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Biofilms of *Candida glabrata* Could Be the Shelter for Diplococci Against Antibacterial Agents

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Candida glabrata is emerging yeast in hospitals. This specie can contaminate catheters on which is able to form biofilms. These structures are, for other bacterial species, considered as places where they can be protected of antibiotic treatments. Microbial biofilms are formed of a single or mixed species which include a set of bacteria and/or fungi. Several studies have revealed the coexistence of bacterial species and *Candida spp*. within a biofilm. The aim of this study was to highlight the cohabitation, synergistic or antagonistic effects, between *Candida glabrata* which form the biofilm and *Diplococcus spp*. which does not have that ability. According to the results, *Diplococcus spp*. was sensitive to chloramphenicol, but within biofilm of *C. glabrata* where it lived in cohabitation, it curiously escaped the same antibiotic.

Introduction

Though intravascular catheters provide necessary vascular access, their use puts patients at local and systemic infectious complications with an increased risk of fungal colonization [1]. Some opportunistic pathogenic yeast like *Candida* spp. is responsible for systemic fungal infections [2, 3]. Indeed, the frequency of invasive candidiasis has been growing steadily in the hospital thereby confirming the results of fungal infectivities of the catheters [3, 4].

Candida spp. has the ability to adhere to the catheter and form biofilms; these structures constitute a permanent source of the infectious agent and frequently escape the antifungal treatment and immune defenses of the host [5, 6]. According to some authors, under some conditions, there may be a synergistic effect among *Candida* species and bacteria which are resistant to antibiotics in mixed biofilms [7, 8]. These studies showed synergistic or antagonistic effects between *Candida spp*. and bacteria that have all the ability to form biofilms which partly explains some therapeutic failures.

Main Body

Among the samples taken in the general surgery department of the hospital of Maghnia - Algeria, one catheter was removed

after an exposure time of 4 d from a female patient aged 56 y, who has underwent abdominal surgery and suffered from meningitis. The antibiotic treatment administered to this patient was chloramphenicol.

Two culture media were used for cultivation of the species; Sabouraud agar containing 0,5 mg/mL chloramphenicol (pH 5, 7) and nutrient broth (pH 7, 2) were used.

To observe biofilm structure, the catheter was cut into two pieces; the first was preserved with glutaraldehyde fixative (Sigma Chemical Co., St. Louis, Mo.), the second was agitated one minute at the vortex [10] to detach biofilms formed on the surface which were then fixed with glutaraldéhyde. The samples were sent to "Dennis Kunkel Microscopy, Inc." for scanning electron microscopy (SEM).

Finally, the ability to form biofilms was assessed, *In Vitro*, by crystal violet to each one of the isolated strains according to the method of O'Toole (2011) [11]. Briefly, to spread the planktonic cells and/or non-adherent cells, the medium was aspirated and the wells of the microplate were washed 3 times with sterile PBS (0,1 M - pH 7, 4). 100µL of methanol were added to the wells and incubated for 15 min at room temperature; thereafter, wells were washed and 100µL of crystal violet were added and incubated for 20 min at room temperature. After that, 150 µL of acetic acid (33%) were added to each well. The optical density was measured at 570 nm using a microplate reader (Biotek, ELx800).

The identification of strains isolated from the colonies formed on the agar containing chloramphenicol revealed only *C. glabrata*. From nutrient broth, two species were identified, *C. glabrata* and *Diplococcus* spp; curiously, this result demonstrates the diplococci that were isolated from the patient to whom chloramphenicol was administered, were sensitive to the same antibiotic added to Sabouraud agar medium.

However, the SEM images revealed a mixed biofilm composed of cells of *C. glabrata* and dipolococci (Fig. 1). In addition, the *In Vitro* biofilm formation was demonstrated only for the fungal specie (*C. glabrata*). This means that these bacteria show no ability to form biofilms. These results may partly explain the therapeutic failure in the treatment of systemic infections caused by biofilms.

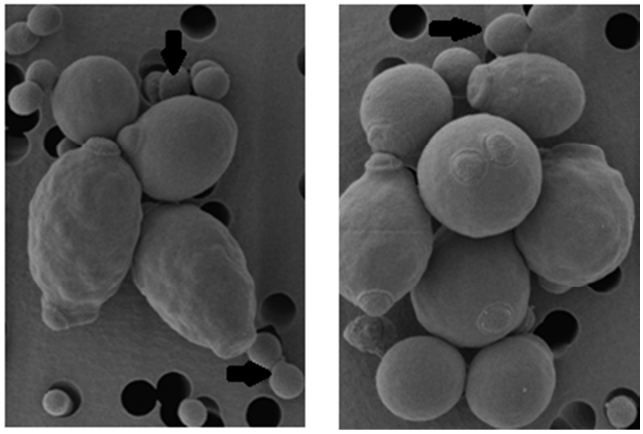

Fig. 1. *Detached cells from mixed biofilm formed on the surface of a catheter removed from a female patient in General Surgery; arrows indicate diplococci (Magnification ×10000).*

The use of catheters has considerably increased for diagnostic and/or therapeutic acts; however catheters related biofilms infection are a public health problem due to their resistance to antimicrobial agents and the host immune defenses. The results of this research were unexpected; they were in both new and extremely surprising.

According Chandra et al. (2008) [12], the biofilm is the most widespread form of microbial life in the nature; it may be formed by a single microbial species or a mixture of bacterial and/or fungi species. Furthermore, nosocomial infections can occur at any time during the use of catheters which are liable to be altered by biofilms [13].

On the other hand, biofilms are a major problem because the dose required to eradicate them is higher than the therapeutic doses [14]. Therefore, the biofilms associated infections are difficult to treat and to eradicate with standard treatment regimens [15].

Antimicrobial resistance has been observed in several species forming mixed biofilms; fungal cells may modulate the action of antibacterial agents [8]. However, synergy is not the only form of interaction between these germs, the antagonism effect between *Pseudomonas aeruginosa* and *Candida* spp. was demonstrated [16]. The mechanisms by which bacterial and yeast species coexist in harmony in mixed biofilms requires a careful balance that switches synergy to antagonism according to the

surrounding conditions [17].

Conclusion

Catheters are often contaminated by microorganisms such as *Candida* and bacteria like diplococci species and this can lead to the formation of biofilms. According to this study, *Diplococcus* spp. did not have the ability to form biofilm and was sensitive to chloramphenicol, but within biofilm of *Candida glabrata* where it lived in cohabitation, it curiously escaped the same antibiotic. *C. glabrata* biofilm seems to be a shelter for such bacteria.

As a conclusion, the ability to form biofilms seem to be not necessary for diplococci species as they can cohabit with *C. glabrata* biofilms where are protected against antibiotics treatment. Consequently, the seriousness of this type of cohabitation could be observed in unexplained therapeutic failures.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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