

Fungal Contamination of Small Surgical Instruments at the Sterilization Units of a Tertiary University Hospital in Abidjan, Côte d'Ivoire, West Africa

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Background: Reusable surgical equipment can be a source of healthcare-associated infections. In Côte d'Ivoire, West Africa, data on fungal contamination of small surgical instruments are limited.

Objective: To assess fungal carriage in small surgical instruments at a university hospital in Angré, Côte d'Ivoire.

Methods: This cross-sectional study was conducted using small surgical instruments in six departments of a tertiary university hospital in Angré from February to July 2024. Samples were collected by swabbing instruments before sterilization and after opening random sterile sets. Direct examination and culture on Sabouraud dextrose agar medium containing chloramphenicol were performed. Fungal spores and hyphae were identified macroscopically and microscopically, whereas yeasts were identified using chromogenic medium and VITEK 2.

Results: Of 313 samples, 70 yielded positive culture (22.4%). Fungi on surgical instruments were identified most frequently at the ear, nose and throat service (34.8%), followed by the dental service (30.4%) and intensive care unit (28.6%). The most frequently isolated mold species was *Aspergillus niger* (47.2%), followed by *Aspergillus flavus* (30.6%), *Mucor* spp. (21.3%), and *Aspergillus fumigatus* (0.9%). Among the yeasts, the most frequently identified was *Candida albicans* (50%), followed by *Candida krusei* (25%) and *Candida parapsilosis* (25%). Fungi identification was more frequent before sterilization (64.3%) than after opening sterile sets (35.7%).

Conclusion: This study revealed the presence of fungi on small surgical instruments before sterilization and after opening random sterile sets. Implementing advanced environmental control, improving sterilization steps, and monitoring may help reduce nosocomial fungal infections from surgical instruments.

Key Words: *Aspergillus* spp., *Candida* spp., Fungal carriage, Surgical instruments

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INTRODUCTION

The hospital environment being an ecological niche for microorganisms can be a public health concern. Contamination varies among healthcare centers and is related to services, patients, healthcare, and pathogen virulence¹. Nosocomial infections can increase hospital morbidity and mortality rates and become burdensome, especially in high-risk departments, for patients who are extremely vulnerable to contamination^{2,3}.

Hospitals offer an ecosystem conducive to the spread of microorganisms, particularly microscopic fungi, which pose a threat to immunocompromised patients^{4,5} and can be a challenge for clinicians and microbiologists. Moreover, reusable surgical instruments are a potential source of healthcare-associated infections⁶. Decreased risk of surgical instrument-related infections depends on washing and sterilization procedures. Indeed, staff behavior, poor hygiene, and inadequate training in good hospital hygiene practices can lead to nosocomial fungal infections⁷. Small surgical instruments used in medical care must be free of microorganisms to avoid nosocomial infections⁸. The fungi frequently implicated in nosocomial infections are *Aspergillus* and *Candida* species^{5,9}. Disinfection and sterilization techniques can reduce pathogen contamination¹⁰. Furthermore, an increased incidence of nosocomial fungal infections has been reported worldwide, most likely due to the widespread use of aggressive treatments. The emergence of abundant fungi and variable resistance to antifungal drugs can lead to failure in patient treatment^{7,11}. The effectiveness of control practices depends on the quality of chemical products and compliance with manufacturer's instructions¹². In Côte d'Ivoire, data and knowledge of fungal contamination of small surgical instruments are scarce. This study aimed to assess fungal carriage in small surgical instruments at a tertiary university hospital in Angré, Côte d'Ivoire.

MATERIALS AND METHODS

1. Study design and setting

This descriptive cross-sectional study was carried out for 6 months from February 2024 to July 2024 at a tertiary university hospital, which caters to patients from all municipalities of Abidjan and nearby cities in Angré in Côte d'Ivoire and boasts high-quality technical facilities, modern healthcare infrastructure, and a wide range of services. Samples were collected from small surgical equipment from six services, including gynecology and obstetrics; intensive care unit (ICU); ear, nose, and throat (ENT) service; dental service; operating block;

and surgical emergency, and brought to the parasitology-mycology laboratory for further analyses.

2. Sample collection

Each small surgical instrument, such as scissors, forceps, retractors, and delivery and episiotomy sets, from the sterilization unit of each service was sampled using two sterile swabs. Swabbing was performed during disinfection, particularly during the washing stage. After sterilization, one sterile set from each service was randomly selected; swabbing was performed on selected instruments in a sterile hood to avoid contamination. The method used was pressure steam sterilization, which is commonly used for reusable medical devices and is suitable for medical devices, such as textiles, stainless steel devices, plastics, and glass. Using an enclosed autoclave, steam is produced by boiling water under pressure to raise the temperature to 134°C for 18 min. Steam hydrolyzes bacteria and fungi, while heat and humidity destroy pathogens by coagulation.

3. Mycological analysis

1) Direct examination and isolation

Two sterile swabs were used to obtain samples for direct examination and culture, respectively. After swabbing the sample on a slide, a drop of physiological water was placed before placing a coverslip for direct examination of fungal elements using light microscopy. Culture was performed on Sabouraud dextrose agar (SDA) medium with chloramphenicol and incubated at 37°C for 2–5 days, which was sufficient to determine the level of fungal contamination.

2) Fungal identification

Fungal species were identified macroscopically, microscopically, and by an automatic machine in difficult cases. Yeast colonies were whitish, smooth, hairless, moist, shiny, or matte. Mold colonies were downy, wooly, or cottony and exhibited varying colors, depending on the species. Yeasts were identified using chromogenic medium (Chromagar™ *Candida*) and, if with diagnostic difficulty, VITEK 2 Compact® (Biomerieux), according to the manufacturers' protocols. The molds isolated from positive cultures were identified based on microscopic characteristics on lactophenol blue staining between the slide and coverslip. The *Aspergillus* genus was identified using the conidiophore characteristics of the species with *Aspergillus* conidial heads (uniseriate or biseriate conidial

heads), phialides, and conidia.

4. Data analysis

All statistical analyses were conducted using IBM SPSS Statistics version 21 (Armonk, NY, USA). A patient was considered positive when at least one colony was detected on SDA medium containing chloramphenicol. The variables were analyzed using chi-square and Fisher's exact tests, with

an alpha risk of 5%. A p -value of <0.05 was considered statistically significant.

RESULTS

1. Characteristics of surgical instruments

A total of 313 samples were collected from small surgical

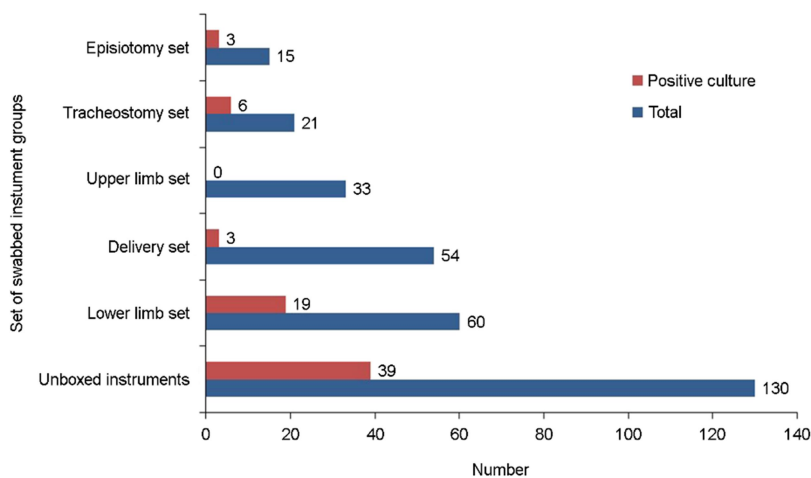


Fig. 1. Distribution of swabbed small surgical instrument sets and positivity rate

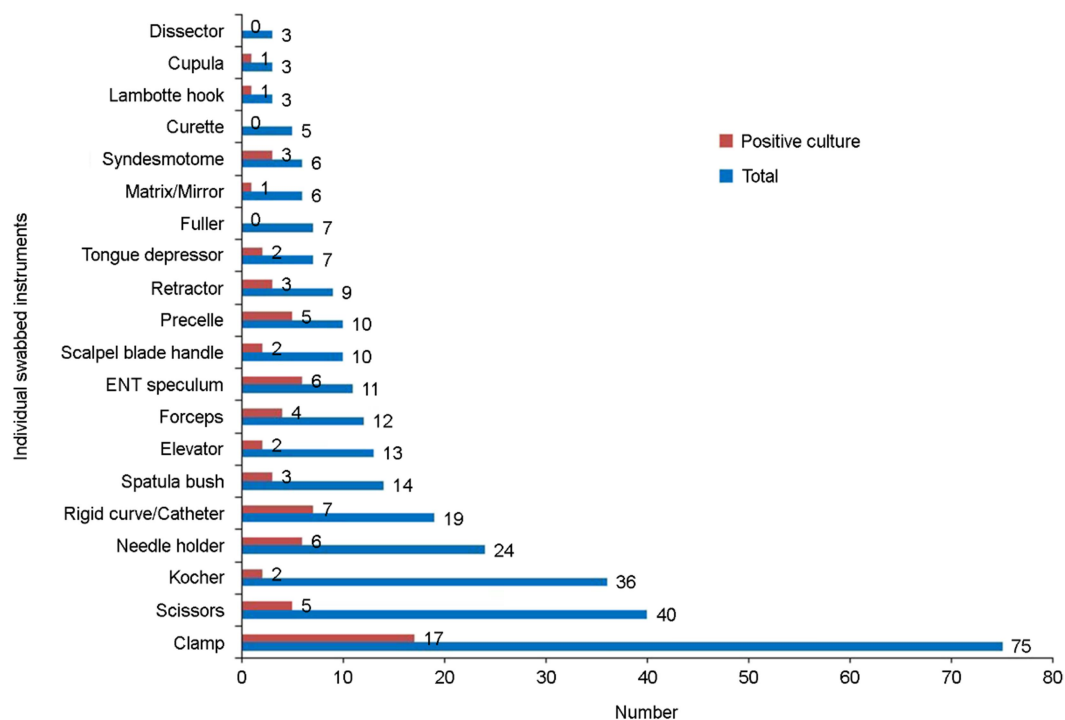


Fig. 2. Distribution of swabbed individual small surgical instruments and positivity rate

instruments in the sterilization units of the services. Samples were unevenly distributed among the services, with the operating block having the largest proportion (n = 93, 29.7%), followed by the dental service (n = 79, 25.2%) and gynecology and obstetrics service (n = 69, 22.0%). The surgical emergency, ENT, and ICU services had the lowest proportions at 8.9%, 7.3%, and 6.7%, respectively. According to timing, samples were collected before sterilization (n = 217, 69.3%) and after opening random sterile sets (n = 96, 30.7%).

Among the small surgical instrument groups, the proportion of collected samples was the highest in the unboxed group (surgical instruments from ENT, dental, and surgical emergency services, n = 130, 41.5%), followed by the lower limb group (n = 60, 19.2%) (Fig. 1). The instruments included in this study were clamps (n = 75, 24.0%); scissors (n = 40, 12.8%); Kocher forceps (n = 36, 11.5%); and needle holders (n = 24, 7.7%) (Fig. 2).

Table 1. Global distribution of fungal species identified among the hospital services

Services	Fungal species	Total n	Before sterilization n (%)	After sterilization n (%)	p-value
Operating block	<i>Aspergillus flavus</i>	11	6 (54.5)	5 (45.5)	0.670
	<i>Aspergillus fumigatus</i>	1	1 (100)	0	–
	<i>Aspergillus niger</i>	13	9 (69.2)	4 (30.8)	0.0499
	<i>Mucor</i> spp.	9	8 (88.9)	1 (11.1)	< 0.001
	<i>Candida albicans</i>	1	1 (100)	0	–
	Total	35	25 (71.4)	10 (28.6)	
Dental service	<i>Aspergillus flavus</i>	10	8 (80.0)	2 (20.0)	0.007
	<i>Aspergillus niger</i>	21	19 (90.5)	2 (9.5)	< 0.001
	<i>Mucor</i> spp.	11	10 (90.9)	1 (9.1)	< 0.001
	<i>Candida krusei</i>	1	1 (100)	0	–
	Total	43	38 (88.4)	5 (11.6)	
Obstetrical gynecology	<i>Aspergillus flavus</i>	1	1 (100)	0	–
	<i>Aspergillus niger</i>	4	3 (75.0)	1 (25.0)	0.160
	<i>Candida parapsilosis</i>	1	1 (100)	0	
	Total	6	5 (83.3)	1 (16.7)	
Ear, nose, and throat	<i>Aspergillus flavus</i>	8	6 (85.7)	2 (14.3)	0.0455
	<i>Aspergillus niger</i>	3	2 (66.7)	1 (33.3)	0.160
	<i>Mucor</i> spp.	3	2 (66.7)	1 (33.3)	0.160
	Total	14	10 (71.4)	4 (28.6)	
Intensive care unit	<i>Aspergillus flavus</i>	2	1 (50.0)	1 (50.0)	1
	<i>Aspergillus niger</i>	5	2 (40.0)	3 (60.0)	0.152
	Total	7	3 (42.9)	4 (57.1)	
Surgical emergency	<i>Aspergillus flavus</i>	2	2 (100.0)	0	–
	<i>Aspergillus niger</i>	5	3 (60.0)	2 (40.0)	0.530
	<i>Candida albicans</i>	1	0	1 (100)	–
	Total	8	5 (62.5)	3 (37.5)	

2. Mycological aspects

Of 313 samples, 70 were culture-positive with an overall colonization rate of 22.4%. Among the positive samples, the sampling timing was before sterilization in 64.3% and after opening random sterile sets in 35.7%. Non-boxed samples had the highest number of positive samples ($p = 0.0008$) (Fig. 1). In total, 70 small surgical instruments showed fungal contamination before sterilization and after opening random sterile sets. The contaminated instruments included clamps ($n = 17$, 22.7%); catheters ($n = 7$, 36.8%); needle holders ($n = 6$, 25.0%); ENT speculums ($n = 6$, 54.5%); and precelle instruments ($n = 5$, 50.0%). No fungi were identified on Fuller forceps, curettes, and dissectors (Fig. 2). Furthermore, among the sterile sets opened randomly, the contamination rates among the different services were 37.0% ($n = 10$) for

the operating block, 14.8% ($n = 5$) for dental service, 14.8% for ICU, 22.2% ($n = 3$) for ENT, 22.2% ($n = 3$) for surgical emergency, and 0.4% ($n = 1$) for obstetrics and gynecology.

The most frequently isolated fungi were *Aspergillus* genus ($n = 85$, 83.3%), followed by *Mucor* genus ($n = 13$, 12.7%) and yeast ($n = 4$, 4.0%). The most frequently isolated fungal species were *Aspergillus niger* and *Aspergillus flavus*. *Aspergillus fumigatus* was isolated from one instrument (2.9%) from the operating block before sterilization. In the yeast group, *Candida parapsilosis* (20.0%), *Candida albicans* (2.9%), and *Candida krusei* (2.6%) were most frequently identified in instruments from the operating block, dental, and obstetrics and gynecology services, respectively. *C. albicans* was isolated from one instrument (33.3%) in the sterile set opened randomly from the surgical emergency service (Table 1). As shown in Table 2, clamps were the most frequently

Table 2. Distribution of fungal species identified among small surgical instruments

Individual instruments	<i>A. niger</i>	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>Mucor</i> spp.	<i>C. albicans</i>	<i>C. krusei</i>	<i>C. parapsilosis</i>	Total
Clamp	10	7	1	3	1	0	1	23
Scissors	2	2	0	0	1	0	0	5
Kocher	2	0	0	0	0	0	0	2
Needle holder	5	3	0	1	0	1	0	10
Rigid curve/catheter	6	3	0	4	0	0	0	13
Spatula brush	3	1	0	0	0	0	0	4
Elevator	1	2	0	0	0	0	0	3
Forceps	4	2	0	2	0	0	0	8
ENT speculum	3	5	0	1	0	0	0	9
Scalpel blade handle	2	0	0	2	0	0	0	4
Precelle	5	1	0	2	0	0	0	8
Retractor	1	2	0	1	0	0	0	4
Tongue depressor	0	2	0	2	0	0	0	4
Fuller	0	0	0	0	0	0	0	0
Matrix/mirror	1	0	0	1	0	0	0	2
Syndesmome	3	1	0	2	0	0	0	6
Curette	0	0	0	0	0	0	0	0
Lambotte hook	1	0	0	1	0	0	0	2
Cupula	1	1	0	0	0	0	0	2
Dissector	0	0	0	0	0	0	0	0
Total	50	32	1	22	2	1	1	

contaminated small surgical instruments by *A. niger* (n = 10, 43.5%); *A. flavus* (n = 7, 30.4%); and *Mucor* spp. (n = 3, 13.0%); catheters and needle handles were second and third, respectively.

DISCUSSION

This cross-sectional study revealed an unequal distribution of fungal carriage in small surgical instruments among the clinical services of a tertiary hospital in Côte d'Ivoire. Microbial contamination of surgical instruments can have serious consequences for patients in healthcare facilities¹³. Among the various microorganisms that may contaminate surgical instruments, fungi are a real major concern for healthcare establishments and practitioners. These pathogens thrive in warm and humid environments, which are common in operating blocks and healthcare rooms. Hence, they pose a threat of soiling and infection from surgical instruments. Despite the cleaning and sterilization of surgical instruments, fungal contamination remains a problem in healthcare establishments worldwide^{14,15}. This study revealed an overall fungal contamination rate of 22.4%.

Similar findings of fungal contamination of surgical instruments from operating theaters at the time of opening of surgical sets have been reported¹⁶. These results underscore the importance of continuous monitoring for fungal contamination in clinical environments. Contamination can be due to several factors, including disinfection practices, equipment storage time, and surgical environment. Inadequate cleaning procedures often explain the variations in fungal contamination rates, underscoring the need to improve existing protocols¹⁷.

This study revealed fungal colonization before sterilization and after random opening of sterile sets. Unbalanced sample sizes could lead to misinterpretations of the distribution of fungi among the services; those with smaller sample sizes may appear to have higher contamination rates. This result raises concerns about the effectiveness of sterilization methods. Furthermore, some sterilization methods may not effectively eliminate all types of fungi, particularly resistant spores¹⁸.

At our institution, surgical instruments are sterilized using steam with pressure or moist heat sterilization, which is commonly used for reusable medical devices. The efficacy of sterilization depends on effective destruction of living organisms¹⁶. Similar to a previous study¹⁹, our study used a combination of chemical products, including chlorhexidine gluconate and ethanol, for sterilization in order to prevent dissemination of infectious pathogens. Factors, such as residual

humidity, inappropriate handling after sterilization, and lack of pre-disinfection steps, among the services can enhance fungal resistance^{12,20,21}. This could have significant clinical consequences, such as increased risk of nosocomial infections. Therefore, sterility should be maintained from sterilization to handling and use of instruments in surgical settings.

Our study revealed a predominance of molds in surgical instruments. These pathogens are common in hospital environments because of their resistance and ability to grow in favorable conditions. Previous studies have shown a high presence of molds in ____, highlighting the need for control strategies to reduce contamination^{13,17,22}. Notably, molds can have significant consequences on patient health by increasing the risk of nosocomial infections.

Among the fungal species identified, *A. niger* was the most frequently isolated among all services in this study. Other studies have reported that mold species were commonly found in hospital environments^{9,13}. Another identified contaminant was *A. fumigatus*, which can cause invasive respiratory tract infections in >80%. It plays an important role in opportunistic nosocomial infections, frequently affecting the lungs because of the small size of spores, which can easily reach pulmonary alveoli^{23,24}. The highest rates of fungal contamination were observed in the operating block and dental services, suggesting that these clinical areas were more prone to fungal growth. Departments with frequent handling and circulation of surgical instruments are particularly vulnerable to contamination, particularly when hygiene and sterilization practices are suboptimal¹⁶.

In terms of yeast contamination, *C. albicans* was frequently identified among the clinical services. Previous studies classified *C. albicans* as a fungal species that has been mostly isolated from nosocomial candidemia^{25,26}. However, over the last decade, we have witnessed the emergence of nonalbicans species^{27,28}. Indeed, the epidemiology of candidiasis has changed considerably in recent years, with the appearance of nonalbicans species in hospitals²⁶. *C. parapsilosis*, which was also identified in our study, has become one of the main causes of nosocomial candidemia over the last decade^{26,29}. It is a saprophytic yeast on the skin and is characterized by its affinity for catheters and other small instruments, leading to a wide range of infections with dreadful prognoses. The presence of these yeasts in hospital environments may be associated with their ability to survive on plastic surfaces for extended periods and the use of reusable medical devices¹⁶. *Candida auris* was not identified in this study. This yeast is capable of causing nosocomial infections in immunocompromised persons; it is associated with candidemia with high mortality rate and presents a serious global health threat³⁰.

The most frequently contaminated surgical instruments were clamps, catheters, needle holders, and speculums. Similar results for surgical instruments in the operating rooms of hospitals in Iran were recently reported¹⁶. The main reason for contamination of these instruments could be prolonged contact with the operating room environment after opening the sterile packs or the frequency of use. These instruments are often unused and may be exposed to environmental contamination by airborne fungal spores, which can deposit on surfaces and instruments over time^{31,32}. This can increase the rate of various diseases, including allergic reactions; infectious diseases, such as aspergillosis; toxicosis; and respiratory ailments, such as asthma, hypersensitivity, and pneumonitis. The impact of air quality on fungal contamination due to suspended particles is a major source of air pollution^{33,34}. Reducing the infectious risk associated with reusable medical devices requires the implementation of effective and rigorous treatment and procedures by trained personnel.

Members of the staff who are primarily involved in disinfection and sterilization need appropriate qualifications, including continuing education at regular intervals and periodic competency assessment³⁵. Sterilization is one of the effective processes for eliminating pathogenic microorganisms from reusable medical devices. This process is described as special, because the sterility of a product cannot be verified without compromising it. The fact that the survival of a microorganism after sterilization is highly unlikely calls into question the procedure for the various stages of sterilization. Compliance with instructions in the predisinfection zone and distribution of instruments in the machine are important steps to guarantee better sterilization of surgical instruments³⁶.

This study had some limitations. The methods of identification were microscopy for molds and chromogenic medium and VITEK 2 for yeasts. Therefore, emerging pathogens, such as nonalbicans *Candida* species (e.g., *Candida auris*) and related species (e.g., *Candida duobushaemulonii* and *Candida haemulonii*), might have been missed. To improve the precision of fungal identification, MALDI-TOF MS and PCR-based methods are necessary. However, these techniques were not used in this study.

CONCLUSION

The findings of this study highlighted an important aspect of handling surgical instruments beyond the initial sterilization process. *A. niger* and *C. albicans* were the most commonly identified species. Prevention of fungal contamination of surgical instruments requires the implementation of control

strategies and microbiological surveillance. Moreover, improving sterilization techniques and regular monitoring in healthcare facilities could enhance patients' security against nosocomial fungal infections transmitted by surgical instruments.

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CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ETHICAL APPROVAL STATEMENT

The study was approved by the Institutional Review Board of (IRB No. N°008/MSHPCMU/CHUA/DMS/bgbn). This study was conducted in accordance with the principles of the Declaration of Helsinki.

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