Journal of Hospital Infection 102 (2019) 120–124

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Short report

Antifungal activity of octenidine dihydrochloride and ultraviolet-C light against multidrug-resistant *Candida auris*

P. Ponnachan [a,1], V. Vinod [b,1], U. Pullanhi [c], P. Varma [d], S. Singh [e], R. Biswas [b], A. Kumar [d,*]

[a] *Amrita School of Biotechnology, Amrita Vishwa Vidyapeetham, Amritapuri, Clappana, Kollam, Kerala, India*
[b] *Center for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi, Kerala, India*
[c] *Department of Microbiology, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi, Kerala, India*
[d] *Department of Molecular Biology, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi, Kerala, India*
[e] *Department of Infection Control, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Ponekkara, Kochi, Kerala, India*

ARTICLE INFO

Article history:
Received 19 July 2018
Accepted 11 September 2018
Available online 25 September 2018

Keywords:
Ultraviolet-C
Octenidine dihydrochloride
Candida auris
Colonization
Disinfection

SUMMARY

Outbreaks due to multidrug-resistant *Candida auris* have emerged as a large threat to modern medicine. Since skin colonization and environmental contamination have been identified as a precursor for outbreaks, we evaluated the antifungal activity of ultraviolet-C light using mercury vapour lamp with a peak emission of 254 ± 2 nm and octenidine dihydrochloride against *C. auris* clinical isolates. Octenidine dihydrochloride was found effective at significantly lower concentrations (0.00005–0.0004%) than those currently used in the clinical setting (0.05–0.1%). Scanning electron microscopy images show destruction of the organism within 6 h of exposure to 0.0005% octenidine dihydrochloride. Ultraviolet-C light could kill all *C. auris* with 15 min exposure.

Introduction

The multidrug-resistant yeast *Candida auris* is an emerging threat to modern medicine, causing frequent outbreaks in hospitals [1]. Crude mortality due to *C. auris* fungaemia is between 28% and 66% among different patient populations and

* Corresponding author. Address: Department of Microbiology, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Peeliyadu Road, Ponekkara, Edappally, Kochi, Kerala 682041, India. Tel.: +91 484 2801234 x8015; fax: +91 484 2802020.
E-mail address: vanilkumar@aims.amrita.edu (A. Kumar).
[1] Authors contributed equally.

https://doi.org/10.1016/j.jhin.2018.09.008

hospitals [1]. Two studies from India on candidaemia and deep-seated infections showed the emergence of clonal strains of *C. auris* in three hospitals [1]. Amplified fragment length polymorphism analysis of our *C. auris* isolates from a previous study showed that they were clonal and clustered together [2]. A neonatal ICU survey reported >5% candidaemia due to *C. auris* in Indian hospitals [1]. The most worrisome aspects of *C. auris* are the nosocomial acquisition and spread of clonal strains, which are not necessarily characteristics of other *Candida* species [1]. The problem is further complicated by the fact that there are no clinical breakpoints available to determine the susceptibility of *C. auris* to antifungal drugs, and no standard treatment regime for bringing about successful cure [1]. Previous studies on *C. auris* from our institute have reported 90% resistance to fluconazole (32–64 mg/L) and elevated minimum inhibitory concentration (MIC) (>2 mg/L) for itraconazole and voriconazole in 6.3% and 15%, respectively. Additionally, rates of echinocandin (>8 mg/L) and amphotericin B (>2 mg/L) resistance were 2.5% and 8%, respectively. The most common combination for multidrug resistance (two or more classes of drugs) was azole and amphotericin B in 8.2% and azole and echinocandins in 2.2% isolates [3]. Colonization of the skin and body sites by *C. auris* for weeks to months has been seen after the initial infection [4]. Nasal colonization of healthcare workers has also been reported in an outbreak in London [4]. It has been postulated that the environmental contamination occurs due to shedding from colonized patients, leading subsequently to contamination of high-touch surfaces such as monitors, key pads, trolleys and floor surfaces [4]. Previous studies have shown that *C. auris* may remain viable for 28 days on surfaces such as steel and plastics and may survive as biofilms [5]. Transmission from environmental surface to hands of healthcare workers occurs fairly easily. The cornerstone for preventing *C. auris* transmission is implementation of strict infection control measures such as hand hygiene, isolation and contact precautions. Since the hospital environment and colonized patients may act as reservoirs of *C. auris* during outbreaks, effective disinfection of surfaces and decolonization of patients play a pivotal role in reducing transmission and managing outbreaks [5]. Although octenidine dihydrochloride (OCT) has broad-spectrum antibacterial activity, its antifungal activity is limited to yeast isolates only [6]. OCT acts on microbial cell membrane and cell wall in a non-specific manner [6]. OCT MIC for *Candida albicans* has been reported to be 1 μg/mL [7]. The residual activity of OCT is similar to that of chlorhexidine, resulting in an antimicrobial depot effect on wound tissue and skin [7]. High antibiofilm activity has been demonstrated against isolates from catheter-related and orthopaedic implant-related infections. OCT is being used for wound care as an irrigation solution without preservatives (Octenidin; Schulke & Mayr, GmbH, Germany) or as antiseptic in combination with phenoxyethanol (Octenisept; Schulke & Mayr) [6].

Germicidal activity of ultraviolet (UV) light (especially UVC with a wavelength range of 240–280 nm) has been known for the last 100 years [8]. Unlike UVB light (290–340 nm) and UVA (340–400 nm), UVC has extremely limited penetration through the stratum corneum [9]. The UVC-induced damage results from dimerization of adjacent pyrimidine (particularly thymine) molecules in DNA chains [10]. Once dimerized, nucleic acid replication is impaired, and, even if it replicates at all, it results in defects making the cell non-viable. Since mammalian cells have efficient DNA repair systems, any UVC-induced damage to host tissue will be readily repaired [9]. Previous studies have shown that UVC irradiation may reduce bacterial burden in meticillin-resistant *Staphylococcus aureus*-infected chronic ulcers [11]. They have also been used to sterilize the inner surface of catheters contaminated with bacterial biofilms [11]. Two recent studies have demonstrated fungicidal activity of UVC against *Trichophyton rubrum* and *C. albicans* in infected tissue [11]. The effectiveness of OCT and UVC have not yet been evaluated for *C. auris*. The aim of our study was to determine the antifungal activity of OCT and UVC light against *C. auris* clinical isolates.

Methods

The fungicidal activity of OCT (Schulke & Mayr, GmbH, Germany) and UVC was evaluated against 32 C. *auris* isolates obtained from a variety of clinical samples, including bronchoalveolar lavage, tissue, blood, urine samples, axillary swab, and groin swabs. All *C. auris* isolates were confirmed by polymerase chain reaction using previously published specific primers [12]. *C. albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019 were used as control organisms. For MIC determination of OCT, all isolates were grown on Sabouraud dextrose agar (SDA) plates for 18–24 h at 35 ± 2°C; thereafter a single colony was used to prepare a suspension of 10^5 cfu/mL in 1 mL of Muller–Hinton broth. Tests were performed in 96-well microtitre plates by adding 100 μL of test organism to 100 μL of serial doubling dilutions of OCT. Fungal growth was indicated by measuring the turbidity after incubation for 24 h. The lowest concentration of OCT that reduced the initial inoculum by >50% was taken as the MIC value for a particular strain. Minimum fungicidal concentration (MFC) was determined for all *C. auris* isolates by sub-culturing from wells around the threshold of turbidity on to an SDA plate and evaluating for growth after 48 h incubation at 35 ± 2°C. The effect of OCT on the growth of *C. auris* was studied by scanning electron microscopy (SEM) (Figure 1). Isolates were incubated in the presence of 1 μg/mL, 2 μg/mL and 5 μg/mL of OCT for 6 h and 24 h. After incubation the cells were pelleted, washed with saline and fixed in 2% glutaraldehyde. This was followed by dehydration of the yeast cells in increasing concentrations of ethanol. Images were obtained using scanning electron microscopy (SEM; JEOL JSM-6490LA).

For evaluating antifungal activity of UVC, UVC light (Kira, Lattice Innovations Pvt Ltd, Delhi, India) was delivered using a low-pressure mercury vapour lamp (Osram HNS, 15 W) with a peak emission of 254 ± 2 nm. The Kira UVC system emits 0.8 W UVC total output with peak irradiance of 1.25 mW/cm^2 at 100 cm. *C. auris* isolates and *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were grown on SDA plates for 18–48 h at 35 ± 2°C. A loop full of organism from a suspension with cell density adjusted to 0.5 McFarland standard ($1–5 \times 10^6$ cfu/mL) was streaked on SDA plates and exposed to UVC (0.8 W) in triplicates for 5, 15, and 30 min at a distance of ~100 cm from the source. Plates were held parallel to the UVC source throughout the period of exposure. For each experiment, one UVC unexposed *C. auris*, *C. albicans* ATCC 90028, and *C. parapsilosis* ATCC 22019 plate was used as growth control. Plates were incubated for seven days at 35 ± 2°C and examined daily for yeast colonies. Viability of yeast cells was

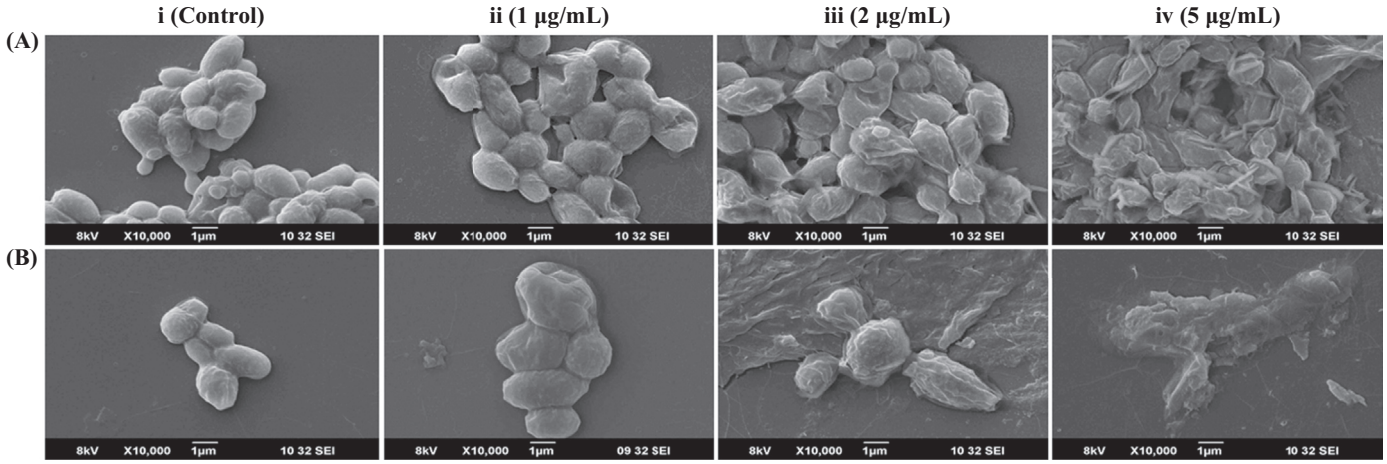

Figure 1. Scanning electron microscope images of *Candida auris* incubated for 6 h (A) and 24 h (B) in presence of (i) 0 µg/mL, (ii) 1 µg/mL, (iii) 2 µg/mL, and (iv) 5 µg/mL concentrations of octenidine dihydrochloride.

assessed by producing visible colonies as growth versus no growth. The fungal growths on the plates exposed to UVC and unexposed control plates were compared.

Results

Almost 45% of isolates had an OCT MIC of 2 µg/mL followed by 36% with 1 µg/mL, 14% with 0.5 µg/mL, and 5% with 4 µg/mL. MFC of 40% isolates was 4 µg/mL followed by 24% with 8 µg/mL, 20% with 1 µg/mL, and 16% with 2 µg/mL. OCT MIC/MFC of *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 was 0.5/1 µg/mL and 0.125/0.25 µg/mL, respectively. On SEM after 6 h incubation, with 1 µg/mL OCT, cells showed weakening in the cell envelope with indications of cell collapse in some (Figure 1A(ii)). These effects were more pronounced at 2 µg/mL (Figure 1A(iii)) and 5 µg/mL (Figure 1A(iv)) OCT. After 24 h incubation, extensive structural damage with extrusion of cellular contents was evidenced in yeast cells at 2 µg/mL OCT (Figure 1B(iii)), whereas complete cell lysis was observed at 5 µg/mL (Figure 1B(iv)) of OCT. Control cells without OCT (Figure 1A(i) and B(i)) were spherical or ovoid in shape with smooth walls and presented clear evidence of budding.

Exposure to UVC demonstrated complete inhibition of growth up to 72 h incubation of all *C. auris*, *C. parapsilosis*, and *C. albicans* isolates at 15 and 30 min exposure, whereas unexposed control strains produced confluent growth overnight (Figure 2A). A 'time of exposure-dependent' anti-fungal activity of UVC against *C. auris* was observed. After 5 min

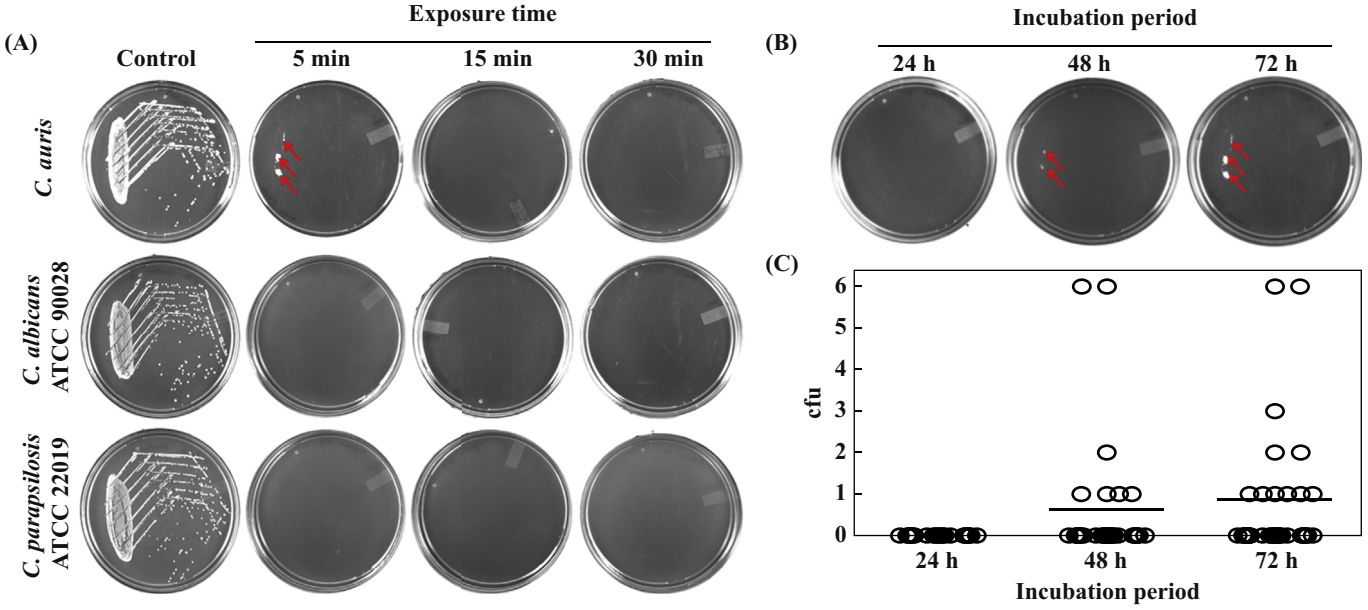

Figure 2. (A) Sabouraud dextrose agar (SDA) culture plates incubated for 72 h following exposure to ultraviolet-C (UVC) for 5, 15, and 30 min. Control plates unexposed to UVC radiation show confluent growth whereas exposed plates show no growth at 15 min and 30 min exposure. (B) SDA agar plates after 5 min UVC exposure and (C) corresponding graphical representation of residual colonies of *Candida auris* after 24, 48, and 72 h incubation.

exposure to UVC, residual colony growth was observed in 34.37% (11/32) *C. auris* isolates, after 48–72 h of incubation. However, fungal load was significantly reduced from ~10,000 cfu to as low as 1–6 cfu (Figure 2B,C). The rest of the 21 isolates did not show any growth on the plates after 5 min UVC exposure at the end of 72 h incubation.

Discussion

Octenidine dihydrochloride has broad-spectrum antibacterial activity with MIC ranges for Gram-positive and -negative bacteria of 1.4–9 and 1.5–3 μg/mL, respectively [6]. Its antifungal activity is limited to only yeast isolates with MIC for *C. albicans* of 1 μg/mL [6]. Disinfectants such as sodium hypochlorite have been used for terminal disinfection during *C. auris* outbreaks [5]. Recent studies have recommended the use of chlorine-releasing agents at 1000 ppm for routine cleaning thrice daily around patient bed areas and 10,000 ppm for terminal environmental cleaning when the patient has vacated the bed [5]. Such high-strength chlorine-based reagents are toxic to skin, respiratory tract, and eyes. Moreover, chlorine-based solutions should not be used for metal-containing materials due to the possibility of corrosion. Delicate equipment such as pressure pumps, echocardiogram monitors, and temperature probes are difficult to clean manually using liquid-based surface disinfectants. A previous study reported that routinely employed H_2O_2 vaporization can effectively kill *C. auris* and other *Candida* species, and therefore may be used for surface decontamination in hospitals [5]. The major limitation of using H_2O_2 vaporization is that it is costly and time-consuming. Only chlorhexidine has been previously used for *C. auris* skin decolonization to reduce transmission during outbreak [5]. Use of chlorhexidine is limited by the fact that it cannot be used in neonates who have one of the highest incidences of *C. auris* candidaemia and colonization. A recent study showed that chlorhexidine gluconate on its own failed to eliminate *C. auris* during in-vitro testing with a contact time of <2 min, and therefore it may be responsible for persistent colonization despite daily chlorhexidine gluconate washes [13]. However, chlorhexidine gluconate with isopropyl alcohol could reduce *C. auris* fungal load to undetectable levels within 2 min [13].

Our results demonstrate that OCT exhibits potent mycostatic and mycocidal activities against *C. auris* isolates. OCT MIC values were between 0.5 and 4 μg/mL (0.00005–0.0004%), indicating that it is highly effective at significantly lower concentrations than those currently used in the clinical setting (0.05–0.1%). They also show that it has a significant effect on cell growth and integrity as evidenced in SEM. The gross changes in the cell envelope and the leakage of cell contents are consistent with activity at the level of cell wall or plasma membrane. OCT has been previously used for skin disinfection in premature newborns and is known not to cross into the bloodstream [14].

Unlike UVC (240–280 nm), UVB light (290–340 nm) and UVA (340–400 nm) used in most germicidal bulbs is harmful to both skin and eyes [9]. UVC from germicidal fixtures has extremely limited penetration, therefore will not harm equipment, furnishings or occupants, because these produce no ozone or secondary contaminants [8]. Crazing and weakening of plastics have been reported with 254 nm radiation [8]. In acute cases, UV radiation causes redness of the skin, and skin cancers may be caused with chronic exposure [11]. Photokeratitis is known to occur on acute overexposure of cornea and conjunctiva [11]. Our study also highlights the in-vitro efficacy of UVC in killing *C. auris*. UVC could kill all *C. auris* with 15 min exposure. However, we are aware of several limitations, most notably the UVC testing of *C. auris* on solid media plates rather than in microdilutions. Nonetheless we believe that growth on solid media plates would best represent surface contamination when compared to microdilution. Many variables such as distance, presence of organic matter, and frequency need to be evaluated. Since environmental contamination and colonization plays a major role in transmission during *C. auris* outbreaks, combined use of OCT and UVC is a safe and effective way of preventing outbreaks.

In conclusion, the findings from our study may not translate to the clinical environment as OCT and UVC were tested against the more susceptible planktonic cells rather than those attached to the surface. However, to our knowledge this is the first report to investigate antifungal activity of OCT and UVC against *C. auris*. Further studies need to be done to evaluate the activity of OCT and UVC at shorter time-intervals and on *C. auris* biofilms on surfaces such as steel and polymer.

Acknowledgements

We thank Lattice Innovations Pvt Ltd, Delhi, India for supplying the Kira UVC system for evaluation. We would also like to thank Schulke & Mayr, GmbH, Germany for supplying octenidine dihydrochloride for evaluation.

Conflict of interest statement
None declared.

Funding sources
None.

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