Development of a new fast antifungal susceptibility test for *Candida auris*

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Introduction

Antimicrobial resistance of fungi poses a global health threat, as the incidence of resistant fungi continues to rise. Current strategies to tackle these infections, including available antifungal agents and conventional antifungal susceptibility tests, remain insufficient. **The conventional antifungal susceptibility tests are time-consuming**, highlighting the urgent need for innovative and new rapid testing approaches to improve diagnosis and treatment. This study focuses on the **validation of the IPAC2 AR (figure 1), an ultra-sensitive particle counter, as a novel, rapid and reliable antifungal susceptibility test for *Candida auris*** since it has been categorized, by the WHO, in the 'critical group' of the fungal priority pathogens list.

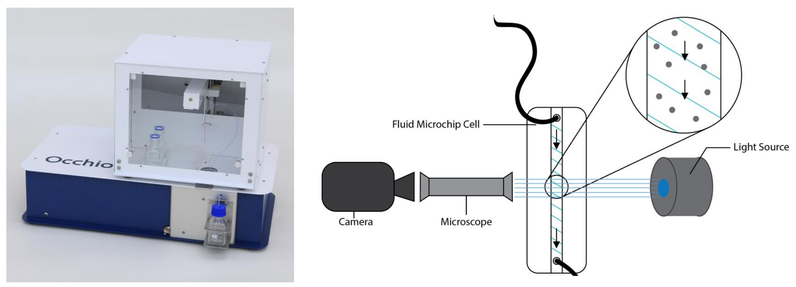

Figure 1: The IPAC2 AR and it's working mechanism.

Experimental work

The experimental work with the IPAC2 AR was performed with five different *Candida auris* strains based on the EUCAST method and the CDC resistance breakpoints. Below, the results of one strain (*Candida auris* CBS 12373) are shown, but in the conclusion the summarized results for the other strains can be found.

IPAC2 AR correlates linearly with CFU

To validate the IPAC2 AR for the quantification of *Candida auris*, serial dilutions (1:2 to 1:10^6) of 0.5 McFarland *Candida auris* were made and analyzed using the agar plate method (CFU/mL) and the IPAC2 AR (particles/mL).

A good linear correlation (figure 2: $R^2 > 0.95$) was found between the measured concentrations with the IPAC2 AR and the practical concentrations of *Candida auris*.

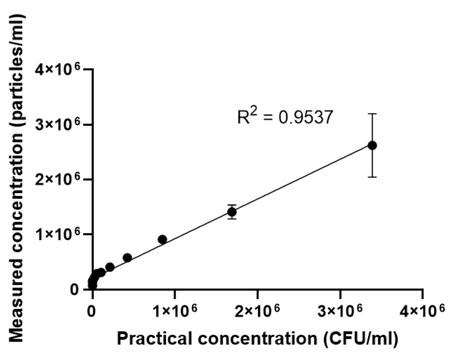

Figure 2: Linear response measured with the IPAC2 AR of *Candida auris* CBS 12373.

Fast growth detection

Candida auris growth was quantified by the IPAC2 AR in the presence and absence of several antimycotics (figure 3: caspofungin, fluconazole and amphotericin B). Concentrations of the antimycotics were based on the current breakpoint guidelines by the CDC (table 1).

Table 1: CDC resistance breakpoints

CDC	R ≥ (mg/l)
Caspofungin	2
Fluconazole	32
Amphotericin B	2

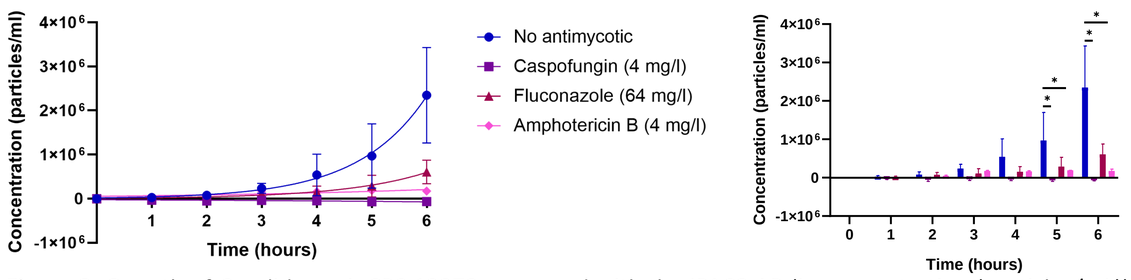

Figure 3: Growth of *Candida auris* CBS 12373 measured with the IPAC2 AR (in concentration (particles/mL)) in function of time (hours). Statistical analysis (2-way ANOVA: * $p < 0.05$; $n \geq 3$) shows that growth can be detected after 5 hours.

For all *Candida auris* strains tested, exponential growth was detected with the IPAC2 AR after 5 to 7 hours.

Rapid and reliable MIC

To determine the minimal inhibitory concentrations (MICs) of the antimycotics for the *Candida auris* strains, 2-fold serial dilutions of the antimycotics were made (concentrations ranging between 64 mg/L and 0.016 mg/L).

Growth of *Candida auris* CBS 12373 gets partially inhibited (figure 4) in 0.016-0.06 mg/L caspofungin. The MIC of 0.125 mg/L was found after ±5 hours, leading to the conclusion that *Candida auris* CBS 12373 is susceptible to caspofungin.

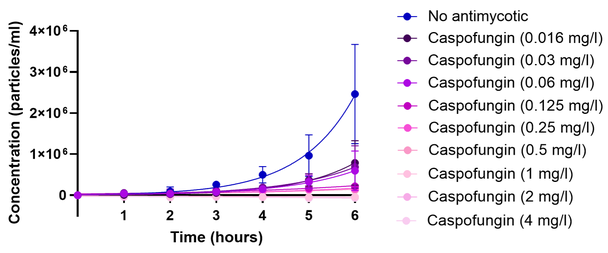

Figure 4: MIC determination of caspofungin with the IPAC2 AR for *Candida auris* CBS 12373.

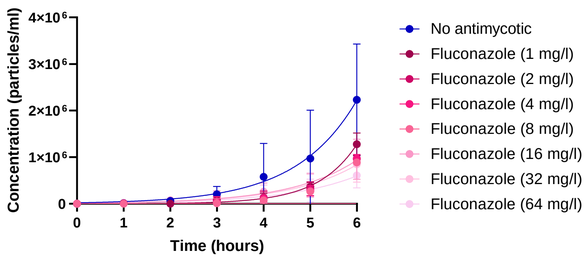

Figure 5: MIC determination of fluconazole with the IPAC2 AR for *Candida auris* CBS 12373.

For *Candida auris* CBS 12373 growth was observed in all the fluconazole concentrations (figure 5). This indicates that the MIC is larger than 64 mg/L, showing resistance of the *Candida auris* CBS 12373 strain for fluconazole.

In the presence of amphotericin B growth of *Candida auris* CBS 12373 is inhibited (figure 6) in all tested concentrations. The MIC is <1 mg/L (found after ±5 hours), whereby *Candida auris* CBS 12373 is susceptible to amphotericin B.

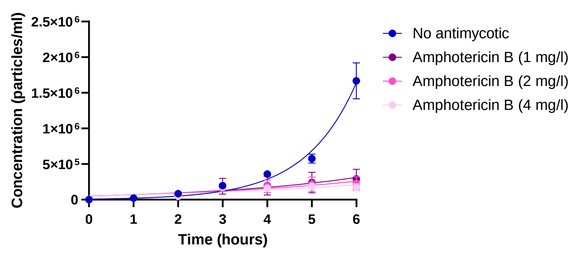

Figure 6: MIC determination of amphotericin B with the IPAC2 AR for *Candida auris* CBS 12373.

With the IPAC2 AR, all tested *Candida auris* strains demonstrated comparable results (susceptible (S) or resistant (R)) with conventional techniques (table 2).

Conclusion

After 5-7 hours the IPAC2 AR could determine reliable MICs of different antimycotics for the *Candida auris* strains, significantly faster than the conventional techniques (24 hours). The further development and use of this test could lead to a faster diagnosis and better treatment for patients.

Table 2: Comparison of different techniques for the determination of the susceptibility (S = susceptible and R = resistant) of the *Candida auris* strains for caspofungin (C), fluconazole (F) and amphotericin B (A). † After 48 hours S becomes R. Δ Plate used: YeastOne YO10.

	C. auris B11220			*C. auris* B11224			*C. auris* B11230			*C. auris* CBS12373			*C. auris* B11222			**Time (hours)**
	C	F	A	C	F	A	C	F	A	C	F	A	C	F	A	
IPAC2 AR (Occhio)	S	S	S	R	R	S	S	R	S	S	R	S	S	R	S	**5-7**
Broth dilution test	S	S	S	R	R	S	S	R	S	S	R	S	S	R	S	**24**
E-test (Biomerieux)	S	S	S	R	R	S	S	R	S	S	R	S	S	R	S	**24**
Sensititre Vizion Δ	S	S	S	R	R	S	S	S †	S	S	R	S	S	R	S	**24**