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Sampling efficiency of *Candida auris* from healthcare surfaces: culture and nonculture detection methods

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Abstract

Sponges and swabs were evaluated for their ability to recover *Candida auris* dried 1 hour on steel and plastic surfaces. Culture recovery ranged from <0.1% (sponges) to 8.4% (swabs), and cells detected with an esterase activity assay revealed >50% recovery (swabs), indicating that cells may enter a viable but nonculturable state.

Candida auris has emerged as an often-misdiagnosed multidrug-resistant organism causing mortality rates of 30%–60% in hospitalized patients.[1–3] It has been shown to persist on surfaces ≥2 weeks.[4,5] Transmission via fomites within healthcare facilities has been reported[1,2] but standard sampling methods are lacking. We evaluated the influence of sampling devices, mucin in organic matrix, and drying time on cell recovery efficiency of 2 *C. auris* strains from steel and plastic surfaces. We also compared the detection of recovered cells using culture (colony forming units, CFU) and an alternate viability assay.

Methods

Steel coupons (S-180 grade, T-304; Stewart Stainless Supply, Suwanee, GA) and plastic coupons (0.80 in. thickness, P1 haircell texture, Kydex-T, Bloomsburg, PA) (322 cm^2) were cleaned with a nonantimicrobial detergent, rinsed with ultrapure water, then rised with 70% ethanol. Steel was autoclaved at 121°C for 20 minutes and plastic was sterilized with ultraviolet (UV) light for 1 hour.

We obtained 2 *C. auris* isolates from the Centers for Disease Control and Prevention (CDC); B11103 (clade I) and AR0385 (clade IV). Yeast colonies were cultured on Sabouraud dextrose agar (SDA) for 48 hours at 37°C then suspended in a body fluid simulant; artificial test soil (ATS), or ATS with mucin (ATS-M) (Healthmark Industries, Fraser, MI). Coupons were inoculated with 500 μL cell suspension (10^5 CFU/mL), spread, and dried for 1 hour at

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ambient temperature and humidity in a closed BSC (light and fan off). Evaluations were also conducted by sampling 0, 20, and 40 minutes after inoculation.

Coupons were sampled across the entire surface with either a cellulose sponge (cellulose) (3M Sponge-stick, St Paul, MN) or a polyurethane sponge (polyurethane) (Hygiena, Camarillo, CA), both moistened with neutralizing buffer, using a standard method[6] modified to 322 cm^2 sample area. Sponges were held 1 hour at ambient temperature to simulate transport time and were then transferred to a stomacher bag containing 45 mL PBS with 0.02% Tween 80 (PBST) for processing. Cells were eluted from sponges using a Stomacher 400 Circulator (Seward Ltd, Worthing, UK) at 260 rpm for 1 minute. The eluate was concentrated by centrifugation, and the final volume was recorded and then cultured on SDA (48–72 hours, 37°C).

To determine the portion of cells remaining on the coupons after sampling, and to investigate the presence of viable but non-culturable cells, smaller steel coupons (26 cm^2) were inoculated as described above using 100 μL 5×10^6 CFU/mL inoculum in ATS-M and sampled with a foam swab (swab) (Puritan; Guilford, ME). The swab was vortexed in 5 mL PBST, the coupon was sonicated in 15 mL PBST, and both eluates were cultured on SDA. Using a solid phase cytometer (ScanRDI, bioMerieux, Durham, NC) and the instrument's standard protocol,[7] 1 mL of each swab and coupon eluate was analyzed for cells exhibiting metabolic esterase activity. Cells with esterase activity cleave a fluorophore creating fluorescent events that can be detected by the solid-phase cytometer and verified visually by fluorescent microscopy.

Percent recovery (%R) was calculated relative to inoculum CFU or cell count via ScanRDI. Significance was determined using the Student *t* test, with significance set at $P < .05$. Solid-phase cytometry fluorescent events were verified visually by fluorescent microscopy as viable cells per swab or coupon and were compared to CFU per swab or coupon.

Results

The mean %R CFU varied with sampling device, surface type, and inoculum matrix, although %R for *C. auris* B11103 was consistently <1% (Table 1), and for AR0385 was <3%. Using cellulose for sampling, the %R was significantly greater when *C. auris* was suspended in ATS-M compared to ATS ($P < .01$) from both surfaces (Table 1). All subsequent studies used the ATS-M formula. The sampling mean %R was significantly higher using polyurethane than cellulose, regardless of surface type. Combining data for steel and plastic using B11103 in ATS-M, the mean %R for polyurethane was 0.41% (SD, 0.32%) and for cellulose was 0.15% (SD, 0.12%). For directly inoculated cellulose and polyurethane (positive controls), the %R ranged between 46% and 82% with significantly higher %R from polyurethane ($P < .01$). The mean %R decreased with increased drying time from both surfaces. When sampled with cellulose at 0, 20, and 40 minutes, mean %R from steel and plastic dropped from ~50% to <1%, representing a CFU decrease of 2.5–3 $\log_{10}$. Furthermore, the variability in %R was also significant ($P < .01$) between the 2 strains evaluated on plastic surfaces using cellulose (Table 1). When examining eluates from swabs using ScanRDI, 1 $\log_{10}$ more *C. auris* cells were detected than by culture (Table 2).

Although the %R was higher for swabs than for sponges, the surface area sampled was much smaller (26 cm^2) and the results were much more variable (higher SD).

Discussion

Candida auris has been found on surfaces in healthcare facilities[1,3,5] and has shown persistence extending ≥2 weeks.[4,5] We found recovery by traditional culture methods to be low after only 40 minutes drying time, yet patient shedding and/or fomite contamination can occur throughout the day[2] and can continue drying on surfaces for much longer than 1 hour. Our findings suggest that the actual contamination on surfaces may be 1–2 log_{10} greater than what is reflected by CFU counts. The mean %R by CFU from directly inoculated sponges was >60%, indicating that neither sampling device nor processing method influenced CFU or induced viable but nonculturable cells in *C. auris*. We also noted that the directly inoculated polyurethane yielded a higher mean %R than cellulose, indicating less cellular adherence to polyurethane, also noted by West-Deadwyler et al.[8] Furthermore, %R improved slightly from both steel and plastic surfaces using the polyurethane sponge, indicating better recovery of *C. auris*. The CDC recommends using swabs for sampling surface areas <26 cm^2 and sponges for 645 cm^2.[6]

Although %R varied between *C. auris* strains, the low %R for both strains was unanticipated because the same methods applied to antimicrobial-resistant bacterial pathogens yielded higher %R, ranging from 7.7% (SD, 5.2%) for carbapenemase-producing *Klebsiella pneumoniae* to 58.9% (SD, 12.7%) for *Clostridium difficile*.[9]

Background particle fluorescence from sponge materials prevented ScanRDI assay comparison to culture, but from swabs, we observed almost 50% cell recovery by esterase activity and only 9% by culture. Thus, although many cells are recovered, they are nonculturable. These results support previous findings that yeasts may become viable but nonculturable,[4,10] though surprisingly, this occurred after only 1 hour of drying. If this holds true for other *C. auris* isolates, contamination on environmental surfaces could be 1 log_{10} higher than detected by culture and culture could mis-represent actual contamination by 2–3 log_{10}. The question of cell resuscitation potential and subsequent transmission from healthcare surfaces is worth investigating. We are not aware of published evidence supporting *C. auris* resuscitation, though it has been reported in other yeasts.[10]

This study was limited to 2 *C. auris* strains, 2 simulated body fluids, and 3 sampling devices, and each of these can influence recovery efficiency. However, the data presented here contribute to creating a standard method for *C. auris* surface sampling and to interpreting results obtained in the context of an outbreak investigation or in evaluating environmental infection control interventions.

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Table 1.

Percent Recovery of *C. auris* From 322 cm^2 Steel and Plastic Coupons as Determined by Culture (CFU)[a]

Surface	Isolate	Drying Time (min)	Sampling Tool	Matrix	No. of Coupons Inoculated[b]	Mean %R (SD)	*P* Value
Steel	B11103	60	CS	ATS	9	0.04 (0.05)	<.01
			CS	ATS-M		0.16 (0.11)	
Plastic	B11103	60	CS	ATS	9	0.13 (0.09)	<.01
			CS	ATS-M		0.26 (0.2)	
Steel	B11103	60	CS	ATS-M	9	0.11 (0.08)	<.01
			PS	ATS-M		0.43 (0.37)	
Plastic	B11103	60	CS	ATS-M	6	0.24 (0.12)	<.05
			PS	ATS-M		0.37 (0.22)	
Plastic	B11103	60	CS	ATS-M	6	0.65 (0.31)	<.01
	AR0385		CS	ATS-M		2.27 (0.81)	
Steel and Plastic	B11103	60	CS	ATS-M	12	0.15 (0.12)	…
			PS	ATS-M	12	0.41 (0.32)	…
Steel	B11103	0	CS	ATS-M	4	47.11 (6.28)	…
		20	CS	ATS-M	4	1.82 (1.42)	…
		40	CS	ATS-M	4	0.75 (0.68)	…
Plastic	B11103	0	CS	ATS-M	4	51.79 (4.36)	…
		20	CS	ATS-M	4	0.77 (0.40)	…
		40	CS	ATS-M	4	0.58 (0.42)	…
Directly inoculated sampling tools		N/A	CS	ATS-M	7	55.28 (8.82)	<.01
		N/A	PS	ATS-M	7	69.36 (11.9)	

Note. SD, standard deviation; CS, cellulose sponge; PS, polyurethane sponge; ATS, artificial test soil; ATS-M, artificial test soil with mucin (both formulations contain hemoglobin, proteins, carbohydrates, albumin, lipids, and vitamins to simulate body fluids).

[a]Variables tested were isolate, dry time, sampling tools and suspension matrix. Percent recovery was calculated relative to the inoculum (~5 log/coupon).

[b]Experiments conducted on2 or 3 independent days.

Table 2.

Recovery of *C. auris* From 26 cm^2 Steel Coupon[a]

Sample Type	CFU (SD)	%R CFU (SD)	ScanRDI Cell Counts (SD)[b]	%R ScanRDI (SD)[c]
Inoculum[d]	4.52×10^5	NA	3.62×10^5	NA
	(8.42×10^4)		(1.47×10^5)	
Swab eluate	3.77×10^4	8.94	1.78×10^5	49.97
	(5.40×10^4)	(12.17)	(7.04×10^4)	(27.80)
Coupon eluate	1.28×10^3	0.26	5.16×10^3	1.87
	(2.21×10^3)	(0.43)	(4.06×10^3)	(1.74)

Note. CFU, colony-forming units; SD, standard deviation; NA, not available.

[a]Cells detected by culture (CFU) and esterase activity (ScanRDI) from the inoculum suspension, from swabs after sampling the coupon, or from the coupon after sampling. Data represents mean of n=18 replicates.

[b]ScanRDI indicates the cells determined as viable by metabolic esterase activity.

[c]%R ScanRDI indicates the percent of viable cells determined by ScanRDI, relative to the ScanRDI inoculum.

[d]Titer of inoculum placed on coupons: 100 µL of 4.52×10^6/mL suspension.