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Differential susceptibility of *Candida* (*Candidozyma*) *auris* clades to surface disinfectants

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SUMMARY

Background: *Candida* (*Candidozyma*) *auris* is a globally emerging multidrug-resistant fungal pathogen that causes nosocomial outbreaks in healthcare facilities.
Aim: To evaluate the susceptibility of different *C. auris* clade strains (I to V) and *C. albicans* ATCC 10231 strain to commonly used disinfecting agents and commercially available ready-to-use disinfecting wipes.
Methods: Standardized tests (EN 13624:2013 and EN 16615:2015).
Findings: Suspension tests under EN 13624:2013 guidelines revealed that the ethanol-based disinfectant at 40% concentration effectively achieved a 4 $\log_{10}$ reduction for all tested *Candida* strains. Quaternary ammonium compound (QAC)-based disinfectants, however, displayed greater inter-species and inter-clade variations. When tested under the EN 16615:2015 four-field method, the ethanol-based commercial disinfectant wipe did not meet the required criteria ($\geq$4 $\log_{10}$ reduction, $<$50 cfu transfer) for *C. albicans* and *C. auris* under dirty conditions, with the exception of the clade II strain. The QAC-based product showed uniform efficacy across all *C. auris* strains under dirty conditions, while *C. albicans* test data did not meet the EN standard requirements. Propanol/QAC-based disinfectant wipes achieved effective inactivation of *C. auris* strains under clean conditions, but not the *C. albicans* ATCC strain. Hydrogen peroxide-based wipes failed to reach the necessary reduction factor across all tested *Candida* strains under dirty conditions.

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Conclusion: These results emphasize the variability in the disinfecting efficacy of commercially available products with yeasticidal claim between *C. auris* clades and *C. albicans* in practice-like tests. Further research is needed to determine effective disinfection strategies against the various *C. auris* clades.

Introduction

In recent years, *Candida* (*Candidozyma*) *auris* has garnered special attention in the scientific and medical communities due to its emergence as a cause of nosocomial outbreaks in healthcare facilities around the world [1—5]. It has been included in the World Health Organization (WHO) list of fungal priority pathogens as a pathogen of the highest priority group and is considered an emerging public health threat by the US Centers for Disease Control and Prevention (CDC) [6,7]. The rapid spread, the potential to cause invasive nosocomial infections with high mortality rates in vulnerable populations, and its intrinsic resistance to many therapeutic and preventative measures make the management of *C. auris* a particular challenge in healthcare settings with considerable future relevance [8—12].

Different potential reservoirs, clinical or environmental, have been discussed for *C. auris*, and aquatic habitats have been postulated as possible environmental sources [13]. Interestingly, however, whole-genome sequencing studies of the geographically distributed *C. auris* isolates suggest that they arose simultaneously yet independently in different areas of the world, and clustered into distinct clades: clade I (South Asian), clade II (East Asian), clade III (South African), clade IV (South American), clade V (Iranian), and, recently, clade VI (Indomalayan) [14—16]. However, this attribution of *C. auris* clades to specific geographic regions may prove unreliable in the future, as several clades have occurred in areas other than their initial origin, suggesting their dispersal and mixing [17,18]. While isolates within these clades appear clonal, differences between clades are made up of many thousands of single nucleotide polymorphisms (SNPs) [14,19]. Clinically important differences between clades can include the severity and outcome of clinical presentation of infection, as well as susceptibility to antifungal agents and surface disinfectants [20,21].

C. auris shows strong environmental resilience and colonization potential on patient skin, implanted foreign bodies, as well as external abiotic surfaces for prolonged periods of time. This appears to be, in part, explained by the possession of *C. auris*-specific adhesion factors, the ability to form biofilms, and a strong resistance to drying [22—25]. All these factors facilitate surface colonization and may, therefore, contribute to healthcare-associated transmission events.

Currently, different inactivating agents and physical inactivation protocols for surface disinfection are available, but their efficiency against the different *C. auris* clades remains poorly understood. A growing body of recent research data suggests that there are significant differences in sensitivity to disinfectants and other substances between the various clades [26—29]. Previously, the effectiveness of disinfectants against *C. auris* strains has been compared to a widely used *C. albicans* surrogate strain using European standards (EN) protocols, deeming the surrogate suitable for determining yeasticidal activity against *C. auris* [30,31].

According to the regulations provided by the European Committee for Standardization (CEN), we performed standardized inactivation tests, including suspension tests (EN 13624:2013) and four-field tests (EN 16615:2015) [32]. The latter method mimics the real-world process of manual surface disinfection as it occurs in healthcare settings for decontamination of patient-surrounding environments and materials. Using commercially available ready-to-use wipes with commonly used active ingredients — alcohol, quaternary ammonium compounds (QAC), or hydrogen peroxide (H_2O_2) — we aimed to determine whether (a) inter-clade differences exist in the susceptibility of *C. auris* to different inactivating procedures and agents and (b) whether the *C. albicans* surrogate strain is suitable for determining yeasticidal activity against *C. auris* strains of different clades.

Methods

Test organisms

The *Candida albicans* (ATCC 10231) reference strain and the *C. auris* isolates used in this study, including one reference strain (DSM 21092), are detailed in Table I. *C. auris* isolates originated from clinical specimens of five different clades. For EN 13624:2013 testing, *C. albicans* and all *C. auris* isolates were

Table I
Candida albicans and *Candida auris* strains included in EN 13624:2013 and/or EN 16615:2015 testing

Strain	Supplier designation	Country of origin	Clade
C. albicans	ATCC 10231	Unknown	—
C. auris[a]	10051259	India	I
C. auris[a]	10111019	NL (India)	I
C. auris[b]	CDC387	Pakistan	I
C. auris[a]	10031064	Japan	II
C. auris[a]	10031062	Korea	II
C. auris	DSM 21092	Japan	II
C. auris	CDC383	South Africa	III
C. auris[a]	10051549	South Africa	III
C. auris[a]	10080101	Venezuela	IV
C. auris[a]	CDC385	Venezuela	IV
C. auris[b]	CDC386	Venezuela	IV
C. auris[b]	10111018	Iran	V

All strains have been recovered from clinical specimens and have been typed by microsatellite genotyping or next-generation sequencing [33].
[a] Strains were only used in EN 13624:2013.
[b] Strains were only used in EN 16615:2015.

included except for one *C. auris* clade V isolate which was not available at the time the experiments were conducted. For EN 16615:2015 testing, the *C. albicans* reference strain and one *C. auris* isolate from each clade were used.

European test method EN 13624:2013 – suspension test

Under EN 13624:2013, yeasticidal activity can be claimed for a surface disinfectant when reduction rates after a set contact time reach at least four decimal logarithms (log_{10}). Yeasticidal activity was evaluated using ethanol (Product 1) and a commercially available quaternary ammonium compound (QAC)-based disinfectant (Product 2) in a quantitative suspension test (detailed in Table II). Exposure time for Product 1 was 5 min, with concentrations ranging from 25% v/v to 50% v/v, diluted doubly distilled water. Test concentrations for Product 2 ranged from 0.025% to 0.15% with a 5 min contact time. Experiments were conducted under low organic soiling conditions (0.3 g/L bovine serum albumin (BSA); 'clean conditions') as defined in EN 13624:2013, across three independent test runs. As neutralizer for ethanol 3% tween 80, 3% saponin, 0.3% lecithin, 0.1% histidine in doubly distilled water were incorporated. All tests – including growth assessment of the strains, validation of the test conditions, and verification of the neutralizer efficacy – were conducted in accordance with EN 13624:2013 as described previously [34].

European test method EN 16615:2015 – four-field test

Commercially available, ready-to-use wipes with commonly used active ingredients – low alcohol, quaternary ammonium compounds, a combination of low alcohol and QAC, and hydrogen peroxide (H_2O_2) (Table II) – were tested for yeasticidal activity using the four-field test (EN 16615:2015) [32]. Soiling conditions and contact times were based on pre-existing yeasticidal efficacy claims and are detailed in Table II. According to EN 16615:2015, a disinfectant meets the standard requirements if the reduction of test micro-organisms in field 1 reaches at least four decimal logarithms (log_{10}) and the average number of cells in fields 2 to 4 does not exceed 50 cfu. The test principle as previously described is performed on a PVC surface on which four 5×5 cm fields are marked [35,36]. In this study, we used PVC plate free foam (Forex classic; 3A Composites GmbH, Singen, Germany), which has been validated before as an equivalent alternative to PVC under EN 16615, as this material is often used to cover patient-near surfaces [37]. Field 1 was contaminated with a standardized inoculum of test organisms. After visible drying, drying losses of the yeast inocula and drying controls are measured to ensure proper experimental conditions, as described in EN 16615:2015 (see Supplementary Figure A1). In this study, drying times ranged from 20 to 25 min. The impregnated wipes listed in Table II were then placed under a unitary weight of 2.5 kg and wiped across all four fields and back in a continuous motion for 1 s in each direction, starting from field 1. After the procedure and a given contact time, remains of the test organisms were recovered from each field with a swab and colony-forming units (cfu)/25 cm^2 determined. In the dirty condition, experiments were performed in the presence of organic soiling consisting of a mixture of 3 mL/L sheep erythrocytes and 3 g/L of bovine albumin solution. Experiments were performed in two parallel test runs. Neutralization was performed using 30 g/L polysorbate 80, 30 g/L saponin, 3 g/L lecithin, 1 g/L histidine, 5 g/L sodium thiosulphate.

Table II
Active ingredients, manufacturers' recommended test conditions, and contact times of products used for EN 16615:2015 testing

Product no.	Active ingredient	Test condition	Contact time (min)
1	Ethanol (25–50%)	Clean	5
2	DDAC (6.9%)	Clean	5
3	Ethanol (<50%)	Dirty	2
4	DDAC (≥0.1 to <0.25%) ADBAC (≥0.1 to <0.25%)	Dirty	2
5	Propan-1-ol (<20%) DDAC (<0.25%)	Clean	1
6	Hydrogen peroxide (≥1 to <2.5%)	Dirty	1

DDAC, didecyldimethylammonium chloride; ADBAC, alkyl(C12-18) dimethyl-benzylammonium chloride.

Data analysis

Data analysis was performed using Microsoft Excel 2013 (Microsoft Corp., Redmond, WA, USA) and GraphPad Prism (version 10.2.1).

Results

In the EN 13624:2013 suspension test, Product 1 (ethanol) reached the 4 log_{10} reduction required for labelling the compound as yeasticidal (EN 13624:2013) at a concentration of 40% and a contact time of 5 min for the *C. albicans* surrogate strain ATCC 10231 and all tested *C. auris* strains belonging to clades I–IV (Figure 1). At lower concentrations than 40% ethanol, the *C. albicans* surrogate strain appeared less susceptible to inactivation than the tested *C. auris* strains. Product 1 reached a 3 log_{10} reduction for *C. auris* DSM strain 21092 (clade II) at a concentration of as low as 30%, and a 4 log_{10} reduction at 35% ethanol, implying greater susceptibility of the *C. auris* clade II strain to ethanol at lower concentrations compared to the *C. albicans* surrogate strain. Similarly, *C. auris* strains belonging to clades I, III, and IV also showed considerable reduction rates – albeit below the threshold at around 3 log_{10} – at concentrations of 35% ethanol, whereas *C. albicans* required a concentration of 40% ethanol to reach substantial reduction levels.

The use of a QAC-based disinfectant (Product 2) appeared to show more substantial inter-species and inter-clade differences (Figure 2). *C. albicans* ATCC strain was less susceptible to inactivation than the *C. auris* DSM strain 21092 (clade II) (Figure 2B), requiring a concentration of 0.075% QAC to reach a 4 log_{10} reduction (vs 0.05% for *C. auris* DSM 21092). However, it appeared to reach a 4 log_{10} reduction with lower concentrations than *C. auris* strains of clades I (0.125% QAC), III (0.125% QAC) and IV (0.1% QAC), respectively, implying greater relative susceptibility (Figure 2A, C, D). In our test, clade I strains appeared most resistant to inactivation, requiring higher concentrations of QAC to reach 2–3 log_{10} orders of

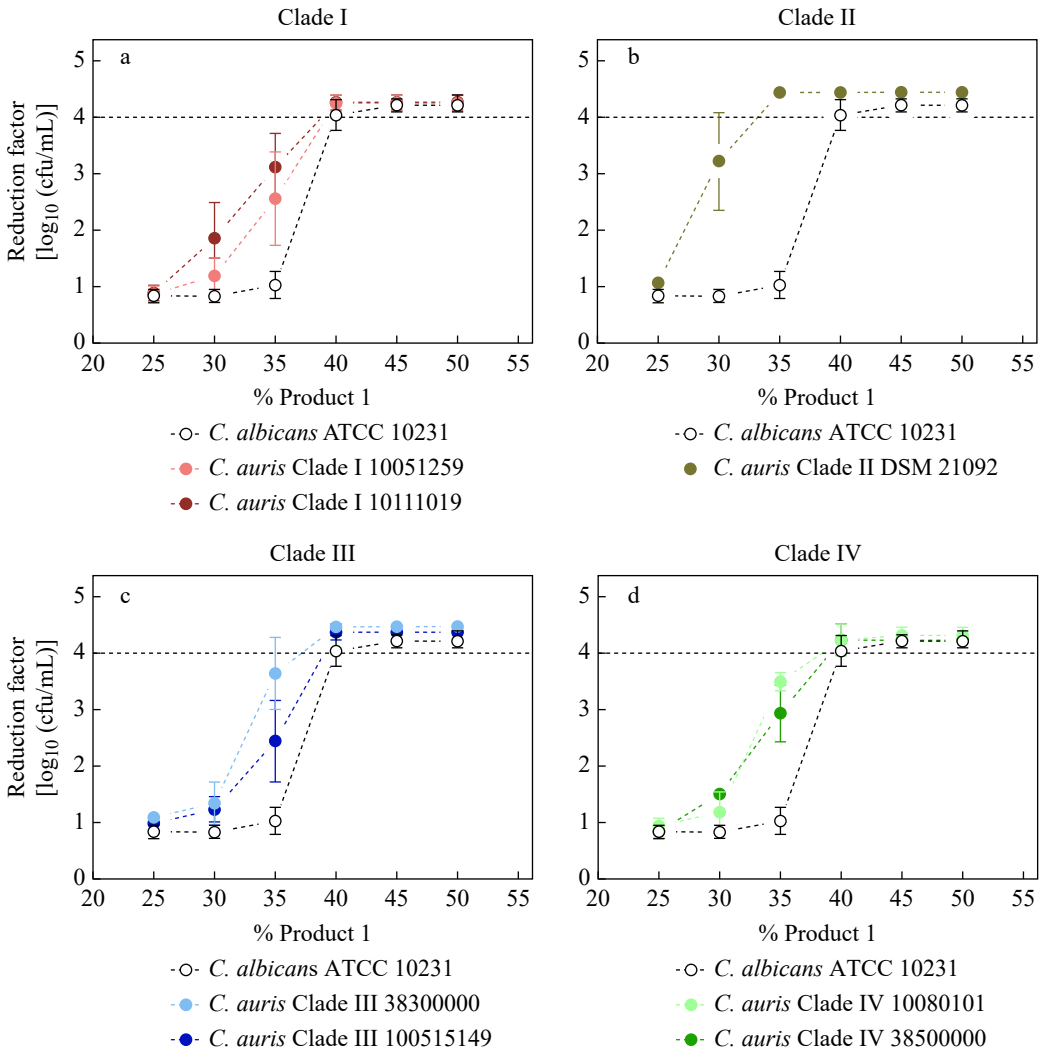

Figure 1. Susceptibility of *C. auris* strains of clades I–IV compared to *C. albicans* ATCC 10231 to an ethanol-based disinfectant in the EN 13624:2013 suspension test. Reduction factors (log_{10} [cfu/mL]) are shown for *C. auris* strains belonging to clades I–IV (A–D) for ethanol concentrations ranging from 25–50% with a contact time of 5 min under clean conditions (0.3% bovine albumin), compared to the *C. albicans* ATCC reference strain. The required 4 log_{10} reduction for determining yeasticidal activity is indicated by the dotted line.

magnitude reduction levels (0.1%) than any of the other clades or the surrogate *C. albicans* strain.

Next, we investigated the inactivation efficacy of four commercially available products with claimed yeasticidal activity under the EN 16615:2015 four-field method guidelines (Figure 3). These products differ in the contained active agents and were tested under dirty or clean conditions with differing contact times (Table II), according to the manufacturers' instructions. Product 3 (ethanol-based) was tested under dirty conditions with a contact time of 2 min. Here, a 4 log_{10} reduction of cfu/25 cm^2 could be achieved for *C. auris* clades I, II, III, and V. The *C. albicans* ATCC strain and *C. auris* clade IV strain did not reach the required 4 log_{10} reduction (Figure 3A). Furthermore, carryover to fields 2–4 exceeded the threshold of 50 cfu/25 cm^2 in all *Candida* strains, with the exception of *C. auris* clade II 21092 (Table III). Transfer was particularly high for *C. albicans* and the two *C. auris* clades which exhibited a higher resistance to drying within our experiments: clades III and IV (Supplementary Figure A1). Taken together, reduction and transfer data showed that Product 3 met the standard requirements for only one strain, *C. auris* clade II 21092. No other strains passed EN 16615:2015 testing (Table IV).

Product 4 (QAC-based) achieved a 4 log_{10} reduction for all tested *C. auris* strains under dirty conditions and a contact time of 2 min, showing no major differences between the *C. auris* clades. *C. albicans*, by contrast, was not sufficiently reduced (Figure 3B), and carryover of *C. albicans* to fields 2–4 substantially surpassed the limit of 50 cfu/25 cm^2 (Table III). These data indicate insufficient activity of the QAC-based disinfectant wipe against the *C. albicans* reference strain under the given conditions, while EN 16615:2015 criteria were uniformly met for *C. auris* of various clades (Table IV).

Product 5 (containing a combination of propanol and QAC) achieved the necessary 4 log_{10} reduction of cfu/25 cm^2 for all tested *C. auris* clades, but not for *C. albicans*, under clean conditions and a contact time of 1 min (Figure 3C). Carryover for all species and clades remained under 50 cfu/25 cm^2 (Table III).

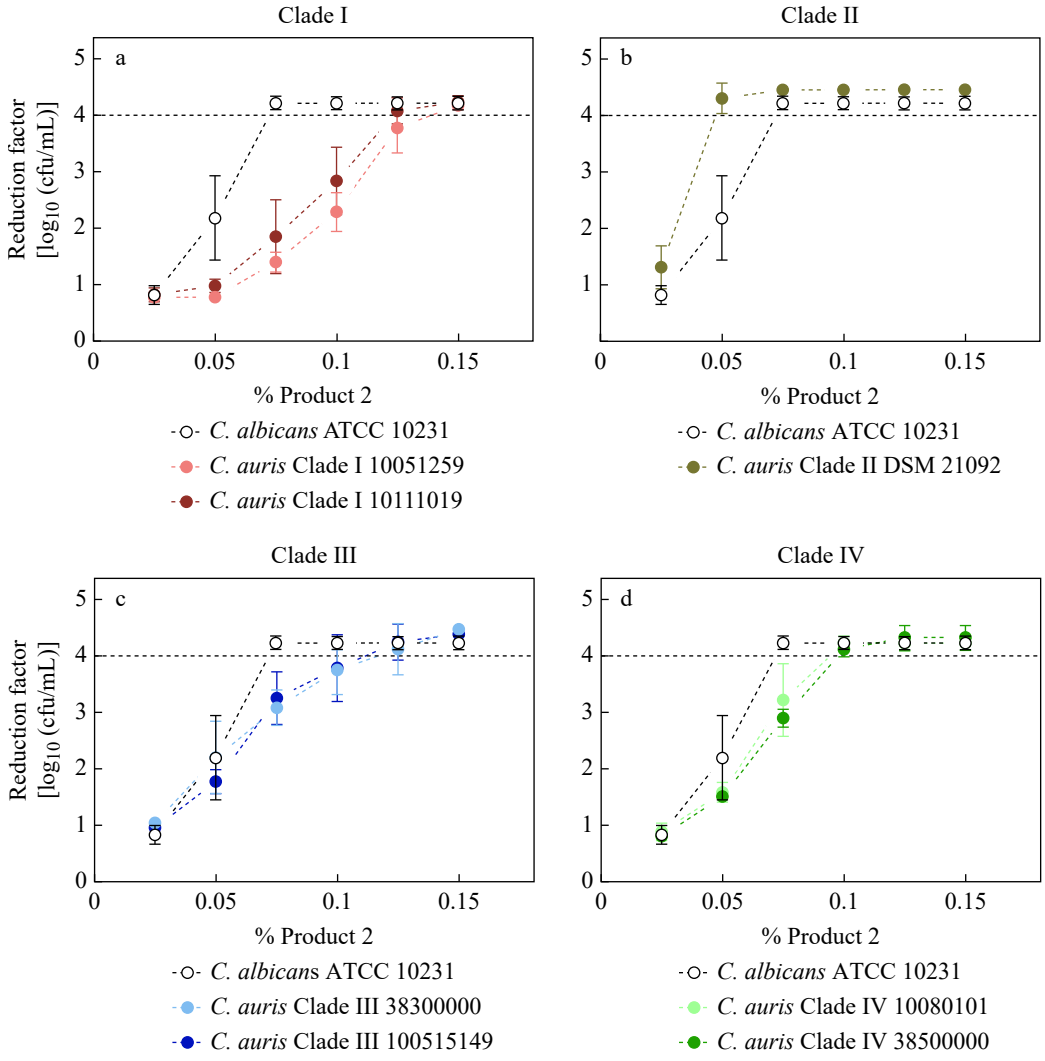

Figure 2. Susceptibility of *C. auris* strains of clades I–IV compared to *C. albicans* ATCC 10231 to a QAC-based disinfectant in the EN 13624:2013 suspension test. Reduction factors (log_{10} [cfu/mL]) are shown for *C. auris* strains belonging to clades I–IV (A–D) for QAC concentrations ranging from 0.025–0.15% with a contact time of 5 min under clean conditions (0.3% bovine albumin), compared to the *C. albicans* ATCC reference strain. The required 4 log_{10} reduction for determining yeasticidal activity is indicated by the dotted line.

Finally, Product 6, the hydrogen peroxide-based wipe, failed to achieve the necessary logarithmic reduction across all tested species and clades when used under dirty conditions and a contact time of 1 min (Figure 3D). Carryover remained $<$50 cfu/25 cm^2 for all strains. Taken together, EN 16615:2015 requirements were not met under the given experimental conditions.

Discussion

C. auris is easily transmitted in healthcare settings and therefore represents a significant threat to public health worldwide. In the medical environment, commercial ready-to-use disinfecting wipes are often used for decontamination of patient-near surfaces and medical equipment. To measure the bactericidal or yeasticidal efficacy of such products, standardized test protocols are available. In Europe, these are provided by the CEN [32]. The EN 16615:2015 standard is used to simulate the manual disinfection process as it occurs in healthcare facilities, as the wiping process itself is crucial for decontamination efficiency of high-touch environmental surfaces to prevent nosocomial transmission or infections [38]. Another standard for surface disinfection testing is the American ASTM Standard E2967-15 [39]. In contrast to the EN 16615:2015, this method relies on an automated, circular wiping movement on one spot, as opposed to the manual straight movement across a surface of the former. Other differences include the wipe selection, the duration of the process and the applied wiping pressure. Both methods have been compared before, and while both have been deemed well-suited for evaluating surface disinfectants, the EN standard is believed to more accurately reflect the real-world application [40].

C. albicans has been described as a suitable surrogate for determining yeasticidal activity against *C. auris* when being tested under such standardized conditions [30]. There, suspension tests (EN 13624:2013) and four-field tests (EN

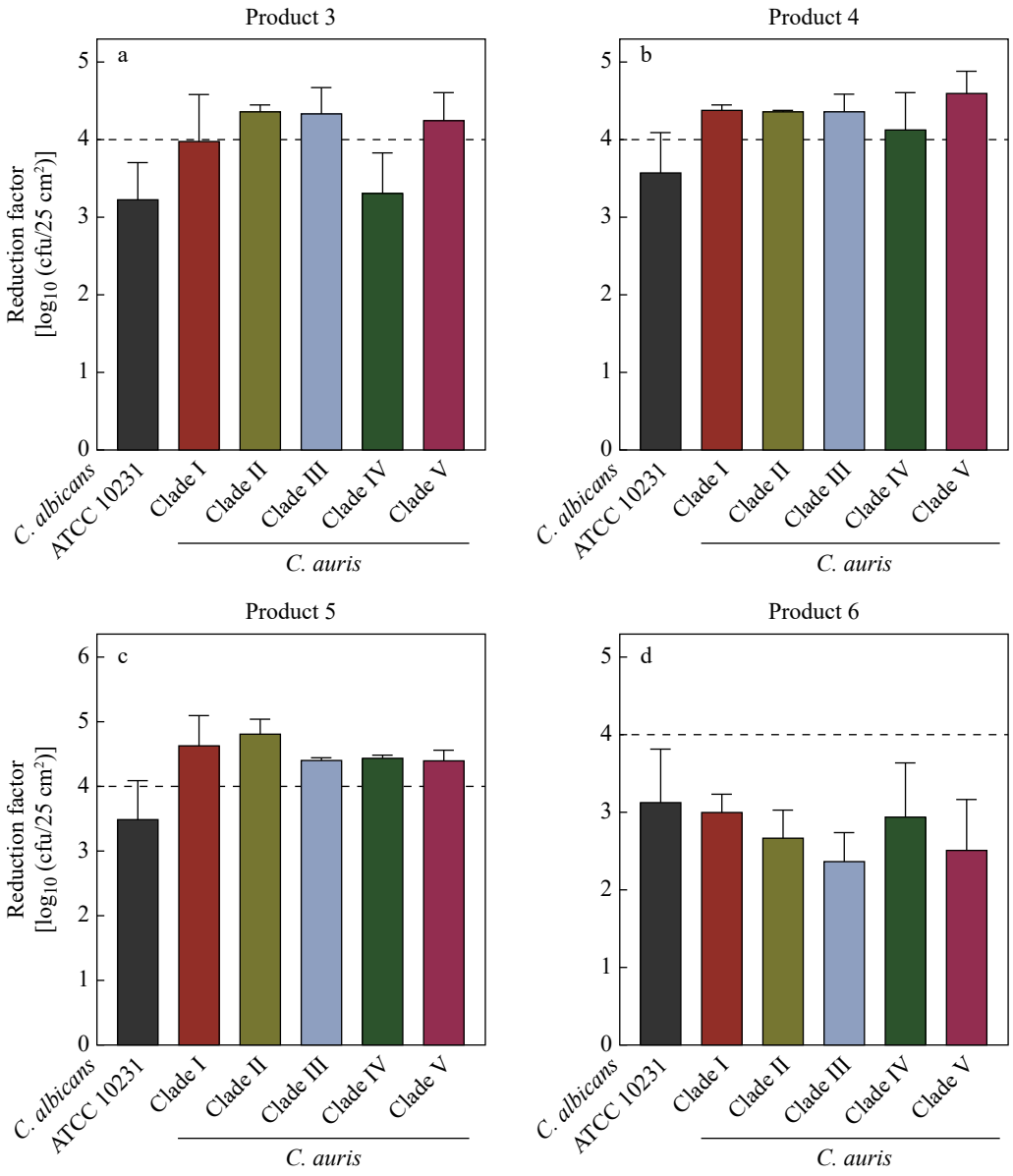

Figure 3. Standardized EN 16615:2015 four-field test using commercially available disinfection products with claimed yeasticidal activity. Reduction factors (log_{10} [cfu/25 cm^2]) are shown for *C. auris* strains of clades I—V and the *C. albicans* ATCC reference strain 10231 for each commercial disinfecting product (A—D) under varying conditions according to manufacturers' instructions. The required 4 log_{10} reduction for determining yeasticidal activity is indicated by the dotted line.

16615:2015) have shown that *C. auris* strains were more susceptible to inactivation by alcohol and QAC-based disinfectants than the *C. albicans* surrogate strain. Differences between *C. auris* clades, however, were not investigated.

An increasing amount of evidence indicates that *C. auris* clades are not uniformly susceptible to different inactivating agents and procedures [26—29]. In line with this, the data obtained in our study using standardized test designs suggest that there might be inter-species and inter-clade differences in inactivation susceptibility to widely used disinfecting agents and products. Suspension tests showed that the *C. albicans* ATCC strain appeared less susceptible to an ethanol-based disinfectant than the tested *C. auris* counterparts at concentrations below 40% ethanol. A similar observation has been described before, where *C. albicans* ATCC test strains appeared more resistant to inactivation than clinical *Candida* strains [34]. In such cases *C. albicans* could be deemed a suitable surrogate organism, predicting efficacy against *C. auris*, consistent with previous reports [30,31]. As for QAC, *C. albicans* appeared more susceptible to inactivation than the tested *C. auris* strains, with the exception of *C. auris* DSM strain 21092 (clade II), which appeared most susceptible. Because of the observed inter-species variabilities in concentrations needed for sufficient reduction as well as the differing reduction dynamics even within *C. auris* clades, it could be argued that susceptibility of *C. albicans* to these substances might not always be a reliable measure to predict susceptibility to QAC-based products for all circulating *C. auris* clade strains. It should be noted, however, that within our suspension tests, *C. auris* clades V and VI were not investigated. Thus, further

Table III
Mean carryover to fields 2—4 [cfu/25 cm^2] in EN 16615:2015 four-field testing

Strain	Product 3	Product 4	Product 5	Product 6
C. albicans ATCC 10231	1084.17	611.39	28.89	19.72
C. auris CDC387 (clade I)	387.22	19.45	5.83	45.28
C. auris DSM 21092 clade II	17.78	9.17	0.28	43.34
C. auris CDC383 (clade III)	727.22	43.61	5.00	15.00
C. auris CDC386 (clade IV)	1527.50	12.22	2.22	3.89
C. auris 10111018 (clade V)	66.25	18.61	0.84	30.00

Table IV
Summary of EN 16615:2015 efficacy testing as determined by reduction factor ($\geq$4 log_{10} [cfu/25 cm^2]) and carryover ($<$50 cfu/25 cm^2) to fields 2—4

Strain	Product 3	Product 4	Product 5	Product 6
C. albicans ATCC 10231	No	No	No	No
C. auris CDC387 (clade I)	No	Yes	Yes	No
C. auris DSM 21092 (clade II)	Yes	Yes	Yes	No
C. auris CDC383 (clade III)	No	Yes	Yes	No
C. auris CDC386 (clade IV)	No	Yes	Yes	No
C. auris 10111018 (clade V)	No	Yes	Yes	No

Products marked with 'yes' achieved a $\geq$4 log_{10} reduction as well as minimal carryover of $<$50 cfu/25 cm^2 to fields 2—4 when tested with the respective strains under EN 16615:2015 guidelines. Products marked with 'no' did not meet one or both of the requirements.

data is needed to determine and compare the susceptibility of strains belonging to these clades. Furthermore, these results would need to be corroborated using tests closer to the real-world application of disinfecting agents in a healthcare setting.

In the EN 16615:2015 four-field test, a more practice-like test which simulates a practical approach to manual surface decontamination, we found that the QAC-based product inactivated all tested *C. auris* strains, despite existing data on their insufficient activity against *C. auris* [26,41,42]. Data for the *C. albicans* reference strain, however, did not meet the standard requirements, despite a pre-existing yeasticidal claim. Similarly, the product containing a mixture of alcohol and QAC did not sufficiently inactivate and reduce the *C. albicans* ATCC strain, while all *C. auris* clade strains met EN 16615:2015 criteria. The use of hydrogen peroxide-based wipes, however, did not result in the required logarithmic reduction of pathogen load for either the *C. albicans* ATCC 10231 strain, or any of the *C. auris* strains under the given conditions. In contrast to these results, hydrogen peroxide-based disinfection procedures have been reported to exhibit sufficient disinfection efficiency for *C. auris* [43,44]. Hydrogen peroxide-containing products are included in the US Environmental Protection Agency (EPA) list of products effective against *C. auris* and are recommended for use by the European Centre for Disease Prevention and Control (ECDC) [45,46]. Whether the obtained results were due to insufficient activity of the agent or rather a consequence of test conditions, including contact time or organic soiling, is subject to further studies. The use of an ethanol-only-based product failed to reduce *C. albicans* ATCC strain 10231 and *C. auris* clade IV strain to the necessary degree; all other strains were sufficiently inactivated.

Products 3—6 all failed to pass EN 16615:2015 testing for the *C. albicans* ATCC strain under the given conditions, despite pre-existing yeasticidal claims. The reasons for this are most likely manifold. Possible factors might include culture conditions, media and ingredients, as well as strain variabilities over time. Differences between laboratories in terms of test design and conditions, including the choice of surface material used for the original claim, might influence testing outcomes.

Nosocomial transmission events underscore the urgent need for effective preventive measures. In addition to the usual infection control measures, the ECDC recommends the use of chlorine-based disinfectants, hydrogen peroxide, or disinfectants with fungicidal activity for decontamination of patient rooms after discharge in order to prevent transmission [46]. The CDC recommends the use of EPA-registered products effective against *C. auris* for surface disinfection (listed in List P) [45]. Alternatively, disinfectants effective against *C. difficile* spores are recommended (List K) [47]. WHO recommendations include the use of hydrogen peroxide and sodium hypochlorite for surface disinfection, while QACs are to be avoided [48].

Judging from our data, commercially available products used for surface disinfection differ in their effectiveness in eradicating different yeast species and/or clades. Our study, however, is limited in the fact that not all currently circulating *C. auris* strains were tested. Clade V was excluded from suspension tests, and clade VI was not included in either test, as its first description occurred only later in time. Therefore, data on the susceptibility of these clades cannot be provided.

In conclusion, inter-clade differences exist for *C. auris* in the effectiveness of commercial disinfectants. Furthermore, *C. albicans* may not always be an equally suitable indicator for yeasticidal efficacy against *C. auris* clades. We recognize that further investigation is necessary to obtain more comprehensive data on the effectiveness of hydrogen peroxide-based commercial products and to bring further light to potential inter-clade and inter-species susceptibility differences.

Author contributions

A. Lang: Formal analysis, Writing — original draft. **H. Gabriel:** Data curation, Investigation, Methodology. **T.L. Meister:** Data curation, Formal analysis, Visualization, Writing — review & editing. **J.F. Meis:** Resources. **E. Steinmann:** Data curation, Methodology. **F.H.H. Brill:** Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Writing — review & editing. **J. Steinmann:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing — review & editing.

Conflict of interest statement

The following authors declare a current relationship with Dr. Brill und Partner GmbH Institut für Hygiene und Mikrobiologie, Hamburg, Germany: H. Gabriel (current employee), F. H.H. Brill (Owner and Managing Director), J. Steinmann (Deputy Chairman) and E. Steinmann (Chairman of the Scientific Advisory Board). The other authors declare that they have no known competing financial interests or personal relationships that would influence the work reported in this paper. All procedures were conducted in accordance with relevant guidelines and regulations.

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Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2025.10.003.

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