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Les amibes libres favorisent la survie et la prolifération de *Candida auris* dans l'eau

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Sommaire

Liste des abréviations

ATCC	American Type Culture Collection
CFU	Colony-Forming Unit
DUWL	Dental Unit Waterlines
FLA	Free Living Amoeba
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry
MET	Microscope Électronique à Transmission
MOI	Multiplicity Of Infection
PYG	Peptone, Yeast extract, Glucose
PYNFH	Peptone, Yeast extract, Nucleic acid, Folic acid, Hemin
TEM	Transmission Electron Microscopy

Abstract

Free-living amoebae promote *Candida auris* survival and proliferation in water

Introduction

Candida species are responsible for life-threatening infections in immunocompromised patients. Among them, *Candida auris* is an emerging species discovered in 2009 with recent outbreaks reported worldwide. Its ability to be resistant to most systemic antifungal classes, its identification issues and its capacity to persist in a hospital environment, have led to health concerns. Currently, data about environmental reservoirs are limited but remain essential in control of *C. auris* spread. The aim of our study was to explore the interactions between *C. auris* and two free-living amoeba (FLA) species potentially found in the same environment.

Materials and methods

C. auris was incubated with *Acanthamoeba castellanii* or *Vermamoeba vermiformis* trophozoites or their culture supernatants, in filtered hospital water at 37°C. The number of amoeba trophozoites and yeasts was determined at 48 h, 120 h and 168 h of cocultivation, and transmission electron microscopy (TEM) was performed after 48h. In order to compare the results to another yeast species, same experiments of cocultivation have been carried out with *Candida albicans*.

Results

While *C. albicans* and *C. auris* were not able to survive alone in water, supernatants of the two FLA species promoted yeasts survival and proliferation. *C. albicans* was destroyed by *A. castellanii* trophozoites but survived in the presence of *V. vermiformis* trophozoites. In contrast to *C. albicans*, *C. auris* was able to survive and proliferate in contact with *A. castellanii* or *V. vermiformis* trophozoites. Intact internalization of *C. auris* within both FLA species was also evidenced by TEM.

Conclusion

An environmental reservoir of *C. auris* can therefore be considered through FLAs and contamination of the hospital water networks would consequently be possible.

Keywords: *Candida auris*; Free-living amoeba; hospital tap water; outbreaks

Introduction

Candidiasis are invasive infections due to fungi belonging to *Candida* species (1). Though *Candida albicans* is the leading species involved, non-*albicans* candidemia have been on the uprise for many years (2). Increasing prevalence of colonization and infection with non-*albicans Candida* species may result from increasing use of antifungal agents such as fluconazole (3,4). Among these non-*albicans* species, a new one, *Candida auris,* was recently reported. This yeast was first described in Japan in a patient's ear canal in 2009 (5). In the same year, its pathogenicity was highlighted with three cases of candidemia identified at three university hospitals in South Korea (6). Subsequently, *C. auris* has been isolated from several body localizations in multiple countries on five continents (7), and implicated, similarly to other *Candida* species, in invasive infections associated with high mortality (8–10). Whole-genome sequencing revealed genetically distinct clades of this fungus on different continents and subcontinents, including East Asia, South Asia, South America, and Southern Africa (11). That suggests near-simultaneous emergence of *C. auris* rather than recent spread from a single source. It may be related to new or increasing antifungal selection in humans, animals, or in the environment (12).

One of the issues with *C. auris* is its ability to resist to antifungal drugs. Only three drug classes are available for systemic treatment of *Candidiasis*: azoles (fluconazole, itraconazole, isavuconazole, posaconazole, and voriconazole), polyenes (amphotericin B and its lipid formulations) and echinocandins (anidulafungin, caspofungin, and micafungin). An analysis of fifty-four clinical isolates of *C. auris* isolated from five countries (Pakistan, India, South Africa, Venezuela and Japan), from 2012 to 2015, found 93% of strains resistant to fluconazole, 35% resistant to amphotericin B, and 7% resistant to echinocandins. Moreover, 41% were resistant to 2 antifungal classes and 4% resistant to the 3 classes (11).

Another issue is identification of the yeast. Until recently, it was not identifiable by phenotypical method or matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). In fact, *C. auris* was misidentified as *Candida haemulonii*, *Candida famata* or *Rhodotorula glutinis* (13). A recent update of the MALDI-TOF MS libraries enables more reliable identification (14).

Furthermore, *C. auris* can cause outbreaks. Its diffusion in hospital environments appears superior to that of other *Candida* species. A large-scale outbreak between April 2015 and July 2016 involving 50 patients was reported in a cardiothoracic intensive care unit in London (15). The origin of this outbreak was linked to the persistence of the yeast on skin-surface axillary temperature probes. Several authors have demonstrated that *C. auris* can survive and persist on dry and non-porous surfaces for some weeks (16). In addition, frequently used detergents such as quaternary ammonium show poor activity against *C. auris* (17). The Control Disease Center of Atlanta specified that an Environmental Protection Agency-registered hospital-grade disinfectant effective against *Clostridium difficile* spores must be used for *C. auris* (18).

The reservoir of *C. auris* is not presently known and represents an important focus of research aimed at effectively reducing infectious risk. Environment could be the reservoir of *C. auris* and it must be investigated.

Free living amoebae (FLAs) are saprophytic and ubiquitous protozoa found in domestic water supplies, wells, dental irrigation units, hospital water supplies, or air conditioning units (19–21). They present a two-stage life, switching between a trophozoite form (active) and a cyst form (latent), which resists in hostile environmental conditions such as nutrient depletion, osmotic stress, temperature changes, or pH changes (22). Among FLAs, *Acanthamoeba castellanii* is a commonly species found as a saprophyte in the ear, nose and throat sphere in humans (23), and can also lead to serious corneal infection, primarily in contact lens wearers (24). *Vermamoeba vermiformis*, another FLA species, has only rarely been isolated in human pathology. However, it has been frequently found in natural aquatic environments or recreational waters such as swimming pools (25) and even in bottled mineral water (26). *V. vermiformis* has been more frequently isolated than *Acanthamoeba spp.* from hospital water (27).

It is recognized that FLA can interact with a variety of microorganisms including bacteria, viruses, and fungi (28). This association, when involving human pathogens, can have a significant health impact. For example, many dental procedures produce aerosols that may be contaminated by microorganisms hosted by FLAs forming biofilms in dental unit waterlines (DUWL) (32,33). Some case reports found in the literature have highlighted a link between contaminated DUWL exposure and infection (30–32). In the same way, *V. vermiformis* and *A. castellanii* may serve as hosts for pathogenic bacteria such as *Legionella pneumophila* (33).

Infected FLAs are found in hospital water networks leading to protection of this bacterium from water treatment, and may lead to *L. pneumophila* outbreaks (34,35). Another example is the relationships between FLAs and *C. albicans* which have previously been reported with *V. vermiformis* (36) and *A. castellanii* (37).

Due to the capacity of FLAs to promote the growth of microorganisms and interact with other *Candida* species, we have hypothesized that FLAs can lead to the proliferation and survival of *C. auris* in environmental and hospital water. The aim of this study was then to investigate *in vitro* interactions in water between *C. auris* and FLAs, and to compare them with interactions between these protists and the nosocomial yeast *C. albicans*.

Materials and methods

Strains and cultures conditions

The free living-amoeba (FLAs) used in this study were *Acanthamoeba castellanii* (ATCC 30234) and *Vermamoeba vermiformis* (ATCC 50256). FLAs were grown in 150 cm^2 tissue culture flasks at 27°C, in PYNFH broth for *V. vermiformis* and in PYG medium for *A. castellanii*, as previously described (38). When cells formed a monolayer, two washes were performed with filtered (0.22 µm) hospital tap water. Trophozoites were then harvested in 15 mL of this tap water and diluted for a final concentration of 5 x 10^5 trophozoites/ml.

The yeast strains used in this study were *Candida auris* (clinical strain, kindly provided by Dr E. Bailly, Tours Hospital, France) and *Candida albicans* (ATCC 14053). Yeasts were grown on Sabouraud agar at 37°C, for 24 h, then diluted in filtered hospital tap water to final sample concentration of 1.5 x 10^5 yeasts/mL.

FLA supernatants

A. castellanii and *V. vermiformis* were incubated in 150 cm^2 culture flasks containing 10 mL of hospital-filtered tap water. After 3 days at 27°C, the incubation medium was centrifuged (10 min, 800 g). Resulting supernatant was used for experiments.

Cocultivation of FLA and yeasts

100 µL of FLAs at 5 x 10^5 trophozoites/ml were distributed in a 96-well microplate and incubated for 2 hours at 37°C to promote adherence. Then, 100 µL of yeast suspension (1.5 x 10^5 yeasts/mL) was added leading to multiplicity of infection (MOI) of 0.3, and incubation was performed at 37 ° C.

At 48 h, 120 h and 168 h, FLAs viable trophozoites number was evaluated using Trypan Blue with KOVA hemocytometer.

After 48 h, 120 h and 168 h of incubation, serial dilutions of the cocultures were plated on CAN2 chromogenic medium (bioMérieux, Lyon, France) and incubated at 37°C during 48h

to evaluate the yeasts' colony-forming units (CFU). Controls were carried out by incubating yeasts in filtered tap water without FLA.

Experiments were performed for four combinations: *A. castellanii* and *C. albicans*, *A. castellanii* and *C. auris*, *V. vermiformis* and *C. albicans*, and *V. vermiformis* and *C. auris*. They were carried out in two independent experiments in triplicate.

Cocultivation of FLA supernatant and yeasts

In order to study indirect interactions between FLA and *Candida spp.*, the same experiments as those previously described were performed, but the 100 µL of FLA trophozoites were replaced by 100 µL of FLA supernatants. The two experiments were performed independently in triplicate.

Transmission electron microscopy analysis

FLA were cultured in 150 cm² tissue culture flasks at 27°C as previously described. When cells formed a monolayer, two washes were performed with hospital filtered tap water. Yeasts were then added to obtain a concentration of 1.5×10^5 yeast/mL.

After 48 h of incubation at 37°C, co-culture samples were centrifuged (5 min, 500g), supernatant was removed and pellet was fixed for 1 h with 2.5% glutaraldehyde in 1 mol/L phosphate buffer, pH 7.1. After phosphate buffered saline washes, pellet was post-fixed for 45 min in 1% osmium tetraoxyde in phosphate buffer. Dehydration was carried out using successive incubations of increasing ethanol concentrations (from 70 to 100%). Pellet was then suspended in 100% ethanol and centrifuged at 10,000 rpm for 10 min. Pellet was included in epon epoxy resin and after 24 h of polymerization, 70 nm sections were performed using an ultramicrotome UC6 (Leica). Uranyl acetate (2% in 70% ethanol) and lead citrate were used as contrasting agents for electron microscopy (JEOL 1010 at 80 kV). TEM was recorded using Quemesa camera with iTem® software (Olympus).

Statistical analysis

For statistical analysis, non-parametrical non-matching test of Mann-Whitney and Kruskal-Wallis, and non-parametrical matching test of Wilcoxon were used. They were carried out using GraphPad Prism® 8.0.1 software.

Results

Presence of *C. auris* does not affect the number of FLA trophozoites.

The number of viable amoebae trophozoites was evaluated at 48 h, 120 h and 168 h. For *A. castellanii* (Figure 1), the number of trophozoites was not significantly different between wells containing trophozoites alone and wells containing cocultures of trophozoites and *C. auris* or trophozoites and *C. albicans* ($p = 0.847$). Compared to the initial inoculum, a significant decrease of trophozoites was evidenced for all conditions ($p < 0.05$).

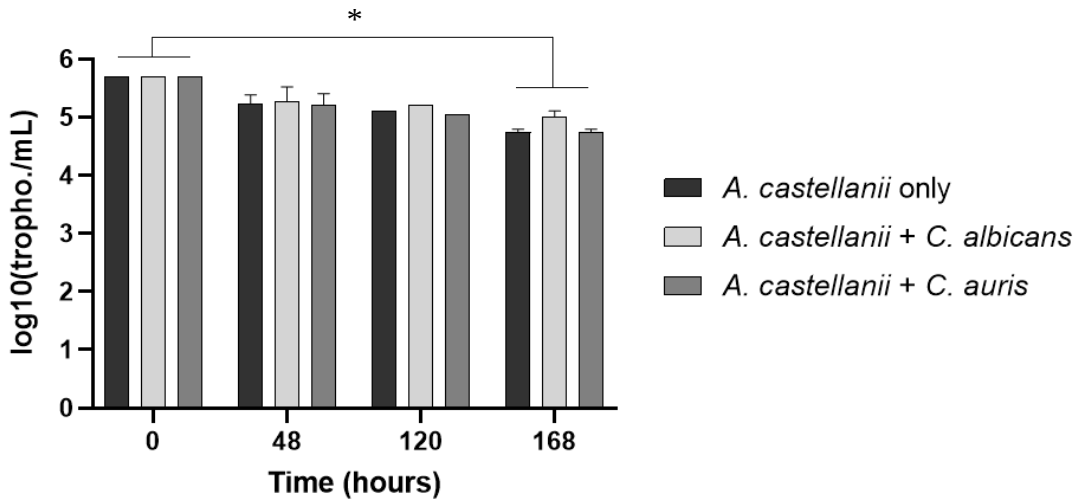

Figure 1: Viability of *Acanthamoeba castellanii* trophozoites alone and in co-cultivation with *Candida albicans* or *Candida auris*. *A. castellanii* trophozoites (5 x 10^5 trophozoites/mL) were incubated alone, with *C. albicans* (1.5 x 10^5 CFU/mL) or *C. auris* (1.5 x 10^5 CFU/mL) in tap-water at 37°C. At 48 h, 120 h and 168 h, *A. castellanii* trophozoites number was evaluated. *tropho.: trophozoites.*

The same results were highlighted for *V. vermiformis* (Figure 2), there was no significant difference between the three conditions (trophozoites alone, trophozoites and *C. auris*, trophozoites and *C. albicans)* ($p = 0.888$) and a significant decrease was evidenced compared to initial inoculum ($p < 0.05$).

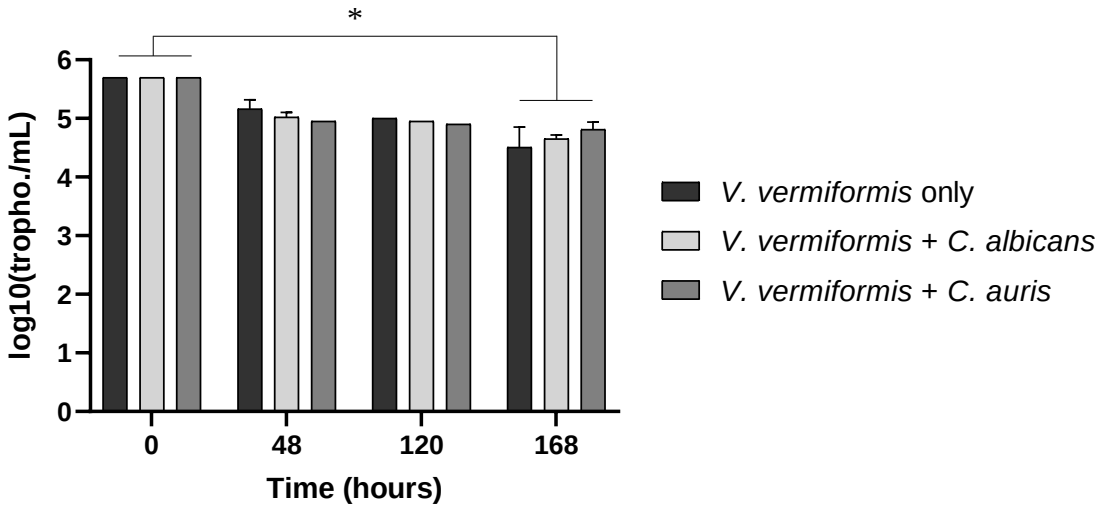

Figure 2: Viability of *Vermamoeba vermiformis* trophozoites alone and in co-cultivation with *Candida albicans* or *Candida auris*. *V. vermiformis* trophozoites (5 x 10^5 trophozoites/mL) were incubated alone, with *C. albicans* (1.5 x 10^5 CFU/mL) or *C. auris* (1.5 x 10^5 CFU/mL) in tap-water at 37°C. At 48 h, 120 h and 168 h, *V. vermiformis* trophozoites number was evaluated. *tropho.: trophozoites.*

C. albicans survival is differently impacted according to FLA species.

C. albicans was not able to grow in filtered water without FLA or FLA supernatants (Figure 3).

When this yeast was incubated with *A. castellanii* trophozoites, the number of fungal CFUs was very low with 47 CFU/mL at 48 h and 120 h and no fungal growth recovered after 168 h of cocultivation (Figure 3). However, after incubation in *A. castellanii* supernatant, the number of CFUs was significantly higher ($p < 0.05$), with 3 x 10^3 CFU/mL at 48h, 1.3 x 10^5 CFU/mL at 120h and 1.5 x 10^5 CFU/mL at 168h (Figure 3), with significant increased growth between 48 h and 120 h ($p < 0.05$).

In contrast, when *C. albicans* was incubated with *V. vermiformis* trophozoites, a high number of CFUs was reported with 3 x 10^4 CFU/mL, 2 x 10^5 CFU/mL, and 10^6 CFU/mL at 48 h, 120 h and 168 h respectively. The number of fungal CFUs was also high in the presence of *V. vermiformis* supernatant with 7 x 10^3 CFU/mL, 6 x 10^4 CFU/mL and 10^5 CFU/mL at 48h, 120h and 168h respectively. Significant growth was highlighted between 48 h and 120 h with FLA trophozoites ($p < 0.05$) or FLA supernatant ($p < 0.05$).

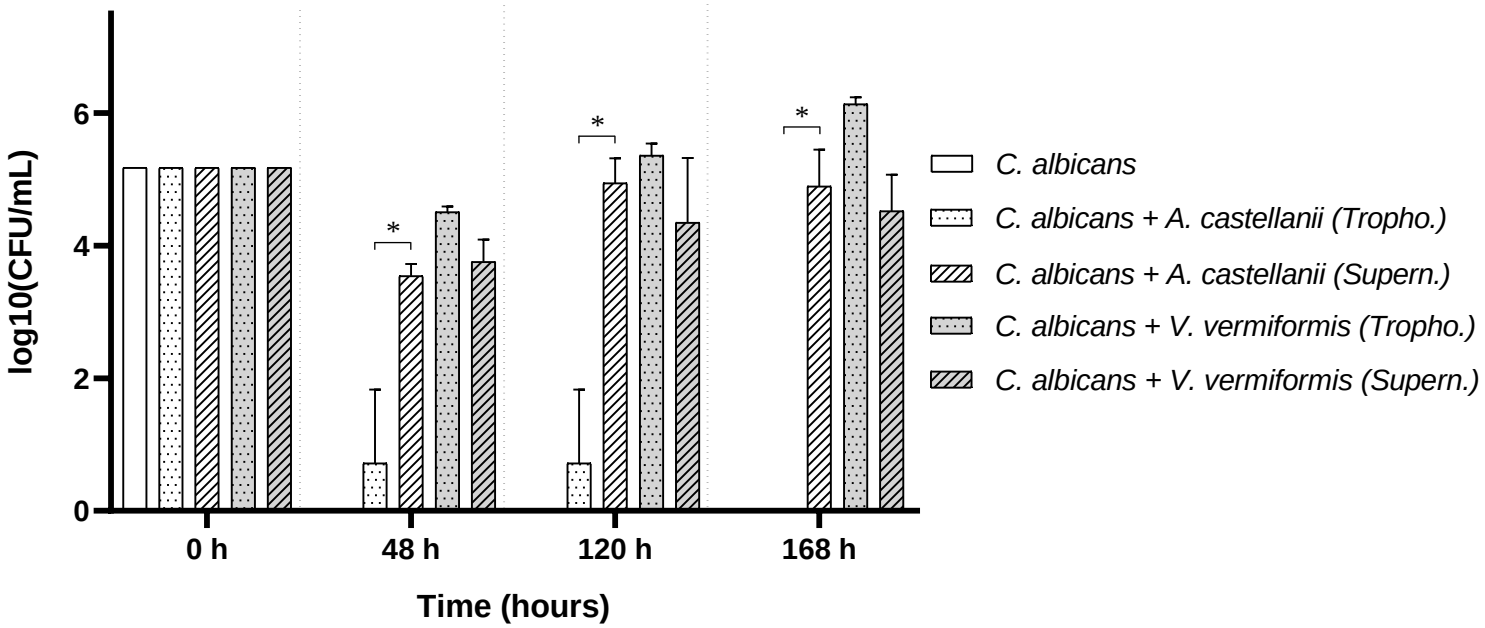

Figure 3: Number of *Candida albicans* CFUs in co-cultivation with FLA trophozoites or FLA supernatant. *C. albicans* (1.5 x 10^5 CFU/mL) was incubated with *A. castellanii* or *V. vermiformis* trophozoites (5 x 10^5 CFU/mL) or *A. castellanii* or *V. vermiformis* supernatant in tap-water at 37°C. At 48 h, 120 h and 168 h cocultures were plated and incubated at 37°C during 48 h to evaluate yeasts' CFUs. Experiment was performed in two independent experiments in triplicate. *Tropho.: trophozoites ; Supern.: supernatant. * $p < 0.05$*

FLA supernatants and trophozoites promote *C. auris* survival and proliferation.

As for *C. albicans*, *C. auris* was not able to grow in filtered water without FLA or FLA supernatants (Figure 4).

When *C. auris* was incubated with *A. castellanii* trophozoites, a high number of CFUs was reported with 2 x 10^4 CFU/mL, 2 x 10^5 CFU/mL and 10^5 CFU/mL at 48 h, 120 h and 168 h respectively (Figure 4). When *C. auris* was incubated with *A. castellanii* supernatant, the same behavior was observed with 2 x 10^5 CFU/mL, 7 x 10^5 CFU/mL and 3 x 10^5 CFU/mL at 48 h, 120 h and 168 h respectively (Figure 4). Moreover, significant growth was highlighted between 48 h and 120 h with FLA trophozoites ($p < 0.05$) or FLA supernatant ($p < 0.05$).

When *C. auris* was incubated with *V. vermiformis* trophozoites, the number of CFUs was 3 x 10^5 CFU/mL, 2 x 10^6 CFU/mL and 1 x 10^7 CFU/mL at 48 h, 120 h and 168 h respectively (Figure 4). When *C. auris* was incubated with *V. vermiformis* supernatant, the same behavior was observed with 10^5 CFU/mL, 7 x 10^6 CFU/mL and 3 x 10^6 CFU/mL at 48 h, 120 h and 168 h respectively. As for *A. castellanii*, significant growth was highlighted between 48 h and 120 h with FLA trophozoite ($p < 0.05$) or FLA supernatant ($p < 0.05$).

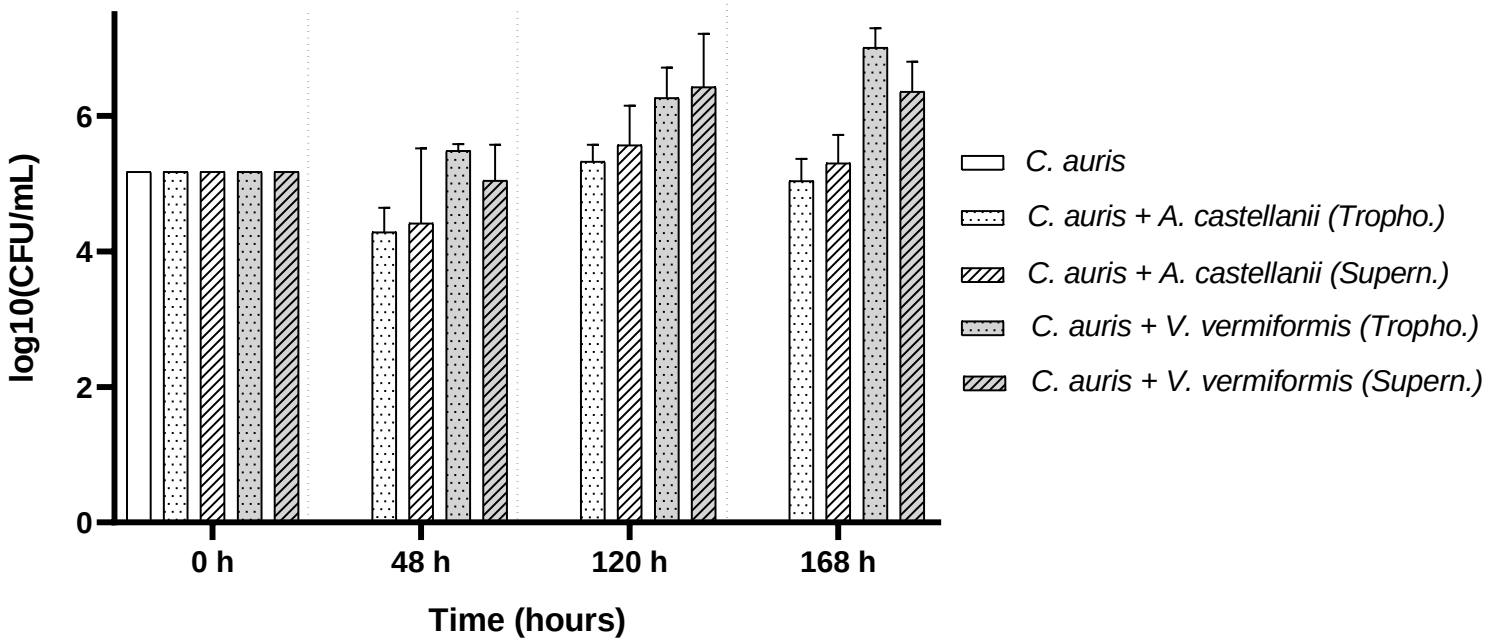

Figure 4: Number of *Candida auris* CFUs in co-cultivation with FLA trophozoites or FLA supernatant. *C. auris* (1.5×10^5 CFU/mL) was incubated with *A. castellanii* or *V. vermiformis* trophozoites (5×10^5 CFU/mL) or *A. castellanii* or *V. vermiformis* supernatant in tap-water at 37°C. At 48 h, 120 h and 168 h cocultures were plated and incubated at 37°C during 48 h to evaluate yeast' CFUs. Experiment was performed in two independent experiments in triplicate. *Tropho.: trophozoites ; Supern.: supernatant.*

***C. auris* is found internalized and undigested in FLA trophozoites.**

In order to visualize interaction between *C. auris* and *V. vermiformis* or *A. castellanii*, co-cultivations were incubated for 2 days at 37°C and TEM was performed. *C. auris* was found internalized in both amoeba species (Figure 5). Most of blastospores were found whole and undigested in phagocytosis vacuoles (Figure 5A, 5B and 5C). No digestion process was highlighted and, some FLA contained several yeasts (Figure 5 A and 5B). Pseudopodia were visible on some pictures (Figure 5D).

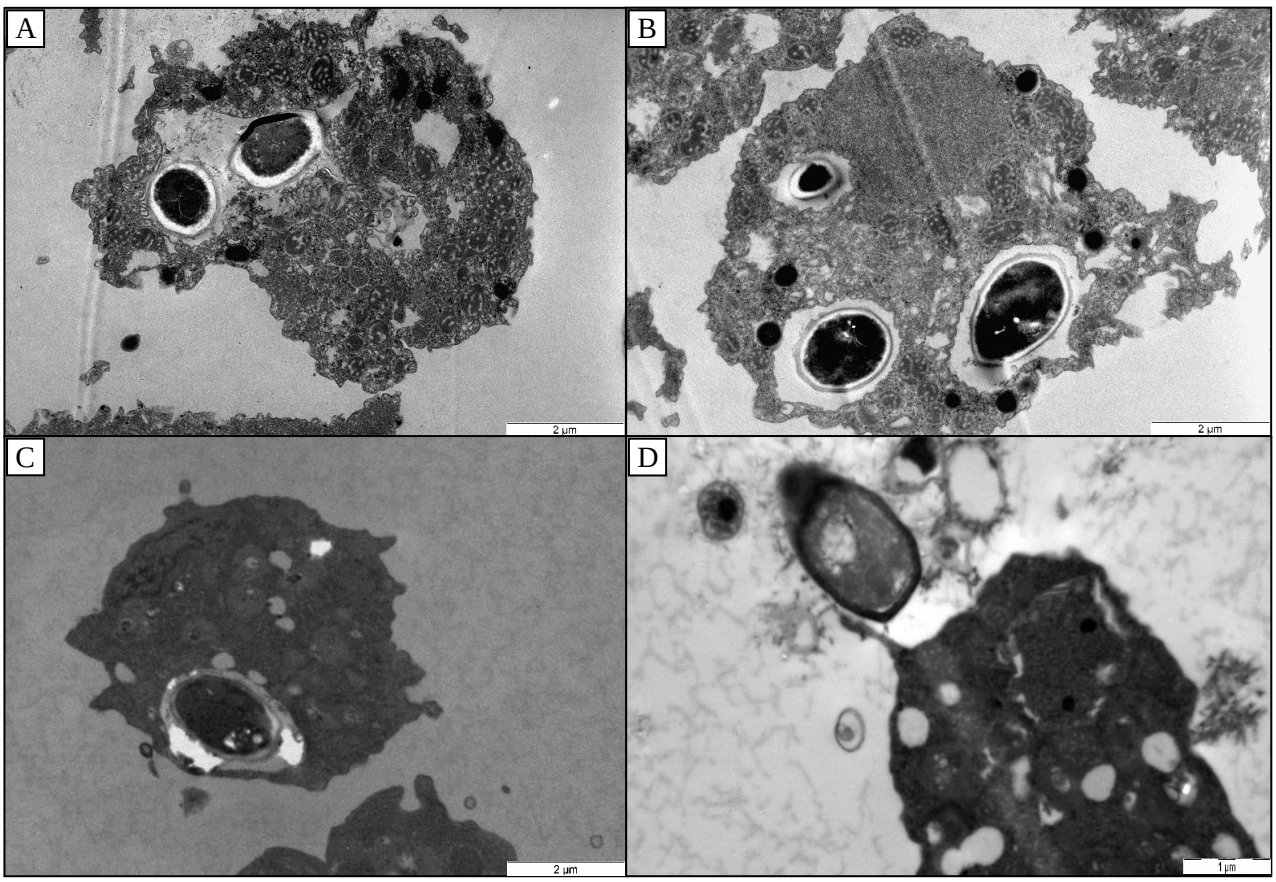

Figure 5: Transmission electron microscopy of *Candida auris* in cocultivation with FLA trophozoïtes. *C. auris* (1.5 x 10^5 CFU/mL) was incubated with *A. castellanii* trophozoites (A, B) or *V. vermiformis* trophozoites (C, D) at 37°C. After 48 h, coculture samples were fixed for MET analysis.

Discussion

Elucidation of *C. auris* reservoir, a new multi-resistant pathogen producing severe infection, may help to prevent dissemination. We have hypothesized that *C. auris* could survive and proliferate in water through interactions with FLAs. In this study, we conducted experiments to investigate interactions between two species of FLA and *C. auris*.

The number of amoeba trophozoites was evaluated at different times of cocultivation to determine the impact of *Candida spp.* on FLA trophozoites (Figures 1 and 2). For both species of FLA, there were no significant differences between groups. The number of amoeba trophozoites was similar between trophozoites alone and trophozoites in cocultures with *C. albicans* or *C. auris*. It would seem that, unlike some bacteria (39,40), these yeasts did not present an adverse effect on FLA. Interactions in the environment could consequently be possible since it is not deleterious to amoebae. Compared to the starting inoculum, 29% of amoeba trophozoites were still present at 48 h and about 12% at 168 hours. The significant overall decrease could be related to the lack of nutrients in the wells in our experimental conditions. However, due to the large number of amoeba trophozoites still present at 168 h, interactions with yeasts remained possible.

In our experimental conditions, *C. albicans* and *C. auris* seemed not able to survive in filtered (0.22 µm) hospital tap water at 37°C. For *C. albicans,* this was previously demonstrated, in the study conducted by Barbot *et al.* in 2012 (36). The authors showed that filtered tap water is not an appropriate environment. The same behavior was observed with *C. auris* in our study. Filtered tap water is a poor nutrient medium and may not be conductive to *Candida* planktonic culture proliferation. However, some *Candida* species, especially *C. auris*, have the ability to produce biofilm on different types of surfaces (41,42). It is therefore not excluded that even without FLA, *C. auris* was able to resist on surfaces, in biofilm condition, in contact with water under real conditions.

Our results show that supernatants of both FLA species leads to survival and proliferation of *C. albicans* and *C. auris*. This same behavior has been found for several other microorganisms such as *Salmonella enterica serovar Typhi* (43), *Acinetobacter baumanii* (44), *Mycobacterium Smegmatis* (45) or *Fusarium oxysporum* (46). FLAs secrete nutrients that could be used by microorganisms for their growth. Gonçalves D. *et al.* have shown that FLAs secrete extracellular vesicles containing free fatty acids (47). It is known that *Candida* species can use

fatty acids as a source of carbon (48,49). The lysis of these extracellular vesicles could enrich the medium and be a source of nutrients.

As regards co-culture experiments, behavior of *C. albicans* is consistent with what has already been described. Steenbergen *et al.* (37) observed that with an initial inoculum of 10^5 *C. albicans*/mL co-cultured with *A. castellanii*, no yeasts survived for 48 hours. In our experiment, when *C. albicans* was co-cultured with *A. castellanii* trophozoites, there was also a rapid decrease in the number of CFU/mL, and no yeasts were found at 168 h. However, with *A. castellanii* supernatant, *C. albicans* was able to survive and proliferate. This data support the ability of *A. castellanii* to phagocyte and digest *C. albicans*. Phagocytosis has been recognized as an important feeding mechanism for FLA (50,51). *A. castellanii* feeds on various microorganisms such as bacteria (52) or yeasts such as *Saccharomyces cerevisiae* (37). Yeast walls are mainly composed of mannose polymers (53). Due to mannose receptors, FLAs are able to recognize and trigger yeast phagocytosis (54). However, phagolysosome effectiveness in destroying yeast could differ between two species of FLA. In our study, when *C. albicans* is co-cultured with *V. vermiformis* trophozoites, no yeast decrease occurred. Barbot *et al.* (36), also observed by TEM that *C. albicans* was internalized unaltered in *V. vermiformis* trophozoites. *V. vermiformis* seemed not able to digest *C. albicans*. It has been noted that *Acanthamoeba* genus present relatively simple nutritional requirements and can be grown in a wide variety of media, unlike the genus *Vermamoeba*, which has more complex nutritional requirements (55). Capacity of microorganism digestion of this two FLA species may be different and could explain the difference in *Candida* phagocytosis. For example, N-acetyl muramidase, involved in bacteriolysis, differs between the two FLA species (56).

In contrast to *C. albicans*, in cocultures with *A. castellanii* or *V. vermiformis*, *C. auris* could survive up to 168 h, even though internalization was observed in both FLA species (Figure 5). There was significant proliferation between 48 h and 120 h of incubation under each condition. Thus, unlike *C. albicans*, *C. auris* presented some capacity to resist destruction by both FLA species. It could be one of the microorganisms, such as several bacteria, which have evolved as to be resistant to FLA predation. These bacteria are collectively referred to as amoeba-resistant bacteria (28). For *Cryptococcus neoformans*, another yeast, normally destroyed by *Acanthamoeba polyphaga* (57), several strains have evolved to resist destruction by FLA (58). Steenbergen *et al.* showed that *C. neoformans* developed a capacity for non-lytic exocytosis by modification of their capsule (59,60). Another example is *L. monocytogenes*,

which, in order to disrupt the phagosomal membrane of phagocytic host cells, secretes listeriolysin and two phospholipases C (61). This bacteria can thereby survive predation by *A. castellanii* (62). *Mycobacterium llatzerense*, a non-tuberculous environmental mycobacterium remains viable and capable of growing by inhibiting phagosomal acidification despite internalization into *A. castellanii* by phagocytosis (63). Observations by Samba-Louaka *et al.* in 2018 (64), suggested that *Mycobacterium avium* could also block the acidification process of the phagosomes of *A. castellanii*. *C. auris* could employ similar resistance mechanisms to escape phagocytic destruction by FLA. This is corroborated by TEM that show the internalization of intact yeasts in the vacuoles of the two FLA species (Figure 5).

In the environment, FLAs are found mainly in water biofilms. Due to beneficial interaction with several bacteria, they act as reservoirs for these pathogens resulting in protection against hostile conditions and promoting bacterial spread in the environment (28,65,66). The mechanism could be the same for *C. auris*. In this case, the external environment where FLAs are present, especially water, could represent an ecological niche for *C. auris*. Moreover, FLA in hospital water systems raises the question of a potential infectious source. Management of hot water contamination systems is a major issue in the control of public health risks. FLA leads to major problems of physical and chemical resistance to the treatments used in water networks, whether with *Acanthamoeba sp.* (67,68) or *Vermamoeba sp.* (69). *C. auris* could benefit from physical protection and colonize water systems.

FLAs can increase internalized microorganisms' virulence, one example being *L. pneumophilia*. In 1980, Rowbotham *et al.* published the first report on the intracellular multiplication of *L. pneumophila* in *Acanthamoeba spp*. and *Naegleria spp*. (70). They showed that intracellular growth within protozoa led to *L. pneumophila* being more able to infect human monocytes (71), particularly by inducing phenotypic modulation (72). These strains were also more resistant to chemical disinfectants, biocides and antibiotics (73,74). Steenbergen JN *et al*. showed that FLA could play a key role in the selection and maintenance of fungal virulence (37). Selection of virulent strains could consequently apply to *C. auris* in the environment, due to the positive interaction with FLA.

In conclusion, we showed that there are positive interactions between *C. auris* and FLA. This is particularly beneficial to *C. auris*, which is able to survive and proliferate in poor environments, probably thanks to diffusible molecules secreted by FLA. There is also physical interaction with internalization of the yeast within the amoeba that could protect it. *A. castellanii*

and *V. vermiformis* being frequently found in outdoor environments and hospital water systems (19,27), hospital tap water could therefore represent a source of contamination with *C. auris* through FLA protection, which would necessitate increased monitoring of the hospital water destined to immunocompromised patients. Fungal and protozoal behaviors could be studied under other conditions (temperature, disinfectants) to continue to explore the presence of *C. auris* in hospital environments.

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Résumé

Les levures du genre *Candida* sont responsables d'infections graves chez les patients immunodéprimés. Parmi celles-ci, *Candida auris* est une espèce émergente découverte en 2009 ayant causé plusieurs épidémies dans le monde. Sa capacité à devenir résistante aux différentes classes d'antifongiques systémiques, les difficultés liées à son identification et son aptitude à persister en milieu hospitalier en font un problème de santé publique. Actuellement, les données sur le réservoir environnemental de *C. auris* sont limitées alors qu'elles sont essentielles afin de contrôler sa propagation. Le but de notre étude était d'explorer les interactions entre *C. auris* et deux espèces d'amibes libres, potentiellement présentes dans le même environnement hydrique.

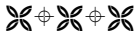
C. auris a été incubé à 37°C avec des trophozoïtes d'*Acanthamoeba castellanii* ou de *Vermamoeba vermiformis,* ou avec leurs surnageants de culture, dans de l'eau filtrée issue du réseau hospitalier. Le nombre de trophozoïtes d'amibes et de levures a été déterminé à 48 h, 120 h et 168 h de coculture. De la microscopie électronique à transmission (MET) a été effectuée à 48 h de coculture. Afin de comparer les résultats avec une autre espèce de *Candida,* les mêmes expériences de coculture ont été réalisées avec *Candida albicans.*

Alors que *C. albicans* et *C. auris* n'ont pas pu survivre seules dans l'eau, les surnageants des deux espèces d'amibes libres ont favorisé la survie et la prolifération des levures. *C. albicans* a été détruit par les trophozoïtes d'*A. castellanii* mais a survécu en présence des trophozoïtes de *V. vermiformis*. Contrairement à *C. albicans, C. auris* a pu survivre et proliférer au contact des trophozoïtes d'*A. castellanii* ou de *V. vermiformis*. L'internalisation de *C. auris* au sein des deux espèces d'amibes libres a également été observée par MET.

Un réservoir environnemental hydrique de *C. auris* peut donc être envisagé en lien avec les amibes libres. De plus, du fait de la présence de ces amibes dans les réseaux d'eau hospitaliers, leur contamination par *C. auris* serait possible.

Mots-clés: *Candida auris* ; amibes libres ; réseaux d'eau hospitaliers ; épidémies

SERMENT



En présence des Maîtres de cette école, de mes chers condisciples et devant l'effigie d'Hippocrate, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité dans l'exercice de la médecine. Je donnerai mes soins gratuits à l'indigent et n'exigerai jamais un salaire au-dessus de mon travail. Admis dans l'intérieur des maisons mes yeux ne verront pas ce qui s'y passe ; ma langue taira les secrets qui me seront confiés, et mon état ne servira pas à corrompre les mœurs ni à favoriser le crime. Respectueux et reconnaissant envers mes Maîtres, je rendrai à leurs enfants l'instruction que j'ai reçue de leurs pères.

Que les hommes m'accordent leur estime si je suis fidèle à mes promesses ! Que je sois couvert d'opprobre et méprisé de mes confrères si j'y manque !