioMerieux, containing substrates for the identification of 19 hydrolases. Enzymatic activity was determined in nanomoles of hydrolyzed substrate, in accordance with the intensity of the color reaction, according to the manufacturer's instructions, always by the same person.

Drug susceptibility was assessed using FUNGITEST® (Sanofi Diagnostics Pasteur) for the analysis of the growth of yeast-like fungi in the presence of six drugs: 5-fluorocytosine, amphotericin B, miconazole, ketoconazole, itraconazole and fluconazole used at two concentrations, in modified RPMI 1640 medium, in the presence of a redox indicator.

The results were interpreted according to the manufacturer's instructions, always by the same person and always with reference to the color of two wells containing the same drug:

- a blue color in both wells indicated an *in vitro* susceptible strain;
- a pink color at lower concentrations and a blue color at higher concentrations indicated an *in vitro* strain with low susceptibility;
- a pink color in both wells indicated an *in vitro* resistant strain.

Numerical characteristics of the evaluated parameters, percentage values, and the Wilcoxon test were used for statistical analysis.

RESULTS

The following was found based on the material collected from the surfaces of mobile phones and the hands of their owners:

- *Candida glabrata* dominated among the strains isolated from the material (89.1% - hands; 74.9% - mobile phones).
- The average number of fungal colonies of the same genera/species was higher in samples collected from hand surfaces than those from mobile phone surfaces.
- The median of the number of fungal colonies was significantly lower than the average, which suggests that few respondents from the study population were identified with a large number of fungal colonies.
- The maximum values were very high for some types of fungal genera (Table 1).
- No fungi were isolated from 0.6% of hand surfaces and 5.7% of mobile phone surfaces.

Table 1. Numbers of *Candida* fungi in samples collected from hand and phone surfaces

Number of fungal colonies	*N*	%	$\overline{x}$	Me	*s*	c_{25}	c_{75}	min.	max.
on the surface of the hand									
Candida albicans	146	83.4%	5.9	3	6.6	2	8	1	30
Candida glabrata	156	89.1%	13.0	8.5	15.7	3	15	1	100
Candida krusei	122	69.7%	12.4	5	19.5	2	12	1	100
Candida tropicalis	9	5.1.%	5.2	4	4.6	2	7	1	15
Candida species	1	0.6%	4.0	4	0	4	4	4	4
on the surface of the phone									
Candida albicans	114	65.1%	3.3	2	3.8	1	4	1	20
Candida glabrata	131	74.9%	8.5	5	8.3	2	13	1	40
Candida krusei	95	54.3%	6.3	3	8.0	2	8	1	43
Candida tropicalis	11	6.3%	1.5	1	1.2	1	1	1	5

The main aim of this study was to do a detailed analysis of the correlations between the hydrolytic activity of certain enzymes and resistance to different types of antimycotics.

Information on the number of drugs to which the isolated strains were resistant was also included.

Statistically significant correlation coefficients are marked with * in the tables (*p* value lower than 0.05). They are additionally highlighted in bold.

We have found that the strength of almost all statistically significant correlations was very low (R rarely exceeded 0.30), which indicates that the practical importance of these correlations may be limited. Nevertheless, they signal if and where any correlations between activity and susceptibility occurred.

Table 2 contains the results of the analysis of correlations between enzymatic activity and susceptibility to antifungal drugs of *Candida albicans* isolated from hand surfaces. We found statistically significant correlations between an increased resistance to 5-fluorocytosine and higher activity of acid phosphatase; an increased resistance to ketoconazole and higher activity of beta-glucuronidase; an increased resistance to miconazole and higher activity of beta-glucosidase; an increased resistance to itraconazole and higher activity of N-acetyl-beta-D-glucosidase, as well as an increased susceptibility to itraconazole and higher enzymatic activity of cystine arylamidase and naphthyl phosphohydrolase. Other correlations were statistically insignificant.

Table 3 contains the results of the analysis of correlations between enzymatic activity and susceptibility to antifungal drugs of *Candida albicans* isolated from mobile phone surfaces.

We found statistically significant correlations between an increased resistance to 5-fluorocytosine and higher activity of naphthyl phosphohydrolase and lipase; an increased resistance to miconazole and higher activity of beta-glucosidase; an increased resistance to fluconazole and higher activity of alpha-glucosidase, alpha-galactosidase and fucosidase.

Other correlations were statistically insignificant. Table 4 contains the results of the analysis of correlations between enzymatic activity and susceptibility to antifungal drugs of *Candida glabrata* isolated from hand surfaces.

We found statistically significant correlations between an increased resistance to 5-fluorocytosine and higher enzymatic activity of alkaline phosphatase, chymotrypsin and alpha-glucosidase; an increased resistance to fluconazole and higher enzymatic activity of esterase and beta-glucosidase as well as an increased susceptibility to ketoconazole and higher enzymatic activity of cystine arylamidase; an increased susceptibility to fluconazole and higher enzymatic activity of mannosidase. Other correlations were statistically insignificant.

Table 5 contains the results of the analysis of correlations between enzymatic activity and susceptibility to antifungal drugs of *Candida glabrata* isolated from mobile phone surfaces.

Table 2. Correlations between enzymatic activity and susceptibility of *Candida albicans* strains isolated from hands

Enzymatic activity (enzyme type)	***Candida albicans* hand Susceptibility to antibiotics**						
	5-fluorocytosine	**amphotericin B**	**miconazole**	**ketoconazole**	**itraconazole**	**fluconazole**	**Number of resistant drugs**
Alkaline phosphatase	0.03 (0.6814)	-0.02 (0.8082)	0.02 (0.8300)	-0.02 (0.8231)	0.16 (0.0547)	-0.07 (0.3846)	-0.01 (0.8700)
Esterase (C4)	0.09 (0.2696)	0.00 (0.9767)	0.00 (0.9649)	0.03 (0.7122)	-0.07 (0.4082)	0.12 (0.1517)	0.03 (0.6992)
Lipase esterase (C8)	-0.02 (0.8372)	-0.14 (0.0948)	0.06 (0.4531)	-0.06 (0.4442)	-0.08 (0.3153)	0.12 (0.1379)	0.03 (0.7589)
Lipase (C14)	-0.13 (0.1185)	-0.15 (0.0757)	0.05 (0.5834)	-0.06 (0.4442)	-0.12 (0.1562)	0.16 (0.0578)	0.04 (0.6355)
Leucine arylamidase	0.03 (0.7298)	-0.03 (0.6995)	-0.08 (0.3222)	0.11 (0.2008)	0.02 (0.8530)	0.04 (0.6690)	-0.05 (0.5508)
Valine arylamidase	0.00 (0.9973)	0.07 (0.4172)	-0.05 (0.5476)	0.01 (0.8678)	0.09 (0.2665)	0.04 (0.5928)	0.14 (0.1021)
Cystine arylamidase	0.06 (0.4674)	-0.02 (0.7987)	0.06 (0.4446)	-0.12 (0.1533)	**-0.23 (0.0063)**	0.03 (0.7151)	-0.06 (0.4439)
Trypsin	-0.01 (0.9463)	0.06 (0.4455)	-0.02 (0.7958)	0.05 (0.5526)	0.02 (0.8062)	0.02 (0.8211)	0.08 (0.3665)
Chymotrypsin	-0.05 (0.5128)	0.11 (0.2013)	-0.06 (0.4534)	-0.05 (0.5864)	0.06 (0.4430)	0.09 (0.3062)	0.03 (0.7049)
Acid phosphatase	**0.19 (0.0233)**	0.13 (0.1297)	-0.13 (0.1198)	0.03 (0.7285)	0.06 (0.4834)	0.12 (0.1478)	0.05 (0.5772)
Naphthyl phosphohydrolase	-0.05 (0.5519)	-0.01 (0.8705)	0.09 (0.2916)	0.05 (0.5525)	**-0.24 (0.0030)**	0.04 (0.6666)	-0.04 (0.6216)
alpha-Galactosidase	-0.09 (0.2963)	-0.05 (0.5431)	0.07 (0.4087)	0.01 (0.9018)	-0.04 (0.6716)	0.03 (0.7542)	0.04 (0.6266)
beta-Galactosidase	0.05 (0.5731)	-0.01 (0.9504)	0.09 (0.2880)	-0.10 (0.2169)	-0.06 (0.5064)	-0.04 (0.6157)	0.00 (0.9808)
beta-Glucuronidase	0.16 (0.0569)	-0.11 (0.1741)	-0.02 (0.8237)	**0.19 (0.0205)**	0.00 (0.9696)	-0.11 (0.1907)	0.06 (0.4868)
alpha-Glucosidase	-0.06 (0.4813)	-0.02 (0.7736)	0.02 (0.8468)	0.05 (0.5761)	-0.02 (0.8273)	0.06 (0.4941)	-0.06 (0.4531)
beta-Glucosidase	-0.06 (0.4868)	0.02 (0.8351)	**0.17 (0.0412)**	0.05 (0.5792)	0.01 (0.9038)	-0.08 (0.3252)	-0.01 (0.9091)

Table 3. Correlations between enzymatic activity and susceptibility of *Candida albicans* strains isolated from mobile phone surfaces

Enzymatic activity (enzyme type)	***Candida albicans*** **phone Susceptibility to antibiotics**						
	5-fluorocytosine	**amphotericin B**	**miconazole**	**ketoconazole**	**itraconazole**	**fluconazole**	**Number of resistant drugs**
Alkaline phosphatase	0.02 (0.8578)	0.11 (0.2627)	0.00 (0.9942)	-0.02 (0.8057)	0.06 (0.5230)	0.03 (0.7723)	0.13 (0.1730)
Esterase (C4)	0.17 (0.0667)	0.07 (0.4662)	0.03 (0.7174)	0.09 (0.3473)	-0.03 (0.7613)	0.08 (0.3739)	0.17 (0.0810)
Lipase esterase (C8)	0.17 (0.0692)	0.06 (0.5160)	0.14 (0.1508)	0.11 (0.2433)	0.07 (0.4374)	0.09 (0.3356)	**0.20 (0.0318)**
Lipase (C14)	**0.21 (0.0301)**	-0.05 (0.6021)	0.17 (0.0685)	0.09 (0.3211)	0.02 (0.8049)	0.08 (0.4260)	**0.20 (0.0319)**
Leucine arylamidase	0.12 (0.2195)	0.06 (0.5275)	0.10 (0.2773)	0.08 (0.4145)	-0.06 (0.5226)	0.04 (0.6543)	0.03 (0.7446)
Valine arylamidase	-0.06 (0.5222)	-0.06 (0.5640)	-0.08 (0.3811)	-0.11 (0.2356)	0.11 (0.2397)	0.01 (0.9448)	0.03 (0.7301)
Cystine arylamidase	0.03 (0.7224)	0.08 (0.4152)	0.08 (0.3735)	0.09 (0.3291)	-0.16 (0.0985)	0.05 (0.6319)	0.12 (0.2043)
Trypsin	-0.07 (0.4430)	-0.01 (0.9166)	-0.15 (0.1246)	-0.09 (0.3245)	0.04 (0.6430)	0.01 (0.9409)	-0.04 (0.7088)
Chymotrypsin	0.02 (0.8201)	0.05 (0.5714)	-0.03 (0.7248)	0.00 (0.9604)	0.09 (0.3209)	0.06 (0.5087)	0.03 (0.7456)
Acid phosphatase	0.13 (0.1568)	0.07 (0.4767)	0.02 (0.8256)	-0.08 (0.4076)	-0.07 (0.4463)	0.14 (0.1399)	0.15 (0.1113)
Naphthyl phosphohydrolase	**0.20 (0.0330)**	0.11 (0.2596)	0.05 (0.6131)	-0.12 (0.2142)	0.02 (0.8201)	0.02 (0.8397)	0.15 (0.1092)
alpha-Galactosidase	0.01 (0.8780)	-0.04 (0.6721)	0.11 (0.2398)	0.16 (0.0921)	-0.15 (0.1157)	**0.22 (0.0175)**	**0.21 (0.0230)**
beta-Galactosidase	0.05 (0.5695)	-0.06 (0.4976)	**0.20 (0.0385)**	0.04 (0.6516)	0.00 (0.9679)	-0.01 (0.9276)	0.11 (0.2503)
beta-Glucuronidase	-0.08 (0.3905)	0.18 (0.0606)	-0.10 (0.2925)	-0.01 (0.9062)	0.02 (0.8341)	-0.15 (0.1081)	-0.08 (0.3909)
alpha-Glucosidase	-0.09 (0.3503)	-0.04 (0.7044)	-0.05 (0.5952)	0.14 (0.1307)	-0.11 (0.2368)	**0.30 (0.0015)**	**0.23 (0.0149)**
beta-Glucosidase	0.07 (0.4519)	0.06 (0.5276)	0.15 (0.1220)	0.18 (0.0622)	0.04 (0.6823)	0.03 (0.7284)	0.00 (0.9810)
N-acetyl-beta-D-glucosidase	-0.11 (0.2326)	0.13 (0.1614)	0.04 (0.6984)	0.10 (0.2767)	0.04 (0.7121)	0.05 (0.5803)	0.01 (0.8912)
alpha-Mannosidase	0.03 (0.7534)	-0.05 (0.5727)	0.08 (0.4212)	0.02 (0.8038)	0.08 (0.4314)	0.06 (0.5383)	0.03 (0.7633)
alpha-Fucosidase	0.02 (0.8110)	-0.14 (0.1495)	0.13 (0.1789)	0.08 (0.4040)	0.03 (0.7829)	**0.21 (0.0262)**	**0.19 (0.0482)**

Table 4. Correlations between enzymatic activity and susceptibility of *Candida glabrata* strains isolated from hands

Enzymatic activity (enzyme type)	***Candida glabrata*** **hand Susceptibility to antibiotics**						
	5-fluorocytosine	**amphotericin B**	**miconazole**	**ketoconazole**	**itraconazole**	**fluconazole**	**Number of resistant drugs**
Alkaline phosphatase	**0.17 (0.0310)**	0.00 (0.9587)	0.02 (0.7898)	0.03 (0.6849)	-0.04 (0.5983)	0.07 (0.3978)	0.05 (0.5310)
Esterase (C4)	0.06 (0.4832)	0.16 (0.0530)	-0.09 (0.2733)	-0.06 (0.4535)	-0.02 (0.7591)	**0.20 (0.0109)**	0.13 (0.1044)
Lipase esterase (C8)	0.09 (0.2458)	0.05 (0.5585)	-0.04 (0.5950)	-0.07 (0.3690)	0.05 (0.5324)	0.08 (0.3310)	0.05 (0.5361)
Lipase (C14)	-0.03 (0.6763)	-0.05 (0.5203)	-0.04 (0.6066)	-0.04 (0.6583)	-0.01 (0.8596)	0.02 (0.8071)	-0.02 (0.8492)
Leucine arylamidase	0.13 (0.1016)	0.15 (0.0621)	-0.11 (0.1749)	-0.05 (0.5007)	0.07 (0.3977)	0.13 (0.0992)	**0.19 (0.0201)**
Valine arylamidase	-0.13 (0.1004)	0.04 (0.6287)	-0.09 (0.2675)	-0.04 (0.6449)	-0.08 (0.3198)	0.03 (0.7133)	0.02 (0.7676)
Cystine arylamidase	-0.08 (0.3184)	-0.08 (0.3477)	0.01 (0.9390)	**-0.17 (0.0325)**	0.00 (0.9606)	0.02 (0.8108)	0.01 (0.8829)
Trypsin	-0.10 (0.2110)	-0.01 (0.9158)	0.05 (0.5054)	-0.06 (0.4898)	-0.10 (0.2327)	0.01 (0.8978)	0.01 (0.9251)
Chymotrypsin	**0.25 (0.0017)**	0.08 (0.3189)	-0.08 (0.3191)	0.03 (0.7258)	0.07 (0.3618)	0.04 (0.6459)	0.12 (0.1285)
Acid phosphatase	0.00 (0.9991)	0.11 (0.1653)	-0.11 (0.1834)	0.04 (0.6498)	0.03 (0.7411)	0.09 (0.2496)	0.06 (0.4490)
Naphthyl phosphohydrolase	-0.05 (0.5032)	0.00 (0.9576)	-0.08 (0.3524)	0.04 (0.6285)	-0.08 (0.2963)	-0.06 (0.4738)	-0.04 (0.6225)
alpha-Galactosidase	-0.10 (0.2147)	0.04 (0.6210)	0.02 (0.7589)	-0.16 (0.0505)	-0.13 (0.1199)	-0.05 (0.5784)	0.04 (0.5923)
beta-Galactosidase	-0.03 (0.7121)	0.02 (0.8381)	0.04 (0.5932)	-0.09 (0.2455)	-0.01 (0.9174)	-0.04 (0.6060)	0.00 (0.9962)
beta-Glucuronidase	-0.01 (0.9191)	-0.10 (0.2034)	-0.05 (0.5366)	-0.08 (0.3199)	-0.01 (0.9314)	-0.01 (0.8617)	0.09 (0.2828)
alpha-Glucosidase	**0.20 (0.0126)**	0.06 (0.4833)	-0.07 (0.3721)	-0.02 (0.8022)	-0.05 (0.5365)	0.12 (0.1257)	0.06 (0.4668)
beta-Glucosidase	0.15 (0.0579)	0.00 (0.9828)	-0.04 (0.5825)	-0.04 (0.6173)	-0.01 (0.9085)	**0.18 (0.0252)**	0.13 (0.1180)
N-acetyl-beta-D-glucosidase	0.14 (0.0903)	0.10 (0.2239)	-0.07 (0.4099)	0.01 (0.9167)	0.04 (0.6014)	0.06 (0.4709)	0.04 (0.6115)
alpha-Mannosidase	0.03 (0.7453)	0.04 (0.5906)	0.05 (0.5098)	-0.05 (0.5386)	0.13 (0.1050)	**-0.17 (0.0320)**	0.00 (0.9826)
alpha-Fucosidase	0.00 (0.9642)	-0.02 (0.8393)	0.04 (0.6141)	-0.13 (0.1177)	0.08 (0.3146)	-0.03 (0.7272)	0.05 (0.5774)

Table 5. Correlations between enzymatic activity and susceptibility of *Candida glabrata* strains isolated from mobile phone surfaces

Enzymatic activity (enzyme type)	***Candida glabrata*** **– phone Susceptibility to antibiotics**						
	5-fluorocytosine	**amphotericin B**	**miconazole**	**ketoconazole**	**itraconazole**	**fluconazole**	**Number of resistant drugs**
Alkaline phosphatase	0.01 (0.9367)	-0.02 (0.7939)	0.08 (0.3549)	-0.01 (0.8891)	0.08 (0.3575)	0.13 (0.1349)	0.00 (0.9759)
Esterase (C4)	0.04 (0.6226)	0.13 (0.1315)	-0.12 (0.1620)	0.06 (0.5314)	0.10 (0.2456)	0.04 (0.6239)	0.13 (0.1446)
Lipase esterase (C8)	0.16 (0.0746)	0.07 (0.4454)	-0.01 (0.9417)	-0.04 (0.6380)	-0.05 (0.5794)	-0.04 (0.6890)	-0.02 (0.7783)
Lipase (C14)	0.10 (0.2613)	-0.02 (0.8595)	0.04 (0.6180)	-0.08 (0.3840)	**-0.18 (0.0375)**	-0.10 (0.2482)	-0.07 (0.4407)
Leucine arylamidase	0.16 (0.0733)	0.06 (0.4802)	**-0.23 (0.0077)**	0.03 (0.7558)	0.13 (0.1540)	0.03 (0.7596)	0.06 (0.5270)
Valine arylamidase	0.02 (0.8492)	-0.16 (0.0640)	0.15 (0.0930)	0.11 (0.2306)	-0.05 (0.6053)	0.10 (0.2700)	0.00 (0.9966)
Cystine arylamidase	0.07 (0.4289)	0.08 (0.3675)	0.02 (0.8614)	0.04 (0.6610)	-0.04 (0.6828)	-0.06 (0.4687)	-0.07 (0.4349)
Trypsin	-0.06 (0.5270)	0.02 (0.8019)	0.05 (0.6105)	0.11 (0.1958)	-0.06 (0.5052)	-0.11 (0.2113)	0.07 (0.4071)
Chymotrypsin	**0.25 (0.0048)**	-0.06 (0.5143)	0.09 (0.2951)	0.08 (0.3589)	-0.15 (0.0984)	0.05 (0.5876)	0.05 (0.5699)
Acid phosphatase	0.03 (0.7678)	0.06 (0.4773)	**-0.21 (0.0184)**	0.03 (0.7752)	-0.09 (0.2863)	0.06 (0.4864)	-0.06 (0.5151)
Naphthyl phosphohydrolase	0.03 (0.7375)	-0.03 (0.7386)	-0.04 (0.6206)	-0.04 (0.6181)	0.03 (0.7279)	0.08 (0.3830)	0.06 (0.4867)
alpha-Galactosidase	0.06 (0.5184)	0.00 (0.9975)	-0.03 (0.7705)	-0.08 (0.3511)	0.00 (0.9722)	0.09 (0.2922)	-0.01 (0.8684)
beta-Galactosidase	-0.03 (0.6963)	-0.11 (0.2103)	0.04 (0.6569)	-0.03 (0.7343)	-0.01 (0.9338)	0.10 (0.2682)	-0.05 (0.5890)
beta-Glucuronidase	0.05 (0.5679)	-0.07 (0.4470)	**0.18 (0.0414)**	0.15 (0.0855)	-0.03 (0.7228)	-0.01 (0.8928)	-0.01 (0.8914)
alpha-Glucosidase	0.04 (0.6754)	-0.02 (0.8287)	0.10 (0.2656)	0.13 (0.1393)	-0.06 (0.5025)	0.07 (0.4146)	0.07 (0.4372)
beta-Glucosidase	0.10 (0.2695)	-0.01 (0.8732)	0.02 (0.8062)	-0.01 (0.8688)	-0.02 (0.8653)	0.09 (0.3069)	0.02 (0.7938)
N-acetyl-beta-D-glucosidase	0.15 (0.0820)	0.11 (0.2017)	0.12 (0.1646)	0.04 (0.6216)	0.01 (0.9450)	0.09 (0.2952)	**0.20 (0.0235)**
alpha-Mannosidase	**0.19 (0.0318)**	-0.02 (0.8180)	-0.05 (0.5692)	-0.07 (0.4422)	0.08 (0.3396)	0.08 (0.3452)	0.07 (0.4285)
alpha-Fucosidase	0.14 (0.1226)	0.03 (0.7053)	-0.05 (0.5652)	-0.08 (0.3584)	-0.03 (0.7657)	0.00 (0.9897)	-0.04 (0.6211)

We found statistically significant correlations between an increased resistance to 5-fluorocytosine and higher enzymatic activity of chymotrypsin and alpha-mannosidase; an increased resistance to miconazole and higher enzymatic activity of beta-glucuronidase; as well as an increased susceptibility to miconazole and higher enzymatic activity of leucine arylamidase and acid phosphatase; an increased susceptibility to itraconazole and higher enzymatic activity of lipase. Other correlations were statistically insignificant.

Table 6 contains the results of the analysis of correlations between enzymatic activity and susceptibility to antifungal drugs of *Candida krusei* isolated from hand surfaces.

Table 6. Correlations between enzymatic activity and susceptibility of *Candida krusei* strains isolated from hands

Enzymatic activity (enzyme type)	***Candida krusei* hand Susceptibility to antibiotics**						
	5-fluorocytosine	**amphotericin B**	**miconazole**	**ketoconazole**	**itraconazole**	**fluconazole**	**Number of resistant drugs**
Alkaline phosphatase	**-0.19 (0.0325)**	0.15 (0.0945)	0.09 (0.3362)	0.01 (0.8917)	0.04 (0.6685)	-0.10 (0.2571)	-0.16 (0.0772)
Esterase (C4)	0.01 (0.9249)	0.12 (0.1966)	-0.04 (0.6594)	0.06 (0.5437)	-0.06 (0.5130)	-0.09 (0.3456)	**-0.19 (0.0356)**
Lipase esterase (C8)	-0.07 (0.4614)	0.01 (0.8920)	-0.13 (0.1618)	0.01 (0.9447)	-0.11 (0.2467)	0.02 (0.8699)	0.05 (0.5737)
Lipase (C14)	-0.05 (0.5647)	0.02 (0.8532)	0.04 (0.6391)	-0.05 (0.5680)	-0.04 (0.6605)	0.16 (0.0826)	0.07 (0.4460)
Leucine arylamidase	-0.18 (0.0537)	0.05 (0.6082)	0.00 (0.9955)	0.02 (0.8016)	0.06 (0.4860)	0.01 (0.8812)	-0.10 (0.2860)
Valine arylamidase	**-0.19 (0.0412)**	-0.08 (0.3760)	0.12 (0.2021)	0.03 (0.7588)	0.17 (0.0552)	0.00 (0.9823)	0.06 (0.4889)
Cystine arylamidase	-0.07 (0.4599)	0.07 (0.4733)	0.07 (0.4445)	**-0.20 (0.0302)**	0.02 (0.8642)	0.13 (0.1666)	0.10 (0.2939)
Trypsin	**-0.18 (0.0464)**	-0.04 (0.6880)	0.00 (0.9716)	-0.11 (0.2503)	0.00 (0.9782)	**0.19 (0.0321)**	0.13 (0.1509)
Chymotrypsin	**-0.19 (0.0393)**	-0.17 (0.0691)	0.14 (0.1149)	-0.12 (0.1868)	0.00 (0.9870)	0.04 (0.6715)	-0.02 (0.8457)
Acid phosphatase	-0.07 (0.4203)	0.04 (0.6305)	0.08 (0.4110)	0.05 (0.6227)	-0.08 (0.3862)	0.03 (0.7691)	0.05 (0.6044)
Naphthyl phosphohydrolase	0.03 (0.7660)	0.06 (0.5454)	-0.05 (0.5653)	0.04 (0.6814)	**0.18 (0.0468)**	0.15 (0.0993)	**0.19 (0.0410)**
alpha-Galactosidase	-0.02 (0.8484)	0.13 (0.1587)	0.06 (0.5058)	0.02 (0.8073)	**0.21 (0.0192)**	-0.08 (0.3617)	0.02 (0.8568)
beta-Galactosidase	0.08 (0.4058)	0.10 (0.2848)	0.05 (0.5779)	0.13 (0.1423)	0.04 (0.6470)	0.00 (0.9702)	0.08 (0.4004)
beta-Glucuronidase	-0.09 (0.3246)	0.08 (0.3969)	0.04 (0.6307)	0.03 (0.7275)	-0.06 (0.4937)	0.03 (0.7072)	0.01 (0.9420)
alpha-Glucosidase	0.02 (0.8035)	0.06 (0.5249)	-0.14 (0.1202)	0.07 (0.4696)	-0.16 (0.0750)	0.06 (0.5096)	0.07 (0.4543)
beta-Glucosidase	-0.04 (0.6692)	0.12 (0.1737)	0.07 (0.4729)	0.12 (0.1977)	-0.07 (0.4405)	**0.19 (0.0384)**	**0.21 (0.0179)**
N-acetyl-beta-D-glucosidase	-0.05 (0.5712)	-0.10 (0.2755)	-0.09 (0.3396)	-0.02 (0.8620)	0.01 (0.8745)	-0.08 (0.3933)	-0.14 (0.1238)
alpha-Mannosidase	-0.06 (0.5410)	0.10 (0.2888)	0.11 (0.2404)	0.02 (0.7902)	0.04 (0.6555)	0.06 (0.4914)	0.12 (0.1852)
alpha-Fucosidase	-0.09 (0.3439)	0.10 (0.2620)	0.15 (0.0925)	0.03 (0.7725)	0.02 (0.8318)	0.02 (0.8392)	0.06 (0.5418)

Table 7. Correlations between enzymatic activity and susceptibility of *Candida krusei* strains isolated from mobile phone surfaces

Enzymatic activity (enzyme type)	***Candida krusei* phone Susceptibility to antibiotics**						
	5-fluorocytosine	**amphotericin B**	**miconazole**	**ketoconazole**	**itraconazole**	**fluconazole**	**Number of resistant drugs**
Alkaline phosphatase	-0.11 (0.3134)	-0.11 (0.3113)	0.05 (0.6756)	-0.06 (0.6068)	-0.16 (0.1610)	-0.09 (0.4362)	-0.11 (0.3227)
Esterase (C4)	0.02 (0.8312)	0.07 (0.5638)	-0.12 (0.2821)	0.13 (0.2609)	-0.21 (0.0586)	-0.15 (0.1991)	-0.11 (0.3307)
Lipase esterase (C8)	0.09 (0.4300)	0.10 (0.3563)	-0.11 (0.3491)	0.01 (0.9288)	-0.19 (0.0938)	-0.11 (0.3193)	-0.12 (0.2737)
Lipase (C14)	0.14 (0.2270)	-0.12 (0.2896)	-0.02 (0.8304)	-0.01 (0.9254)	0.13 (0.2388)	0.04 (0.7197)	0.08 (0.4753)
Leucine arylamidase	-0.05 (0.6790)	0.00 (0.9794)	0.10 (0.3608)	-0.09 (0.4320)	-0.12 (0.3071)	0.06 (0.6143)	0.04 (0.7510)
Valine arylamidase	-0.08 (0.5082)	0.17 (0.1352)	0.10 (0.3958)	-0.21 (0.0602)	**-0.22 (0.0465)**	0.00 (0.9678)	-0.09 (0.4173)
Cystine arylamidase	-0.03 (0.7665)	-0.03 (0.8059)	-0.10 (0.3599)	-0.20 (0.0760)	0.19 (0.0836)	0.16 (0.1656)	0.16 (0.1523)
Trypsin	0.03 (0.7870)	0.01 (0.9176)	0.04 (0.7128)	**0.24 (0.0292)**	0.06 (0.6169)	0.18 (0.1100)	0.15 (0.1747)
Chymotrypsin	-0.09 (0.4074)	-0.07 (0.5653)	0.03 (0.8065)	-0.06 (0.6140)	0.13 (0.2575)	0.07 (0.5357)	0.07 (0.5399)
Acid phosphatase	0.00 (0.9948)	0.14 (0.2134)	0.21 (0.0600)	-0.11 (0.3214)	-0.17 (0.1392)	0.01 (0.9259)	-0.03 (0.7822)
Naphthyl phosphohydrolase	0.06 (0.5724)	-0.08 (0.4621)	-0.04 (0.7168)	-0.19 (0.0987)	-0.15 (0.1740)	0.14 (0.2170)	0.06 (0.6089)
alpha-Galactosidase	0.17 (0.1229)	0.07 (0.5287)	-0.06 (0.6159)	-0.06 (0.5925)	0.01 (0.8960)	0.05 (0.6577)	0.01 (0.9414)
beta-Galactosidase	0.03 (0.7665)	0.12 (0.2999)	-0.07 (0.5334)	**-0.28 (0.0121)**	-0.01 (0.9310)	0.10 (0.3841)	0.08 (0.5032)
beta-Glucuronidase	0.15 (0.1958)	-0.09 (0.4226)	-0.01 (0.9516)	0.15 (0.1817)	-0.08 (0.4646)	-0.19 (0.0853)	-0.18 (0.1169)
alpha-Glucosidase	0.21 (0.0558)	-0.05 (0.6494)	0.04 (0.7265)	-0.17 (0.1420)	-0.04 (0.6939)	0.07 (0.5407)	0.02 (0.8358)
beta-Glucosidase	0.12 (0.2702)	0.04 (0.7466)	-0.07 (0.5364)	**-0.31 (0.0058)**	**0.29 (0.0091)**	**0.24 (0.0306)**	0.18 (0.1036)
N-acetyl-beta-D-glucosidase	0.15 (0.1747)	0.02 (0.8482)	-0.02 (0.8742)	-0.04 (0.7308)	0.08 (0.4802)	0.04 (0.7180)	0.09 (0.4251)
alpha-Mannosidase	**0.23 (0.0404)**	-0.05 (0.6673)	0.02 (0.8826)	-0.21 (0.0643)	0.01 (0.9571)	0.09 (0.4286)	0.08 (0.5081)
alpha-Fucosidase	0.12 (0.2760)	0.08 (0.4562)	0.06 (0.5716)	-0.20 (0.0764)	-0.02 (0.8265)	0.09 (0.4272)	0.09 (0.4082)

We found statistically significant correlations between an increased resistance to itraconazole and higher enzymatic activity of naphthyl phosphohydrolase and alpha-galactosidase; an increased resistance to fluconazole and higher enzymatic activity of trypsin and beta-glucosidase; as well as an increased susceptibility to 5-fluorocytosine and higher enzymatic activity of alkaline phosphatase, valine arylamidase, trypsin and chymotrypsin; an increased susceptibility to ketoconazole and higher enzymatic activity of cystine arylamidase. Other correlations were statistically insignificant.

Table 7 contains the results of the analysis of correlations between enzymatic activity and susceptibility to antifungal drugs of Candida krusei isolated from phone surfaces.

We found statistically significant correlations between an increased resistance to 5-fluorocytosine and higher enzymatic activity of alpha-mannosidase; an increased resistance to ketoconazole and higher enzymatic activity of trypsin; an increased resistance to itraconazole and higher enzymatic activity of beta-glucosidase; an increased resistance to fluconazole and higher enzymatic activity of beta-glucosidase; as well as an increased susceptibility to ketoconazole and higher enzymatic activity of beta-galactosidase and beta- glucosidase; an increased susceptibility to itraconazole and higher enzymatic activity of valine arylamidase. Other correlations were statistically insignificant.

With regard to the strains of Candida tropicalis isolated from the hand surfaces of mobile phone owners, we found statistically significant correlations only between an increased resistance to miconazole and higher activity of esterase ($p=0.014$) and leucine arylamidase ($p=0.008$). With regard to the strains of Candida tropicalis isolated from mobile phone surfaces, we found statistically significant correlations only between an increased resistance to ketoconazole and higher activity of esterase lipase ($p=0.030$).

DISCUSSION

The increasing growth of fungal resistance to commonly used antifungals encourages intensive research that could explain the thus far unexplored mechanisms of fungal virulence and resistance to drugs.

A substantial amount of literature data indicates that *Candida albicans* strains, which are characterized by high proteolytic activity, are also the most pathogenic strains [7-13].

Naglik et al. [7,8] showed that *Candida albicans* strains which release large amounts of proteases are definitely more pathogenic than mutants lacking the ability to produce these enzymes.

Other studies [9] showed that the disruption of the genes responsible for producing proteolytic enzymes resulted in reduced fungal pathogenicity. According to Batura-Gabryel and Młynarczyk [10], there was a statistically significant correlation between the proteolytic and lipolytic activity of the tested strains. Krajewska-Kułak et al. [11] also showed statistically significantly higher activity of proteolytic enzymes among *Candida* strains isolated from patients with chronic recurrent vaginal candidiasis compared with the enzymatic activity of strains isolated from females without clinical symptoms. Other authors observed a similar correlation for other ontocenoses [12,13].

In their study of *Candida* strains isolated from the blood, Ashraf et al. [14] found that the strains showed significantly higher activity of proteolytic enzymes compared with strains isolated from patients without clinical symptoms.

Fekete-Forgas et al. [15] showed that *Candida albicans* strains with induced resistance to fluconazole showed greater ability to produce proteases as well as population invasiveness.

Tyczkowska-Sieroń and Kurnatowski [16] decided to investigate the relationship between hydrolytic enzyme activity and resistance to fluconazole. They included 200 *Candida* strains in their analysis. They assayed hydrolytic enzyme activity using the APIZYM test and drug susceptibility using the disc diffusion method. The authors [16] showed a statistically significant correlation between the enzymatic activity of *Candida* strains and their resistance to fluconazole in the case of some of the enzymes. The high resistance of *C. glabrata* to fluconazole was associated with high activity of acid phosphatase and lack of releasing beta-glucosidasis and N-acetyl-beta-D-glucosidase [16].

Evaluation of susceptibility of isolated fungi as well as the type and activity of enzymes they release allows to determine their pathogenicity.

In our study, we generally showed statistically significant correlations between an increased resistance to 5-fluorocytosine, ketoconazole, miconazole, fluconazole, itraconazole and higher activity of alpha-galactosidase, alpha-glucosidase, leucine arylamidase, beta-glucuronidase, beta-glucosidase, chymotrypsin, esterase, acid phosphatase, alkaline phosphatase, naphthyl phosphohydrolase, N-acetyl-beta-D-glucosidase, and trypsin for isolates from hand surfaces.

In the case of isolates from mobile phone surfaces, we generally showed statistically significant correlations between an increased resistance to 5-fluorocytosine, miconazole, fluconazole, ketoconazole, itraconazole and higher enzymatic activity of alpha-galactosidase, alpha-glucosidase, alpha-mannosidase, beta-glucuronidase, beta-glucosidase, chymotrypsin, naphthyl phosphohydrolase, fucosidase, lipase, and esterase lipase.

The drugs to which the evaluated strains showed resistance were the same for hand and mobile phone isolates. Both groups of strains showed higher enzymatic activity for alpha-galactosidase, alpha-glucosidase, beta-glucuronidase, beta-glucosidase, chymotrypsin, and naphthyl phosphohydrolase.

Unfortunately, there are no reports on enzymatic activity and drug susceptibility of fungal strains isolated from mobile phone surfaces in the literature. Therefore, a more extensive discussion comparing our results with other authors' reports is impossible.

CONCLUSIONS

1. *Candida albicans*, *Candida glabrata*, and *Candida krusei* dominated among the yeast-like fungi isolated from hand and mobile phone surfaces.
2. We found varying correlations between enzymatic activity and drug resistance depending on the site of isolation and the species/genus of fungi.
3. The strength of nearly all statistically significant correlations between enzymatic activity and drug resistance was very low.

Conflicts of interest

The authors declare that they have no conflicts of interest

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