Surface disinfection challenges for Candida auris: an in-vitro study

Kean, R.; Sherry, L.; Townsend, E.; McKloud, E.; Short, B.; Akinbobola, A.; MacKay, W.G.; Williams, C.; Jones, B.L.; Ramage, G.

Published in:
Journal of Hospital Infection

DOI:
10.1016/j.jhin.2017.11.015

Publication date:
2018

Document Version
Peer reviewed version

Surface disinfection challenges for *Candida auris*: an *in vitro* study

Ryan Kean[1,2], Leighann Sherry[1], Eleanor Townsend[1], Emily McKloud[1], Bryn Short[1], Ayorinde Akinbobola[2], William G Mackay[2], Craig Williams[2], Brian L Jones[3] and Gordon Ramage[1]*

[1]School of Medicine, Dentistry and Nursing, College of Medical, Veterinary and Life Sciences, Glasgow; [2]Institute of Healthcare Policy and Practice, School of Health, Nursing, and Midwifery, University of the West of Scotland, Paisley; [3]Microbiology Department, Glasgow Royal Infirmary, NHS Greater Glasgow and Clyde, Glasgow UK.

Running title: Surface disinfection challenges for *Candida auris*

Key words: *Candida auris*, disinfection, surface

*Corresponding Author: Gordon Ramage, Oral Sciences Research Group, Glasgow Dental School, School of Medicine, Dentistry and Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, 378 Sauchiehall Street, Glasgow, G2 3JZ, UK. Phone: +44(0)141 211 9752. e-mail: gordon.ramage@glasgow.ac.uk

Abstract

The emerging pathogenic multidrug-resistant yeast *Candida auris* is an important source of healthcare–associated infections and of growing global clinical concern. The ability of this organism to survive on surfaces and withstand environmental stressors creates a challenge for eradicating it from hospitals. A panel of *C. auris* clinical isolates was evaluated on different surface environments against the standard disinfectant sodium hypochlorite and high level disinfectant peracetic acid. *C. auris* was shown to selectively tolerate clinically relevant concentrations of sodium hypochlorite and peracetic acid in a surface dependent manner, which may explain its ability to successfully persist within the hospital environment.

Introduction

Fungal infections affect more than a billion people, resulting in approximately 11·5 million life-threatening infections and more than 1.5 million deaths annually. There have been significant strides made in tackling these infections over the past decade, but the global impact of these measures has yet to be realized [1]. An important fungus worth consideration in this context is the multidrug-resistant yeast *Candida auris*, which has been increasingly described as a major global concern and cause of major nosocomial outbreaks [2]. The impact on transmission and infection control is substantial, so understanding their mechanisms of spread and survival in the hospital environment is critical, particularly as it is able to persist on plastics and steel, and survive as biofilms [3, 4]. Several recent investigations have established that *C. auris* is capable of prolonged survival on surfaces [4, 5], and that surface disinfection protocols had variable and unsatisfactory outcomes [5]. Given that it has been shown recently that 1000ppm of an active chlorine solution is highly effective against these organisms tested in suspension [6], then it is clear that surfaces play an important role in survival of this pathogen. Our own work confirms this, with *C. auris* biofilms being generally insensitive to a range of key antimicrobial agents, thus prolonging their survival capacity [3]. Therefore, identifying ways to minimise the impact of *C. auris* within the hospital environment is imperative. The purpose of this study was to investigate the general disinfectant sodium hypochlorite (NaOCl), commonly used for terminal cleaning within the hospital environment, and the high level disinfection agent peracetic acid (PA), on different substrate surfaces. These data will support our understanding of how *C. auris* responds to different levels of challenge on surfaces representative of the hospital environment.

Material and Methods

Strains and culture conditions

Throughout this study four *Candida auris* (Ca) isolates obtained from various clinical sites [7], (NCPF 8971, NCPF 8973, NCPF 8977, NCPF 8978) were used, as previously described [3]. All isolates were identified by ribosomal DNA (rDNA) gene sequencing or matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) [7]. *Candida glabrata* (Cg) ATCC 2001 and *Candida*

albicans (Ca) ATCC 10231 was used as reference strains. All strains were stored and maintained on Sabouraud dextrose (SAB) agar (Oxoid, Hampshire, UK) prior to propagation in yeast peptone dextrose (YPD) (Sigma-Aldrich, Dorset, UK) medium overnight at 30°C. Cells were prepared according to a modified version of the British Standards for chemical disinfectants and antiseptics [8]. Briefly, cells were washed by centrifugation in phosphate buffered saline ([PBS] Sigma-Aldrich, Dorset, UK), and standardised to 1 x 10^7 cells/mL in sterile water containing 5% foetal bovine serum to simulate organic material.

Surface disinfection testing

The following test surface substrates were used: cellulose matrix (IPS Converters, Oldham, UK [1.25cm^2]), 304 stainless steel (LaserMaster, Redruth, UK [3.14cm^2]) and Thermanox™ polyester coverslips (Fisher Scientific, Loughborough, UK [1.32cm^2]). Following the adhesion phase, non-adherent cells were removed by washing with 1 mL PBS. Next, each surface was challenged with either NaOCl (1000 and 10000 ppm, [Fisher Scientific, Loughborough, UK]) or PA (2000ppm [Acros Organics, Geel, Belgium]), both agents diluted to their respective working concentrations in sterile water. Following 5 min or 10 min exposure, disinfectants were neutralised with 5% sodium thiosulphate (Fisher Scientific, Loughborough, UK) for 15 min. The neutraliser alone did not have a detrimental impact on *Candida* viability when treated in the absence of a disinfectant (data not shown). Substrate sections were then sonicated at 35 kHz for 10 min in sterile H_2O to remove cells, and serial ten-fold dilutions in sterile water were plated on to SAB agar according to the Miles and Misra plate count method. These were then incubated at 30°C for 48 h. Parallel experiments were also performed to assess the potential for regrowth following disinfection procedures. After treatment and neutralisation as described above, test coupons were replaced in 10mL of fresh YPD media and incubated for 24 h at 30°C with gentle of 100 rpm. Substrate adhered *C. auris* cells treated with sterile water acted as a positive control, with substrates containing no *C. auris* cells included as negative controls throughout this study. After 24 h, the optical density readings were measured at a wavelength of

530nm (OD_{530}) using a microtitre plate reader (FluoStar Omega, BMG Labtech, Aylesbury, UK).

Statistical analysis

Data distribution, statistical analysis and graph production was performed using GraphPad Prism (version 7; La Jolla, CA, USA). Student t-tests were used to compare treated and untreated samples. A one-way analysis of variance and post-hoc Tukey test was used to compare the effectiveness of each disinfectant against the 3 different substrates. All experiments were performed in triplicate on three independent substrates, with the mean of each experiment used for analyses. Statistical significance was achieved if $p<0.05$.

Results

Initially, a standard disinfectant challenge was performed against *C. auris* on different substrates relevant to the hospital environment. A cellulose substrate was included to act as control for porosity. It was shown that all four *C. auris* were significantly killed by NaOCl challenge at 1000 and 10000ppm, irrespective of substrate and strain, though differences were observed between these substrates. Complete eradication (100%) was only achieved on the cellulose substrate (Fig 1A). On the non-porous materials, significant quantities of viable yeast cells were killed on the steel surface following NaOCl at all treatment parameters, with an approximate 2.5 $\log_{10}$ reduction ($p<0.001$), with no significant differences observed at each time point and concentration tested (Fig 1B). Notably, those isolates treated with 1000ppm for 5 min showed significantly more regrowth compared to the other test conditions ($p<0.001$). When *C. auris* was tested on a polymer substrate it was shown that 5 min exposure at 1000ppm was the least effective overall, and although there was significant activity observed (mean $\log_{10}$ reduction = 1.29; $p<0.001$), 4.95 $\log_{10}$ was retained on the surface (Fig 1C). However, following an increased contact time of 10 min or increased concentration of 10000ppm, significantly enhanced activity was observed compared to the 5 min contact time ($p<0.001$), with an approximate overall 3.5 $\log_{10}$ reduction. When comparing both increased treatment parameters, no significant differences were observed between the regimens ($P=0.347$), and no notable regrowth was detected.

Following a standard disinfection challenge, the efficacy of the HDL agent PA was assessed. When tested against 2000ppm of PA, it was shown that all *C. auris* isolates were significantly killed by this agent. However, differences were again detected between substrates. As observed with NaOCl, complete eradication (100%) was achieved on the cellulose matrix (Fig 2A), with this same fungicidal activity also observed on the polymer substrate (Fig 2B). However, compared to the other two substrates, significant quantities of viable cells were recovered from the steel substrate following PA challenge (mean $\log_{10} = 3.19$; $p<0.001$), with an overall 2.70 $\log_{10}$ reduction ($p<0.001$) (Fig 2C). When re-inoculated into media post-challenge, substantial regrowth was recorded from both steel and polymer substrates, with minimal quantities recovered from the cellulose substrate.

For both disinfectants on each of the substrates, no differences were observed between strains, and both exhibited a similar profile to *C. glabrata* and *C. albicans*. Similarly, the presence of BSA was shown to have no effect of any treatments compared to no BSA controls. Liquid suspension tests showed that NaOCl and PA were highly effective at <20ppm and 40ppm, respectively.

Discussion

Although the precise mechanism of *C. auris* nosocomial transmission remains unknown, it is thought to be a multi-factorial process whereby it can colonise the environment and equipment of the healthcare setting. It has been reported to tolerate a number of environmental stressors, including temperature and salt, and some strains appear to have variable aggregative phenotypes that may have a role in persistence within the environment and the host [7, 9]. We therefore set out to investigate how resilient *C. auris* is within a controlled disinfection challenge using clinical isolates from the UK [7]. Here we report for the first time that both standard and high-level disinfection strategies were unable to completely eradicate *C. auris* from non-porous substrates.

Chlorine based disinfectants have variable yeasticidal activity against planktonic *C. auris* [6, 10], though their role in surface disinfection procedures

lacks definitive evidence. Recently, it has been shown that quaternary ammonium compounds were poorly active against *C. auris*, whereas environmental protection agency registered hospital disinfectants, such as NaOCl containing solutions, were fungicidal on surfaces [5]. In a recent UK outbreak, Schelenz and colleagues (2016) implemented chlorine based disinfectants at 1000ppm three times daily for environmental cleaning, and 10000ppm for terminal cleaning [2]. The data presented herein support this clinical guidance, though length of exposure at 1000ppm or an increased concentration is an important factor for consideration to maximize *C. auris* control. This is evident from our analysis of recovery of cultivable cells directly following surface challenge at 1000 ppm for 5 min, which suggests this is an ineffective control strategy.

It was interesting to note that we observed a significant difference in activity when comparing the polymer to steel, which could be explained by *Candida* species general ability to adhere and form biofilm, which are inherently more resistant. This is reinforced by another study investigating these responses on steel, where different exposure times and concentrations were reported to effectively reduce *C. auris* viability by up to 6 $\log_{10}$ [5]. Taken together, these data suggest the standard disinfection procedures are surface dependent, and given the diversity of fomites in the hospital setting then this could pose a problem for disinfection. To this end we decided to explore a representative high-level disinfection protocol. Here PA was used, a disinfectant routinely used for endoscope reprocessing. Contrary to our previous data, it was shown that on plastic polymers this disinfectant challenge was more effective, showing significant reduction compared to stainless steel. This is the first report to investigate this agent, and although it was used to represent a superior disinfectant strategy, it revealed a risk for potential transmission via contaminated endoscopes. *C. auris* has been isolated from a number of clinical sources [9], so it is not unreasonable to suggest that this and other hospital instruments could facilitate transmission.

Recent studies have suggested that *C. auris* has been shown to survive on steel and plastic surfaces for 1 and 4 weeks, respectively [4, 11]. Comparison

of *C. auris* to *C. parapsilosis* persistence on plastics was quantified under controlled hospital conditions (temperature and humidity). Low density test suspensions (10^4) of *C. auris* was shown to remain viable (CFU counts) for up to 14 days, though more sensitive esterase measurements suggested viable activity up to 28 days that was comparable to *C. parapsilosis* [4]. Piedrahita and colleagues (2017) further investigated *C. auris* in comparison to *C. albicans*, *C. glabrata* and *C. parapsilosis*, specifically looking at moist and dry inoculums over 7 days. Here they demonstrated that in moist conditions all species were recovered in near maximum efficiency after 7 days, whereas only 40% of the dried inoculum were recovered on the steel substrates, which was significantly greater than *C. albicans*, though *C. glabrata* and *C. parapsilosis* were recovered by approximately 65% [11]. This is in line with our own findings, showing comparable disinfectant sensitivity profiles for both *C. auris* and *C. glabrata.* Nevertheless, given the multi-drug resistance phenotype of *C. auris* compared to other species then its ability to persist is particularly alarming.

While this study provides a useful insight into potential complications with disinfectant procedures, there are some limitations. We have only been able to test a limited panel of *C. auris* strains, although they did demonstrate similar sensitivity profiles across each tested parameter. Furthermore, we only studied two disinfectants at individual concentrations and contact times. Future studies aim to undertake extensive analysis with commercial products in conjunction with up-to-date infection control guidelines. Overall, this study reveals the potential deficits we have in controlling this emerging fungal pathogen, and only through understanding the biology of this multi-resistant pathogen will assist us in devising new therapeutic and control interventions.

Acknowledgements

We would like to thank the European Society for Clinical Microbiology and Infectious Disease for financial support for LS.

References

[1] Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC Hidden killers: human fungal infections. *Sci Transl Med* 2012; **4**: 165rv13.

[2] Schelenz S, Hagen F, Rhodes JL *et al.* First hospital outbreak of the globally emerging Candida auris in a European hospital. *Antimicrob Resist Infect Control* 2016; **5**: 35.

[3] Sherry L, Ramage G, Kean R *et al.* Biofilm-Forming Capability of Highly Virulent, Multidrug-Resistant Candida auris. *Emerg Infect Dis* 2017; **23**: 328-31.

[4] Welsh RM, Bentz ML, Shams A *et al.* Survival, Persistence, and Isolation of the Emerging Multidrug-Resistant Pathogenic Yeast Candida auris on a Plastic Healthcare Surface. *J Clin Microbiol* 2017; doi 10.1128/JCM.00921-17.

[5] Cadnum JL, Shaikh AA, Piedrahita CT *et al.* Effectiveness of Disinfectants Against Candida auris and Other Candida Species. *Infect Control Hosp Epidemiol* 2017; doi 10.1017/ice.2017.162: 1-4.

[6] Moore G, Schelenz S, Borman AM, Johnson EM, Brown CS Yeasticidal activity of chemical disinfectants and antiseptics against Candida auris. *J Hosp Infect* 2017; doi 10.1016/j.jhin.2017.08.019.

[7] Borman AM, Szekely A, Johnson EM Comparative Pathogenicity of United Kingdom Isolates of the Emerging Pathogen Candida auris and Other Key Pathogenic Candida Species. *mSphere* 2016; **1**.

[8] BSI. Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on nonporous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2). *BS EN 16615:2015,* London: BSI Standards Publication 2015.

[9] Chowdhary A, Sharma C, Meis JF Candida auris: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLoS Pathog* 2017; **13**: e1006290.

[10] Abdolrasouli A, Armstrong-James D, Ryan L, Schelenz S In vitro efficacy of disinfectants utilised for skin decolonisation and environmental decontamination during a hospital outbreak with Candida auris. *Mycoses* 2017; **60**: 758-63.

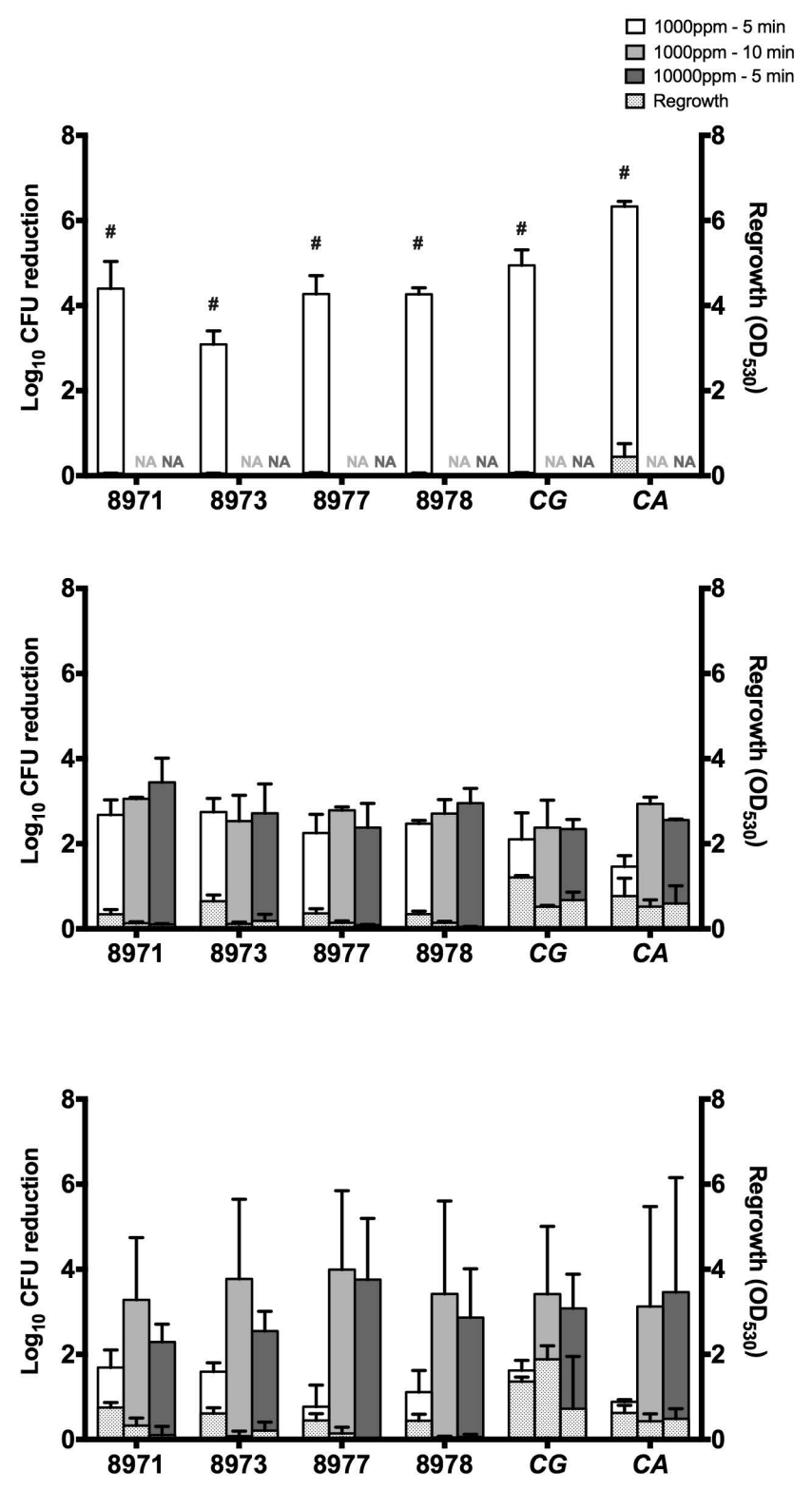
[11] Piedrahita CT, Cadnum JL, Jencson AL, Shaikh AA, Ghannoum MA, Donskey CJ Environmental Surfaces in Healthcare Facilities are a Potential Source for Transmission of Candida auris and Other Candida Species. *Infect Control Hosp Epidemiol* 2017; doi 10.1017/ice.2017.127: 1-3.

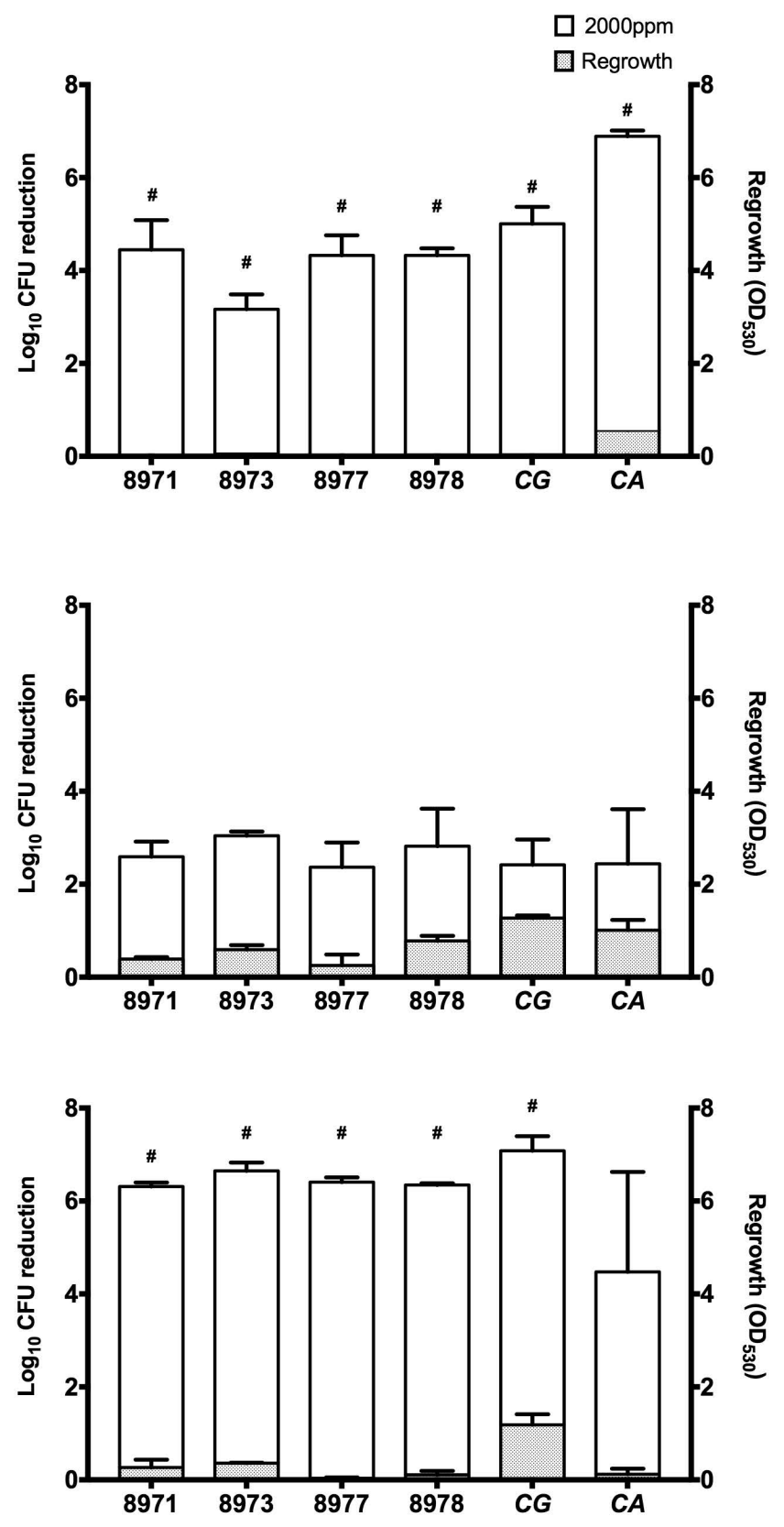
Figure 1. Efficacy of sodium hypochlorite on Candida auris, Candida glabrata and Candida albicans on three different substrates.

Cellulose matrix (A), stainless steel (B) and polymer (C) were inoculated with 1 x 10^7 cells/mL of *C. auris* (NCPF), *C. glabrata* (*CG*) and *C. albicans* (*CA*) for 90 min before being treated with 1000ppm NaOCl for 5 min, 1000ppm for 10 min and 10000ppm for 5 min. Viable cells were then quantified by CFU (left hand y-axis) and regrowth was measured spectrophotometrically (right hand y-axis). Data represents means ± standard deviation of triplicate datasets, with CFU $\log_{10}$ reduction of each test substrate normalised to 1cm^2. # indicates complete eradication compared to untreated control. N.A. = not applicable.

Figure 2. Efficacy of PA on Candida auris, Candida glabrata and Candida albicans on three different substrates.

Cellulose matrix (A), stainless steel (B) and polymer (C) were inoculated with 1 x 10^7 cells/mL of *C. auris* (NCPF), *C. glabrata* (*CG*) and *C. albicans* (*CA*) for 90 min before being treated with 2000ppm PA for 5 min. Cell viability (left hand y-axis) and re-growth (right hand y-axis) were quantified by CFU and spectrophotometric readings, respectively. Data represents means ± standard deviation of triplicate datasets, with CFU $\log_{10}$ reduction of each test substrate normalised to 1cm^2. # indicates complete eradication compared to untreated control.


1000ppm - 5 min
1000ppm - 10 min
10000ppm - 5 min
Regrowth
Log10 CFU reduction
Regrowth (OD530)
#
NA NA
8971
8973
8977
8978
CG
CA


2000ppm
Regrowth
Log10 CFU reduction
Regrowth (OD530)
0
2
4
6
8
#
8971
8973
8977
8978
CG
CA