Antimicrobial Resistance & Infection Control

RESEARCH

Open Access

In vitro long-term exposure to chlorhexidine or triclosan induces cross-resistance against azoles in *Nakaseomyces glabratus*

Kathrin Spettel[1,2†], Dominik Bumberger[1†], Richard Kriz[2,3], Sarah Frank[1], Madita Loy[1], Sonia Galazka[4], Miranda Suchomel[5], Heimo Lagler[3], Athanasios Makristathis[1] and Birgit Willinger[1*]

Abstract

Background Topical antiseptics are crucial for preventing infections and reducing transmission of pathogens. However, commonly used antiseptic agents have been reported to cause cross-resistance to other antimicrobials in bacteria, which has not yet been described in yeasts. This study aims to assess the in vitro efficacy of antiseptics against clinical and reference isolates of *Candida albicans* and *Nakaseomyces glabratus*, and whether prolonged exposure to antiseptics promotes the development of antifungal (cross)resistance.

Methods A high-throughput approach for in vitro resistance development was established to simultaneously expose 96 *C. albicans* and *N. glabratus* isolates to increasing concentrations of a given antiseptic – chlorhexidine, triclosan or octenidine. Susceptibility testing and whole genome sequencing of yeast isolates pre- and post-exposure were performed.

Results Long-term exposure to antiseptics does not result in the development of stable resistance to the antiseptics themselves. However, 50 *N. glabratus* isolates acquired resistance to azole antifungals after long-term exposure to triclosan or chlorhexidine, revealing newly acquired mutations in the *PDR1* and *PMA1* genes.

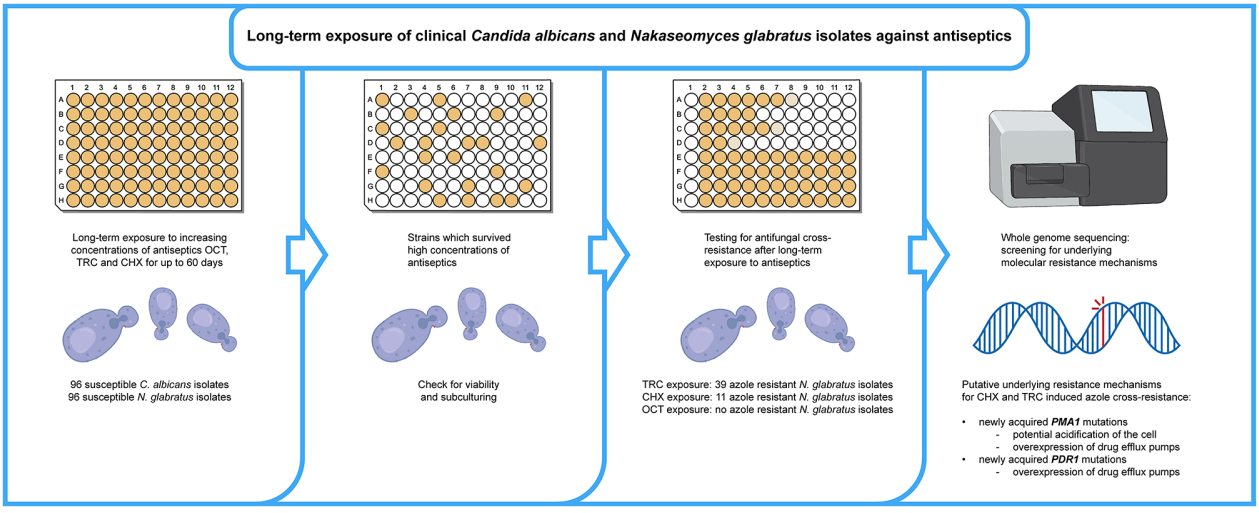
Conclusions Chlorhexidine as well as triclosan, but not octenidine, were able to introduce selective pressure promoting resistance to azole antifungals. Although we assessed this phenomenon only in vitro, these findings warrant critical monitoring in clinical settings.

†Kathrin Spettel and Dominik Bumberger have contributed equally

*Correspondence:
Birgit Willinger
birgit.willinger@meduniwien.ac.at

Full list of author information is available at the end of the article

Graphical Abstract



Keywords *Nakaseomyces*, *Candida*, Cross-resistance, Antiseptics, Chlorhexidine, Triclosan, Octenidine

Background

The impact of fungal infections has been underestimated for a long time, particularly in a growing population of immunocompromised or elderly patients who are susceptible to such opportunistic infections [1]. Indeed, invasive candidiasis accounts for one of the most frequent hospital-acquired fungal infections worldwide [2]. Infections caused by yeasts such as *Candida* spp. can range from rather harmless superficial infections on the skin to severe clinical manifestations, as well as to a life-threatening invasive disease in immunocompromised individuals with a case fatality rate exceeding even 70% in these highly vulnerable patients [3]. To date, there are only four main categories of antifungals available in clinical practice (azoles, echinocandins, polyenes and pyrimidine analogues). This itself already represents a certain limitation of therapeutic options. However, acquired resistance to these antifungals has been reported in different *Candida* species within the last few years [4–6], which emphasizes the importance for antifungal susceptibility testing as well as understanding the underlying molecular resistance mechanisms. In this context, the World Health Organization (WHO) recently published the first-ever "Fungal Priority Pathogens List" due to the worldwide occurrence of increased acquired resistance to antifungal drugs and other critical factors favoring fungal infections, such as climate change, the COVID-19 pandemic and an increasing number of immunocompromised patients [7]. This global effort highlights the emergence of antifungal resistance to become a serious public health threat similar to bacteria highly resistant to antimicrobials. In that regard, indications for the use of antiseptics and disinfectants to prevent nosocomial infections are undisputed, especially in controlling the spread of multidrug resistant organisms in healthcare institutions [8]. Thus, topically applied antiseptics may represent a useful tool to reduce the transmission not only of emerging multidrug resistant bacteria but also of fungi due to their broad spectrum of antimicrobial efficacy. Within the clinical setting, three antiseptics hold particular importance: chlorhexidine (CHX), triclosan (TRC) and octenidine (OCT). The primary mechanism of action for CHX is based on intracellular leakage and increased permeability of the microbial cell membrane subsequently leading to membrane disruption [9]. In contrast, TRC inhibits the fatty acid synthesis, more precisely inhibiting the enoyl-acyl carrier protein reductase FabIp [10]. OCT exerts a rapid and unspecific killing mechanism based on purely physical interactions, targeting critical and evolutionary highly conserved parts of bacterial membranes [11–13]. This effect has been described not only in bacteria but also in *Candida albicans*, specifically regarding its impact on membrane permeability in yeast [14].

However, the so far unique position of antiseptics causing no antimicrobial resistance due to their rather unspecific mode of action, has been challenged recently. Indeed, the meanwhile described manifestations of bacterial resistance to CHX, including even cross-resistance to so called antibiotics of the last resort such as colistin [15, 16], raises serious concerns among healthcare professionals about its use for preventive measures in infection control. Cross-resistance refers to a phenomenon that describes resistance development induced by a given selective pressure to several substances sharing a similar chemical structure, a similar mechanism of action or the

same target [17]. This may potentially result in pathogens being resistant to agents, which they have never encountered before thus posing additional burden for medical health care facilities, especially when last resort agents are affected as previously mentioned. A worst-case scenario, not only in hospital hygiene but also in human medicine in general, is the development of cross-resistance between antiseptic agents on the one hand, which are an essential cornerstone for infection prevention – and antibiotics or antifungals on the other hand, which are indispensable for therapeutic purposes.

The impact of selective pressure exerted by antiseptics, especially the widely used antiseptics CHX and TRC, has been the subject of increased investigation in recent times [15, 18–25]. Several genes were found to be associated with reduced susceptibility to antiseptics or even with (cross-)resistance to antibiotics. In particular, the action of efflux pumps seems to be one of the major mechanisms responsible for resistance to antibiotics in Gram-negative bacteria [15, 26, 27]. There are considerably fewer studies that have also suggested the potential for selective pressure triggered by OCT [28, 29]. However, the observed tolerated concentrations of that antiseptic agent in the investigated bacteria have been far below those applied in clinical settings. Furthermore, Vejzovic et al. demonstrated that different materials and methods used for MIC determination can result in misinterpretations regarding reduced bacterial susceptibility towards OCT [13].

To our knowledge, it has not yet been investigated whether the long-term exposure to CHX, TRC and OCT may induce antiseptic resistance in *C. albicans* or *Nakaseomyces glabratus* (formerly *Candida glabrata*) or if such a selective pressure even coincidently also promotes cross-resistance to antifungals. Thus, the present study analyses the in vitro activity of different antiseptics against susceptible clinical isolates of *C. albicans* and *N. glabratus* as well as the occurrence of resistance to these molecules. Furthermore, we aimed to determine whether long-term antiseptic exposure potentially promotes selective pressure followed by the development of cross-resistance to antifungals.

Materials and methods

Sampling and susceptibility testing of antifungals and antiseptics

Each of 36 *C. albicans* and *N. glabratus* isolates susceptible to commonly used antifungals were obtained from the culture collection stored at the Medical University of Vienna, Department of Laboratory Medicine, Division of Clinical Microbiology, Austria. These selected *C. albicans* and *N. glabratus* isolates were originally recovered from different patients' body sites (blood culture, drainage fluid, swabs (mouth, vaginal, wound), central venous catheter, bronchoalveolar lavage, feces) and were characterized previously for their susceptibility profile. In addition, 60 replicates of the reference strains *C. albicans* SC5314 and *N. glabratus* CBS138 were used as susceptible wild-type references among clinical isolates, respectively. Antifungal susceptibility testing was conducted for all strains, including the control strains *C. parapsilosis* ATCC 22,019 and *C. krusei* ATCC 6258, using the broth microdilution method as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) E.DEF 7.3.2 [30]. Since no guideline for antiseptic susceptibility testing is available to date, the minimum inhibitory concentrations (MICs) of tested antiseptics were determined based on the same EUCAST guideline using broth microdilution for standardization purposes, as previously described [31, 32]. In short, the antiseptic and antifungal serial stock solutions were diluted 1:100 with double strength RPMI 1640 and 100 µL were dispensed into microwell plates, with each row containing one of eight substances (either antifungals or antiseptics) in increasing concentrations. Next, the target inoculum was adjusted to a McFarland standard of 0.5, equating to an approximate cell density of $1–5 \times 10^6$ CFU/mL. This suspension was then thoroughly mixed and diluted 1:10 with distilled water. To verify the cell count, the inoculum of each batch was plated. Subsequently, 100 µL of the fungal suspension was dispensed into each well of the prepared microwell plate, achieving a final cell concentration of $0.5–2.5 \times 10^5$ CFU/mL in 200 µL. The inoculated microwell plates were incubated at 37 °C for 22 to 26 h. After incubation, the plates were evaluated visually and via photometric analysis at the absorbance at 530 nm to obtain the MIC values of the antifungals and antiseptics.

MICs were determined for the antifungals; anidulafungin (0.008-16 mg/L), micafungin (0.008-16 mg/L), caspofungin (0.008-16 mg/L), fluconazole (0.125-256 mg/L), posaconazole (0.016-32 mg/L), voriconazole (0.008-16 mg/L) itraconazole (0.008-16 mg/L), isavuconazole (0.016-32 mg/L) amphotericin B (0.032-16 mg/L), as well as for the antiseptics OCT (0.016-32 mg/L), CHX (0.25–512 mg/L) and TRC (0.016-32 mg/L), where the MIC values in brackets display the MIC concentration ranges which have been used for the antifungal and antiseptic susceptibility testing. Susceptibility testing was performed before and after the long-term exposure to antifungals and antiseptics of *C. albicans* and *N. glabratus*.

High throughput in vitro resistance development approach for long-term exposure to antiseptics

A new high-throughput resistance development model has been established, utilizing a 96-well plate format, to expose various isolates of *C. albicans* and *N. glabratus* to progressively increasing concentrations of antiseptics (CHX, OCT and TRC) and antifungals (micafungin, anidulafungin and voriconazole) over a period of up to 60

days. The incorporation of these antifungal agents as controls was essential to confirm the efficacy of our novel in vitro approach in inducing resistance. This method allows to investigate all *C. albicans* and *N. glabratus* of interest in a microwell plate in parallel using 2% RPMI medium (Sigma-Aldrich, Burlington, MA, USA). Microwell plates with each 60 replicates of the control strain CBS138 and SC5314 as well as each of 36 clinical *C. albicans* and *N. glabratus* isolates were used for the in vitro resistance development model. Initially these isolates were inoculated in Sabouraud broth medium with 2% dextrose (Oxoid Ltd., Basingstoke, UK) in a deep well plate under aerobic conditions at 37 °C for 24 h until growth of all fungal cultures was obtained. Subsequently, 100 µl of fresh overnight culture of all isolates was transferred into the first passage containing a single fixed concentration of a given antiseptic or antifungal in double strength RPMI 1640. This starting concentration for our novel resistance development model was set one dilution below the previously determined MIC of the different antimicrobials. After five days of incubation under aerobic conditions at 37 °C, all strains were transferred into the next passage with double the concentration of the given antifungals or antiseptics. These steps were repeated to slowly increase the concentration and selective pressure of a given antimicrobial over time. At each passage, we tested eight wells from each plate for possible contamination, plating an aliquot onto Columbia agar plates using a multichannel pipette. Table 1 depicts an overview of used antiseptic concentration ranges for the in vitro long-term exposure.

Selection of isolates with newly acquired resistance

After in vitro exposure for a duration of up to 60 days, isolates from the final passage with the highest concentration showing visible growth were plated on Sabouraud Gentamicin Chloramphenicol 2 agar (bioMérieux SA, Marcy l'Etoile, France). Subsequently, the MICs of all viable strains were re-evaluated. After comparing MIC values before and after long-term exposure, a subsample of 12 *N. glabratus* isolates with newly acquired resistance against antifungals according to EUCAST breakpoints were selected for further investigation with WGS. The MIC values of the antiseptics were classified as resistant if they exhibited an increase of ≥ 8-fold (equivalent to three dilution steps) compared to the MIC of the original unexposed isolate. This criteria for resistance classification in antiseptics was adapted according to criteria suggested by Kampf [33].

Table 1 Concentration ranges of antiseptics for in vitro long-term exposure of *C. albicans* and *N. glabratus*

Antimicrobial substance	Drug class	Concentration range (mg/L)
chlorhexidine (CHX)	antiseptic	0.032–64
octenidine (OCT)	antiseptic	0.004–8
triclosan (TRC)	antiseptic	0.016–32

Whole genome sequencing (WGS)

Fungal DNA extraction was performed according to a solution-based protocol with implemented bead beating steps [34]. The total amount of extracted DNA was subsequently measured with a Qubit dsDNA High Sensitivity Assay system (Life Technologies, Carlsbad, CA, USA) and its purity was confirmed with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) by determining the ratios of A_{260}/A_{280} and A_{260}/A_{230}. In this study, WGS was carried out to screen for putative underlying molecular resistance mechanisms of isolates showing newly acquired resistance. DNA sequencing libraries were prepared from extracted DNA according to the Illumina DNA Prep protocol. DNA was then denatured according to the protocol and diluted to a final loading concentration of 8 pM combined with a 5% PhiX spike-in (PhiX Control v3, Illumina) for sequencing on a v3-flowcell 2 × 300 bp on an Illumina MiSeq system (Illumina, San Diego, CA, USA).

Bioinformatic analysis

The quality of the WGS run was evaluated with FastQC 0.11.4 [35]. Low-quality bases were subsequently trimmed using a QC threshold of 20, and all reads under a minimum length of 90 bp were removed using the Trimmomatic 0.35 software [36]. Bowtie2 2.2.7 [37] was used to assemble and align the paired-end reads to the genomic sequences of *Candida* reference strains CBS138 and SC5314 obtained from www.candidagenome.org (accessed on 04.05.2022) [38]. The detection of variants was carried out with SAMtools 0.1.19 and VarScan 2.3.9 [39, 40]. Subsequently, SnpEff 4.270 was used to annotate variants and predict their effects on amino acid sequence [41]. By matching the variants of the original susceptible isolates with the exposed resistant isolates the newly acquired mutations were identified.

Gene expression analysis of drug efflux pump Cdr1p

For gene expression analysis, selected azole resistant isolates were resuspended in 11 mL of Sabouraud broth to reach an optical density of 0.1 at 600 nm (OD600). The cultures were then incubated at 37 °C for five hours with continuous inversion. After the incubation OD600 was measured with expected values of 0.6-1, indicating exponential growth phase, where the gene expression levels are the highest. RNA was extracted post-incubation using the hot phenol acid method. Fungal cells were first resuspended in TES (Tris-EDTA- 0.5% SDS) buffer, followed by the addition of acidic phenol (pH 5) and incubation at 65 °C for 30 min. The aqueous phase was then transferred to a new tube for a second phenol extraction

phase, followed by the addition of chloroform to remove proteins and phenol residues. RNA was precipitated using ammonium acetate (10 M) and absolute ethanol, followed by washing the extracted RNA twice in 70% ethanol and eluting it in Tris buffer.

To avoid inaccuracies in RT-qPCR results, the extracted RNA's quality and quantity were assessed. This involved measuring RNA purity using a Nanodrop 2000c (Thermo Scientific – Waltham, Massachusetts) spectrophotometer, followed by an evaluation of the ratios of A_{260}/A_{280} and A_{260}/A_{230} to determine the presence of proteins and contaminants. The RNA and DNA concentrations were measured using the Qubit RNA HS Assay Kit and Qubit dsDNA HS Assay Kit (Thermo Scientific – Waltham, Massachusetts), distinguishing between RNA and DNA through fluorescence detection. RNA integrity was confirmed via gel electrophoresis using a 1.6% agarose gel with 1x TAE buffer, for evaluation of the intensity of 18 S rRNA and 28 S rRNA bands. The RNA integrity was further verified using the Qubit RNA IQ Assay Kit (Thermo Scientific – Waltham, Massachusetts).

Quantification of transcripts of the multidrug-efflux pump gene *Cdr1* was performed using quantitative Reverse Transcription Real-Time PCR (RT-qPCR), employing the Luna® Universal Probe One-Step RT-qPCR Kit w/o ROX (New England Biolabs - Ipswich, Massachusetts). Separate assays were conducted with two biological duplicates and in technical triplicates. The primers and probes for the target genes were based on Sanguinetti et al. (2005) [42] with some modifications, with probes marked with FAM – TAMRA for *CDR1* and HEX – BHQ1 for the reference gene *UBC13*. The RT-qPCR was performed using the ABI QuantStudio 5 (Applied Biosystems – Waltham, Massachusetts) with a specific cycling program. The qPCR was standardized using 5 ng/µL of extracted RNA to obtain comparable Ct (Cycle Threshold) values. For quantification of drug efflux pumps the mean of the triplet Ct values was calculated for accuracy, followed by computing the difference between the Ct value of the target and reference genes (ΔCt). The study validated the use of *UBC13*, *URA3* and *RDN5.8* as reference genes (data not shown), recognizing *UBC13* as the most stable option, thereby ensuring accuracy and reliability in the experimental results. The values were compared with the corresponding WT strain to obtain ΔΔCt values. Relative Quantification (RQ) was then calculated to determine the fold-change in target gene expression compared to the corresponding reference strain.

Results

MIC values of chlorhexidine, octenidine and triclosan

The initially determined MIC_{50} and MIC_{90} values for CHX, OCT and TRC before in vitro long-term exposure of the wild-type clinical *C. albicans* and *N. glabratus* as well as of the control strains are summarized in Table 2. With an MIC_{50} and MIC_{90} of 0.5 mg/L, OCT showed the lowest value among all tested antiseptics. For CHX, the MIC_{50} was 8 mg/L, and therefore 16-fold higher compared to OCT, whereas the MIC_{90} was further increased to 16 mg/L in *C. albicans*. MIC_{50} and MIC_{90} for TRC were similar to CHX. The MIC distribution for all three investigated antiseptic substances is depicted in Fig. 1 as violine plots.

Selection of resistant isolates due to in vitro long-term exposure to antiseptics

In our high-throughput in vitro resistance development model, using antifungals as a positive control, we were able to generate resistant mutants of *C. albicans* and *N. glabratus* to echinocandins and azoles after extended exposure, thereby validating our approach of in vitro resistance development (data not shown). After extended exposure to antiseptics, *C. albicans* and *N. glabratus* did not show substantially elevated MIC values against all three tested antiseptics, defined as an 8-fold increase (equivalent to three dilution steps higher), compared to the original wild-type isolate. Therefore, resistance to antiseptics did not occur in our in vitro model. Furthermore, long-term exposure to OCT, CHX or TRC did not induce antiseptic cross-resistance. Despite long-term exposure to OCT, all *C. albicans* and *N. glabratus* isolates also maintained their susceptibility to all tested antifungals (anidulafungin, micafungin, caspofungin, voriconazole, posaconazole, fluconazole, itraconazole, isavuconazole, amphotericin B).

However, after long-term exposure to TRC, 39 out of 96 *N. glabratus* isolates (40.6%) developed resistance to the tested azoles: fluconazole, posaconazole, voriconazole, itraconazole, and isavuconazole. Similarly, 11 out of 96 *N. glabratus* isolates (11.4%) exhibited azole resistance following prolonged exposure to CHX.

Table 2 MIC_{50} (interquartile range, IQR) and MIC_{90} (mg/L) before resistance development for tested *C. albicans* and *N. glabratus* isolates. MIC_{50} and MIC_{90} are the concentrations at which 50% and 90% of the population were inhibited respectively

	OCT		CHX		TRC	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
C. albicans	0.5 (0.25–0.5)	0.5	8 (4–8)	16	8 (8–16)	16
N. glabratus	0.5 (0.5–0.5)	0.5	8 (8–8)	8	8 (8–8)	16

OCT: octenidine; CHX: chlorhexidine; TRC: triclosan

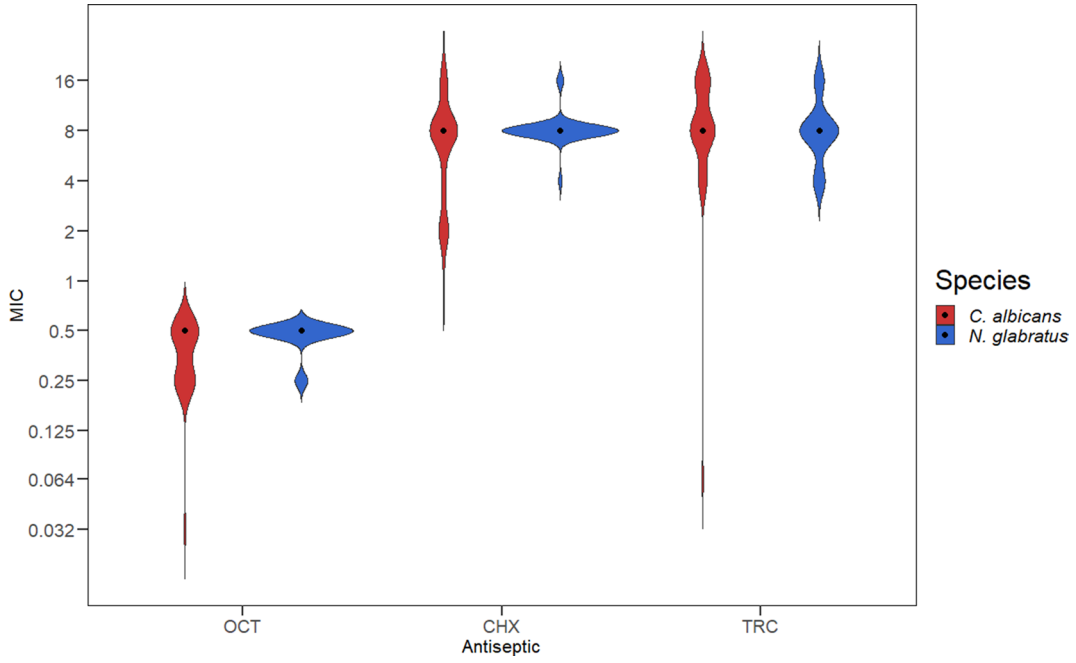


Fig. 1 Violin plots depicting the MIC (mg/L) distribution and MIC_{50} for antiseptics in *C. albicans* and *N. glabratus*. OCT: octenidine; CHX: chlorhexidine; TRC: triclosan. The median (MIC_{50}) is indicated in the violin plot with a dot

These resistant isolates displayed 4 to 512-fold increased MICs against azoles compared to the initially susceptible wild-type isolates and showed resistance by using EUCAST clinical breakpoints (Table 3). Noteworthy, these isolates remained cross-resistant to azole antifungals when removing the selective pressure induced by CHX or TRC even after multiple passages of subculturing on Sabouraud dextrose agar.

To search for the underlying resistance mechanism for newly acquired azole resistance, whole genome sequencing (WGS) was performed on a subset of six TRC and six CHX exposed strains that developed azole resistance. In comparison to the non-exposed wild-type isolates, seven isolates acquired six novel mutations in the *PMA1* gene, associated with maintaining the cell's pH optimum, membrane potential and stress response [43–45]. These mutations resulted in single amino acid substitutions at positions T215I, L347S, A395V (2x), D407G, V594F, and A812V. Additionally, five azole resistant isolates harboured six potentially causal gain of function mutations in the transcription factor *PDR1* (D261Y, C469R, L936S, G943A, D1082G, G1088E), which controls the gene expression of drug efflux pumps Cdr1p, Cdr2p and Snq2p [42, 46, 47]. To evaluate the effects of different mutations in *PDR1* and to ascertain the presence of overexpression, we performed gene expression analysis of the drug efflux pumps Cdr1p. The results demonstrated an overexpression of *CDR1* in all *PDR1* mutants with a median overexpression of 21-fold [9.6–50]. Interestingly, all *PMA1* mutations, except for the two isolates harboring the A395V mutation (Table 3), also demonstrate a high overexpression of the Cdr1p drug efflux pump with a median overexpression of 33.3-fold [0.6–66], with levels notably surpassing those found in the corresponding wild-type isolates. In contrast to *N. glabratus*, none of the 96 *C. albicans* isolate became azole-resistant after exposure to the antiseptics TRC and CHX.

Discussion

Antiseptics and disinfectants are key elements in infection prevention strategies within health-care facilities to increase patient safety in order to prevent the transmission of pathogens and especially multidrug resistant organisms [8]. In our previous research, it was observed that OCT effectively eradicated multidrug resistant isolates of *C. albicans* and *N. glabratus* in vitro, underscoring its high efficacy against clinically relevant fungi [48]. However, reduced antimicrobial susceptibility following exposure to antiseptics as well as the development of antiseptic resistance and cross-resistance to other antimicrobials – including even last-resort antibiotics - has been recently described in Gram-positive as well as Gram-negative bacteria [49, 50]. This underscores the necessity of implementing an antiseptic stewardship program like antibiotic stewardship in clinical settings. Given that these substances appear to induce selective pressure resulting in resistance to antimicrobials, it is crucial to define the appropriate use of antiseptics or disinfectants and to perform monitoring for resistance development.

Table 3 Characteristics of selected *N. glabratus* isolates after in vitro long-term exposure to chlorhexidine (CHX) and triclosan (TRC) including molecular resistance mechanism as determined by whole genome sequencing and changes in MIC values (mg/L) for FLC (fluconazole), PSZ (posaconazole), VCZ (voriconazole), ITR (itraconazole), ISA (isavuconazole), CHX (chlorhexidine), TRC (triclosan) and OCT (octenidine). Overexpression of *CDR1* is given as fold increase compared to the corresponding wild-type isolate. The corresponding wild-type (WT) isolates and the initial MIC values are highlighted in bold

ID	Exposed to	FLC	PSZ	VCZ	ITR	ISA	CHX	TRC	OCT	Resistance genes	Acquired resistance mutation	Putative resistance mechanism	Overexpression of *CDR1*
45 H-WT		**4**	**1**	**0.125**	**1**	**≤0.016**	**4**	**16**	**0.5**				
45 H-D6	**TRC**	256	> 32	4	> 16	4	4	16	0.5	*PDR1*	D261Y	drug efflux pumps	50.23
55 H-WT		**4**	**0.5**	**0.125**	**0.125**	**≤0.016**	**4**	**4**	**0.5**				
55 H-F1	**CHX**	> 256	4	1	2	1	16	16	1	*PMA1*	V594F	drug efflux pumps/ intracellular acidification	65.92
76 H-WT		**4**	**0.5**	**0.25**	**0.5**	**≤0.016**	**8**	**8**	**0.25**				
76 H-F4	**CHX**	64	> 32	> 16	2	0.5	8	16	0.5	*PMA1/ATP1*	L347S/D332G	drug efflux pumps/ intracellular acidification	33.32
105 H-WT		**4**	**0.5**	**0.25**	**0.5**	**≤0.016**	**4**	**16**	**0.5**				
105 H-E5	**CHX**	256	16	8	> 16	8	16	32	0.5	*PMA1*	T215I	drug efflux pumps/ intracellular acidification	38.96
1098 H-WT		**2**	**0.5**	**0.5**	**0.125**	**≤0.016**	**4**	**8**	**0.5**				
1098 H-D7	**CHX**	64	2	2	2	2	8	8	0.25	*PMA1*	A812V	drug efflux pumps/ intracellular acidification	45.74
CBS138-WT		**4**	**0.25**	**0.064**	**0.25**	**0.064**	**4**	**4**	**0.5**				
CBS138-A5	**CHX**	> 256	> 32	8	> 16	4	2	4	0.5	*PMA1*	A395V	intracellular acidification	0.6
CBS138-E12	**CHX**	64	2	0.5	1	0.5	4	4	0.5	*PMA1*	D407G	intracellular acidification	2.32
CBS138-A7	**TRC**	256	16	8	> 16	4	8	32	0.5	*PDR1*	G1088E	drug efflux pumps	23.54
CBS138-B4	**TRC**	256	4	8	> 16	4	8	16	0.5	*PDR1*	G943A	drug efflux pumps	20.18
CBS138-C7	**TRC**	128	4	4	4	4	4	16	0.5	*PDR1*	L936S	drug efflux pumps	20.81
CBS138-E5	**TRC**	128	4	4	2	4	4	16	0.5	*PDR1*	C469R	drug efflux pumps	9.57
CBS138-G10	**TRC**	128	4	2	4	2	4	16	0.5	*PMA1*	A395V	intracellular acidification	2.03

In the present work we determined susceptibility profiles for the antiseptic agents CHX, OCT and TRC in clinical isolates of *C. albicans* and *N. glabratus*, as a first step. Regarding antimicrobial effect, OCT was the most potent agent showing low MIC_{50} and MIC_{90} values of 0.5 mg/L, without species-specific differences for *C. albicans* or *N. glabratus,* indicating a broad-spectrum of antiseptic activity. This is in concordance with other observations on various *Candida* spp. where similar results for OCT have been observed regarding antiseptic efficacy [48, 51–53]. Of note, the MIC_{50} of OCT is 16-fold lower compared to that of CHX and TRC, respectively, which coincides with data presented by Koburger et al. [51]. Also Morrissey et al. proposed an epidemiological cut-off value representing the upper end of the wild-type MIC distribution of 16 mg/L for both TRC and CHX as well as an MIC_{50} value for CHX of 8 mg/L for 200 tested *C. albicans* isolates [54], which is in concordance with our results. For CHX we determined an MIC_{50} of 8 mg/L for *C. albicans* and *N. glabratus*.

In a second step, we explored if selective pressure induced by antiseptics might influence the antifungal susceptibility profiles in various *C. albicans* and *N. glabratus* isolates. By using an antifungal positive control (micafungin, anidulafungin, voriconazole), we were able to induce already described resistance mutations in long-term echinocandin- and azole-exposed isolates validating our resistance development model [55]. Thus, the herein used in vitro experimental setup is suitable to prove the effect of a given selective pressure in *C. albicans* and *N. glabratus*. Noteworthy, no relevant MIC changes have been observed for any of the three tested antiseptic agents. In comparison to antifungals, it was not possible to induce antiseptic resistance (MIC increase to antiseptics by ≥ 8-fold or equivalent to three dilution steps) in any of the tested *Candida* or *Nakaseomyces* isolates after exposure to OCT, CHX or TRC.

In our setup, prolonged exposure to CHX or TRC induced selective pressure favoring cross-resistance to azole antifungals in *N. glabratus*. In contrast, OCT did not demonstrate this effect towards any tested antifungal agents. Recent observations of various Gram-negative bacteria, showed that selective pressure led to the emergence of mutants with reduced susceptibility to CHX and the last-resort antibiotic colistin, which was not observed for OCT [16]. However, to the best of our knowledge the appearance of cross-resistance to azoles induced by selective pressure of antiseptics has not been determined in yeast so far.

In detail, 50 *N. glabratus* isolates have become cross-resistant to all tested azoles (fluconazole, posaconazole, voriconazole, itraconazole and isavuconazole) displaying elevated MICs after long-term exposure to TRC or CHX, respectively. To investigate potential underlying molecular resistance mechanisms, whole genome sequencing (WGS) was conducted on six randomly selected isolates exposed to TRC and six exposed to CHX, all of which had developed azole resistance. The analysis of the sequences before and after exposure revealed that six of these 12 isolates had potential gain-of-function mutations in the transcription factor *PDR1*, with mutations identified at positions D261Y, C469R, L936S, G943A, D1082G, and G1088E. *PDR1* is a key regulator of gene expression for drug efflux pumps Cdr1/2p and Snq2p and is widely recognized for its role in azole resistance, which is often a consequence of the overexpression of these drug efflux pump. Two of the mentioned mutations are located close to the inhibitory domain whereas the other four were identified in the putative activation domain of the transcription factor *PDR1* [56]. We further investigated the effects of various *PDR1* mutations by conducting gene expression analysis of the Cdr1p drug efflux pumps, which revealed a substantial overexpression of *CDR1* across all *PDR1* mutants (a median 21-fold overexpression). Consequently, the novel mutations identified in *PDR1* are likely to constitute the fundamental putative mechanism of resistance, characterized predominantly by the enhanced expression of drug efflux pumps. Moreover, it is crucial to emphasize that this mechanism of resistance has already been observed and well described in clinical *N. glabratus* isolates, underscoring its significance in clinical setting [46, 57].

The other six sequenced azole resistant *N. glabratus* isolates without *PDR1* mutations showed mutations in *PMA1. PMA1* mutants were already described in 1989 by Perlin et al. in *S. cerevisiae* [58]. It has been shown that observed missense mutations at position S368F and P640L can confer resistance to hygromycin B in *S. cerevisiae*, an antimicrobial agent used as a screening tool for plasma membrane ATPase mutants. Under normal conditions, the plasma membrane H+-ATPase Pma1p acts as a major regulator of intracellular pH in fungi and plants [43]. We identified Pma1p amino acid substitutions in azole cross-resistant *N. glabratus* isolates, located at the positions T215I, L347S, A395V (2x), D407G V594F and A812V. Thus, they are in the vicinity of already described ones which conferred resistance in *S. cerevisiae* against hygromycin B. Furthermore, it has been described in more detail that alterations of *PMA1* in *C. albicans* can decrease intracellular cytosolic pH [59]. Thus, potential loss of function mutations in *PMA1* may lead to intracellular acidification. Previous research has indicated that reduced pH levels can compromise the effectiveness of azoles as the MIC increases drastically for azoles in *N. glabratus* under conditions of reduced pH in vitro [60]. Therefore, the subsequent potential dysfunction of proton pumps due to mutations and resulting intracellular

acidification could serve as a potential cause for azole resistance.

Interestingly, isolates harboring *PMA1* mutations also exhibited a marked overexpression of *CDR1* similar to the *PDR1* mutants, indicating that the primary mechanism might also involve an increased activity of drug efflux pumps. All *PMA1* mutants, barring those with the mutation A395V, exhibited a notable increase in *CDR1* expression, with a median overexpression of 33-fold, substantially higher than their wild-type counterparts. The *PMA1* and *PDR1* genes reside on chromosome A, positioned approximately 5 kbp apart. This proximity, coupled with first insights from a limited number of studies, suggests a linkage between *PMA1* and *PDR1* as well as its association in the regulation of the drug efflux pumps [61–63]. It is plausible that a regulatory response could be influencing gene expression, thereby modifying the dynamics of gene interaction and expression of drug efflux pumps. However, the underlying molecular mechanism of why mutations in *PMA1* contribute to the upregulation of these drug efflux pumps, remains unclear.

Further studies investigating especially the detailed *PMA1* function in *N. glabratus* isolates are needed to confirm the proposed mechanism of action for azole cross-resistance. Knockout experiments to generate fungal *PMA1* mutants may be a possible tool to investigate individual gene function in the context of this acquired azole resistance. Furthermore, it must be clarified whether these isolates harboring *PMA1* mutations exhibit the same in vivo pathogenicity as the wild-type isolates.

Our study provides initial insights into the susceptibility profiles of *Candida* and *Nakaseomyces* towards antiseptics and investigates whether exposure to antiseptics leads to selective pressure that promotes the development of antifungal (cross-)resistance. Our findings indicate that clinical isolates of *C. albicans* and *N. glabratus* may not develop resistance to antiseptics over time in vitro. However, we have successfully described, for the first time, a development of acquired cross-resistance to azole antifungals resulting from selective pressure caused by prolonged exposure to the antiseptics CHX or TRC. In contrast long term exposure to OCT did not result in the development of cross-resistance to any of the investigated antifungals. Through WGS, we identified newly acquired *PDR1* and *PMA1* mutations in *N. glabratus* isolates, which are linked to increased expression of drug efflux pumps and potentially reduced drug efficacy due to intracellular acidification. Hence, this study is the first to report that low-level exposure to CHX and TRC has the potential to induce selective pressure promoting antifungal resistance. Although, the observation of this phenomenon was observed in vitro, its potential clinical implications necessitate stringent surveillance within clinical environments, underscoring its importance of patient safety.

Acknowledgements
The authors express gratitude to Michael Reisinger and Maximilian Reutterer for their valuable discussions, support, and advice.

Author contributions
Conceptualization: K.S. and B.W.; methodology: K.S., D.B., S.F. and M.L.; software: R.K.; validation: K.S., D.B. and B.W.; formal analysis: K.S., D.B. and R.K.; investigation: K.S., D.B., S.F. and M.L.; resources: B.W.; data curation: K.S., D.B. and R.K.; writing—original draft preparation: K.S. and D.B.; writing—review and editing: B.W., R.K., S.F., M.L., S.G., M.S., H.L. and A.M.; visualization: K.S., D.B., R.K. and S.G.; supervision: B.W.; project administration: K.S.; funding acquisition: B.W.

Funding
This work was partially supported by Schülke & Mayr GmbH, Vienna, Austria. The sponsor had no role in the design of the study or the collection, analysis and interpretation of data.

Data availability
The sequencing data are available at https://www.ncbi.nlm.nih.gov/ under accession number PRJNA1186917.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Division of Clinical Microbiology, Department of Laboratory Medicine, Medical University of Vienna, Vienna 1090, Austria
[2]Section Biomedical Science, Health Sciences, FH Campus Wien University of Applied Sciences, Vienna 1100, Austria
[3]Division of Infectious Diseases and Tropical Medicine, Department of Medicine I, Medical University of Vienna, Vienna 1090, Austria
[4]Division of Data, Statistics and Risk Assessment, Austrian Agency for Health and Food Safety AGES, Vienna 1220, Austria
[5]Institute for Hygiene and Applied Immunology, Medical University of Vienna, Vienna 1090, Austria

Received: 12 March 2024 / Accepted: 16 December 2024
Published online: 23 January 2025

References

1. Garvey M, Rowan NJ. Pathogenic drug resistant Fungi: a review of Mitigation Strategies. Int J Mol Sci. 2023;24:1584. https://doi.org/10.3390/ijms24021584.
2. Lamoth F, Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother. 2018;73:i4–13. https://doi.org/10.1093/jac/dkx444.
3. Bassetti M, Righi E, Montravers P, Cornely OA. What has changed in the treatment of invasive candidiasis? A look at the past 10 years and ahead. J Antimicrob Chemother. 2018;73:i14–25. https://doi.org/10.1093/jac/dkx445.
4. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole Antifungal Resistance in Candida albicans and Emerging non-albicans Candida Species. Front Microbiol 2017;7. https://doi.org/10.3389/fmicb.2016.02173
5. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, et al. Increasing Echinocandin Resistance in Candida Glabrata: clinical failure correlates with Presence of FKS mutations and elevated minimum

inhibitory concentrations. Clin Infect Dis. 2013;56:1724–32. https://doi.org/10.1093/cid/cit136.
6. Gow NAR, Johnson C, Berman J, Coste AT, Cuomo CA, Perlin DS, et al. The importance of antimicrobial resistance in medical mycology. Nat Commun. 2022;13:5352. https://doi.org/10.1038/s41467-022-32249-5.
7. World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action. Geneva: World Health Organization; 2022.
8. Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Touveneau S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Lancet. 2000;356:1307–12. https://doi.org/10.1016/S0140-6736(00)02814-2.
9. Rzycki M, Drabik D, Szostak-Paluch K, Hanus-Lorenz B, Kraszewski S. Unraveling the mechanism of octenidine and chlorhexidine on membranes: does electrostatics matter? Biophys J. 2021;120:3392–408. https://doi.org/10.1016/j.bpj.2021.06.027.
10. Heath RJ, Rubin JR, Holland DR, Zhang E, Snow ME, Rock CO. Mechanism of Triclosan Inhibition of bacterial fatty acid synthesis *. J Biol Chem. 1999;274:11110–4. https://doi.org/10.1074/jbc.274.16.11110.
11. Malanovic N, Ön A, Pabst G, Zellner A, Lohner K, Octenidine. Novel insights into the detailed killing mechanism of Gram-negative bacteria at a cellular and molecular level. Int J Antimicrob Agents. 2020;56:106146. https://doi.org/10.1016/j.ijantimicag.2020.106146.
12. Malanovic N, Buttress JA, Vejzovic D, Ön A, Piller P, Kolb D, et al. Disruption of the cytoplasmic membrane structure and barrier function underlies the potent antiseptic activity of Octenidine in Gram-positive Bacteria. Appl Environ Microbiol. 2022;88:e00180–22. https://doi.org/10.1128/aem.00180-22.
13. Vejzovic D, Iftic A, Ön A, Semeraro EF, Malanovic N. Octenidine's efficacy: a matter of interpretation or the influence of experimental. Setups? Antibiot. 2022;11:1665. https://doi.org/10.3390/antibiotics11111665.
14. Kakar A, Sastré-Velásquez LE, Hess M, Galgóczy L, Papp C, Holzknecht J et al. The Membrane Activity of the Amphibian Temporin B Peptide Analog TB_KKG6K Sheds Light on the Mechanism That Kills Candida albicans. mSphere. 2022;7:e00290-22. https://doi.org/10.1128/msphere.00290-22
15. Wand ME, Bock LJ, Bonney LC, Sutton JM. Mechanisms of increased resistance to Chlorhexidine and Cross-resistance to Colistin following exposure of Klebsiella pneumoniae clinical isolates to Chlorhexidine. Antimicrob Agents Chemother. 2016;61. https://doi.org/10.1128/aac.01162-16.
16. Lescat M, Magnan M, Kenmoe S, Nordmann P, Poirel L. Co-lateral Effect of Octenidine, Chlorhexidine and Colistin selective pressures on four Enterobacterial species: a comparative genomic analysis. Antibiotics. 2022;11:50. https://doi.org/10.3390/antibiotics11010050.
17. Davin-Regli A, Pagès JM. Cross-resistance between biocides and antimicrobials: an emerging question. Rev Sci Tech. 2012;31:89–104. https://doi.org/10.20506/rst.31.1.2099.
18. Jin M, Liu L, Wang D, Yang D, Liu W, Yin J, et al. Chlorine disinfection promotes the exchange of antibiotic resistance genes across bacterial genera by natural transformation. ISME J. 2020;14:1847–56. https://doi.org/10.1038/s41396-020-0656-9.
19. Pidot SJ, Gao W, Buultjens AH, Monk IR, Guerillot R, Carter GP, et al. Increasing tolerance of hospital Enterococcus faecium to handwash alcohols. Sci Transl Med. 2018;10:eaar6115. https://doi.org/10.1126/scitranslmed.aar6115.
20. Tandukar M, Oh S, Tezel U, Konstantinidis KT, Pavlostathis SG. Long-term exposure to benzalkonium chloride disinfectants results in change of microbial community structure and increased antimicrobial resistance. Environ Sci Technol. 2013;47:9730–8. https://doi.org/10.1021/es401507k.
21. Li D, Zeng S, He M, Gu AZ. Water Disinfection byproducts Induce Antibiotic Resistance-Role of Environmental pollutants in Resistance Phenomena. Environ Sci Technol. 2016;50:3193–201. https://doi.org/10.1021/acs.est.5b05113.
22. Lu J, Jin M, Nguyen SH, Mao L, Li J, Coin LJM, et al. Non-antibiotic antimicrobial triclosan induces multiple antibiotic resistance through genetic mutation. Environ Int. 2018;118:257–65. https://doi.org/10.1016/j.envint.2018.06.004.
23. Hardy K, Sunnucks K, Gil H, Shabir S, Trampari E, Hawkey P, et al. Increased usage of Antiseptics is Associated with reduced susceptibility in clinical isolates of Staphylococcus aureus. mBio. 2018;9:10.1128/mbio.00894-18. https://doi.org/10.1128/mbio.00894-18.
24. Vali L, Davies SE, Lai LLG, Dave J, Amyes SGB. Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant Staphylococcus aureus isolates. J Antimicrob Chemother. 2008;61:524–32. https://doi.org/10.1093/jac/dkm520.
25. Htun HL, Hon PY, Holden MTG, Ang B, Chow A. Chlorhexidine and octenidine use, carriage of qac genes, and reduced antiseptic susceptibility in methicillin-resistant Staphylococcus aureus isolates from a healthcare network. Clin Microbiol Infect. 2019;25. https://doi.org/10.1016/j.cmi.2018.12.036 :1154.e1-1154.e7.
26. Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. J Appl Microbiol. 2002;92. https://doi.org/10.1046/j.1365-2672.92.5s1.4.x. Suppl:65S-71S.
27. Chuanchuen R, Karkhoff-Schweizer RR, Schweizer HP. High-level triclosan resistance in Pseudomonas aeruginosa is solely a result of efflux. Am J Infect Control. 2003;31:124–7. https://doi.org/10.1067/mic.2003.11.
28. Garratt I, Aranega-Bou P, Sutton JM, Moore G, Wand ME. Long-term exposure to Octenidine in a simulated Sink Trap Environment results in selection of Pseudomonas aeruginosa, Citrobacter, and Enterobacter isolates with mutations in Efflux Pump regulators. Appl Environ Microbiol. 2021;87:e00210–21. https://doi.org/10.1128/AEM.00210-21.
29. Bock LJ, Ferguson PM, Clarke M, Pumpitakkul V, Wand ME, Fady P-E, et al. Pseudomonas aeruginosa adapts to octenidine via a combination of efflux and membrane remodelling. Commun Biol. 2021;4:1–14. https://doi.org/10.1038/s42003-021-02566-4.
30. Arendrup MC, Meletiadis J, Mouton JW, Lagrou K, Hamal P, Guinea J et al. EUCAST DEFINITIVE DOCUMENT E.DEF 7.3: Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts. Eucast 2020;1–21.
31. Oliveira Dos Santos C, Kolwijck E, van der Lee HA, Tehupeiory-Kooreman MC, Al-Hatmi AMS, Matayan E, et al. In Vitro Activity of Chlorhexidine compared with seven Antifungal agents against 98 Fusarium isolates recovered from fungal keratitis patients. Antimicrob Agents Chemother. 2019;63:e02669–18. https://doi.org/10.1128/AAC.02669-18.
32. Hao W, Wang Y, Xi Y, Yang Z, Zhang H, Ge X. Activity of chlorhexidine acetate in combination with fluconazole against suspensions and biofilms of Candida Auris. J Infect Chemother. 2022;28:29–34. https://doi.org/10.1016/j.jiac.2021.09.018.
33. Kampf G. Adaptive bacterial response to low level chlorhexidine exposure and its implications for hand hygiene. Microb Cell. 2019;6:307–20. https://doi.org/10.15698/mic2019.07.683.
34. Spettel K, Galazka S, Kriz R, Camp I, Willinger B. Do Candida albicans isolates with Borderline Resistant Micafungin MICs Always Harbor FKS1 Hot Spot. Mutations? JoF. 2021;7:93. https://doi.org/10.3390/jof7020093.
35. Andrews S. FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc 2010.
36. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. https://doi.org/10.1093/bioinformatics/btu170.
37. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9. https://doi.org/10.1038/nmeth.1923.
38. Skrzypek MS, Binkley J, Binkley G, Miyasato SR, Simison M, Sherlock G. The Candida Genome Database (CGD): incorporation of Assembly 22, systematic identifiers and visualization of high throughput sequencing data. Nucleic Acids Res. 2017;45:D592–6. https://doi.org/10.1093/nar/gkw924.
39. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9. https://doi.org/10.1093/bioinformatics/btp352.
40. Koboldt DC, Chen K, Wylie T, Larson DE, McLellan MD, Mardis ER, et al. VarScan: variant detection in massively parallel sequencing of individual and pooled samples. Bioinformatics. 2009;25:2283–5. https://doi.org/10.1093/bioinformatics/btp373.
41. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w ^{1118}; iso-2; iso-3. Fly. 2012;6:80–92. https://doi.org/10.4161/fly.19695.
42. Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. Mechanisms of azole resistance in clinical isolates of Candida Glabrata collected during a hospital survey of antifungal resistance. Antimicrob Agents Chemother. 2005;49:668–79. https://doi.org/10.1128/AAC.49.2.668-679.2005.
43. Portillo F. Regulation of plasma membrane H+-ATPase in fungi and plants. Biochimica et Biophysica Acta (BBA) -. Reviews Biomembr. 2000;1469:31–42. https://doi.org/10.1016/S0304-4157(99)00011-8.
44. Lee Y, Nasution O, Lee YM, Kim E, Choi W, Kim W. Overexpression of PMA1 enhances tolerance to various types of stress and constitutively activates

the SAPK pathways in Saccharomyces cerevisiae. Appl Microbiol Biotechnol. 2017;101:229–39. https://doi.org/10.1007/s00253-016-7898-5.
45. Antunes M, Sá-Correia I. The role of ion homeostasis in adaptation and tolerance to acetic acid stress in yeasts. FEMS Yeast Res. 2024;24:foae016. https://doi.org/10.1093/femsyr/foae016.
46. Ferrari S, Sanguinetti M, Torelli R, Posteraro B, Sanglard D. Contribution of CgPDR1-Regulated genes in enhanced virulence of Azole-Resistant Candida Glabrata. PLoS ONE. 2011;6:e17589. https://doi.org/10.1371/journal.pone.0017589.
47. Vermitsky J-P, Earhart KD, Smith WL, Homayouni R, Edlind TD, Rogers PD. Pdr1 regulates multidrug resistance in Candida Glabrata: gene disruption and genome-wide expression studies. Mol Microbiol. 2006;61:704–22. https://doi.org/10.1111/j.1365-2958.2006.05235.x.
48. Spettel K, Bumberger D, Camp I, Kriz R, Willinger B. Efficacy of octenidine against emerging echinocandin-, azole- and multidrug-resistant Candida albicans and Candida Glabrata. J Glob Antimicrob Resist. 2022;29:23–8. https://doi.org/10.1016/j.jgar.2022.01.028.
49. Kampf G. Biocidal Agents Used for Disinfection Can Enhance Antibiotic Resistance in Gram-negative species. Antibiotics. 2018;7:110. https://doi.org/10.3390/antibiotics7040110.
50. KG. Antibiotic resistance can be enhanced in Gram-positive species by some Biocidal agents used for disinfection. Antibiotics. 2019;8:13. https://doi.org/10.3390/antibiotics8010013.
51. Koburger T, Hübner N-O, Braun M, Siebert J, Kramer A. Standardized comparison of antiseptic efficacy of triclosan, PVP–iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. J Antimicrob Chemother. 2010;65:1712–9. https://doi.org/10.1093/jac/dkq212.
52. Friese K, Neumann G, Siebert J. Topical antiseptics as an alternative in the treatment of acute vulvovaginal candidosis. Arch Gynecol Obstet. 2003;268:194–7. https://doi.org/10.1007/s00404-002-0368-6.
53. Moore G, Schelenz S, Borman AM, Johnson EM, Brown CS. Yeasticidal activity of chemical disinfectants and antiseptics against Candida Auris. J Hosp Infect. 2017;97:371–5. https://doi.org/10.1016/j.jhin.2017.08.019.
54. Morrissey I, Oggioni MR, Knight D, Curiao T, Coque T, Kalkanci A, et al. Evaluation of epidemiological cut-off values indicates that Biocide resistant subpopulations are uncommon in natural isolates of clinically-relevant Microorganisms. PLoS ONE. 2014;9:e86669. https://doi.org/10.1371/journal.pone.0086669.
55. Ksiezopolska E, Schikora-Tamarit MÀ, Beyer R, Nunez-Rodriguez JC, Schüller C, Gabaldón T. Narrow mutational signatures drive acquisition of multidrug resistance in the fungal pathogen Candida Glabrata. Curr Biol. 2021;31:5314–e532610. https://doi.org/10.1016/j.cub.2021.09.084.
56. Tsai H-F, Krol AA, Sarti KE, Bennett JE. Candida Glabrata PDR1, a transcriptional regulator of a pleiotropic drug resistance network, mediates azole resistance in clinical isolates and petite mutants. Antimicrob Agents Chemother. 2006;50:1384–92. https://doi.org/10.1128/AAC.50.4.1384-1392.2006.
57. Ferrari S, Ischer F, Calabrese D, Posteraro B, Sanguinetti M, Fadda G, et al. Gain of function mutations in CgPDR1 of Candida Glabrata not only mediate Antifungal Resistance but also enhance virulence. PLoS Pathog. 2009;5:e1000268. https://doi.org/10.1371/journal.ppat.1000268.
58. Perlin DS, Harris SL, Seto-Young D, Haber JE. Defective H+-ATPase of hygromycin B-resistant pma1 mutants from Saccharomyces cerevisiae. J Biol Chem. 1989;264:21857–64. https://doi.org/10.1016/S0021-9258(20)88263-5.
59. Rane HS, Hayek SR, Frye JE, Abeyta EL, Bernardo SM, Parra KJ, et al. Candida albicans Pma1p contributes to growth, pH Homeostasis, and Hyphal formation. Front Microbiol. 2019;10. https://doi.org/10.3389/fmicb.2019.01012.
60. Danby CS, Boikov D, Rautemaa-Richardson R, Sobel JD. Effect of pH on in Vitro susceptibility of Candida Glabrata and Candida albicans to 11 Antifungal agents and implications for Clinical Use. Antimicrob Agents Chemother. 2012;56:1403–6. https://doi.org/10.1128/aac.05025-11.
61. Ułaszewski S. Interactions between the gene products of pma1 encoding plasma membrane H(+)-ATPase, and pdr1 controlling multiple drug resistance in Saccharomyces cerevisiae. Acta Biochim Pol. 1993;40:487–96.
62. Nakamura K, Niimi M, Niimi K, Holmes AR, Yates JE, Decottignies A, et al. Functional expression of Candida albicans Drug Efflux Pump Cdr1p in a Saccharomyces cerevisiae strain deficient in membrane transporters. Antimicrob Agents Chemother. 2001;45:3366–74. https://doi.org/10.1128/aac.45.12.3366-3374.2001.
63. Liu L, Jiang T, Zhou J, Mei Y, Li J, Tan J, et al. Repurposing the FDA-approved anticancer agent ponatinib as a fluconazole potentiator by suppression of multidrug efflux and Pma1 expression in a broad spectrum of yeast species. Microb Biotechnol. 2022;15:482–98. https://doi.org/10.1111/1751-7915.13814.

Publisher's note