



Microbiological monitoring of environment surfaces in a hospital in Fez city, Morocco

Surveillance microbiologique des surfaces de l'environnement d'un hôpital dans la ville de Fès, au Maroc

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Abstract

The hospital environment, colonized by many micro-organisms, is composed of true ecological niches. Contamination by these microorganisms is diffuse. Cumbersome procedures that are complex and costly are needed to master such contamination. The realization of a microbiological control of surfaces in a health facility is part of the policy for preventing nosocomial infections. It allows a better understanding of the microbial ecology for the purpose of conducting preventive and corrective actions. The aim of our study was to isolate bacteria from surfaces in a provincial public hospital in the city of Fez. We analyzed a total of 81 samples of which 3 were negative. The identification of 89% of the bacteria isolated showed a predominance of *Bacillus* sp (30%) and coagulase-negative *staphylococci* (24%). The proportions of the other identified germs were: *Staphylococcus aureus* (9%), *Aeromonas salmonicida* (7%), *Lactobacillus* (6%), *Pseudomonas vesicularis* and *Serratia rubidaea* (3%) each, and *E. coli* (2%). A small proportion of 1% was contained *Photobacterium damsela*, *Stenotrophomonas maltophilia*, *Acinetobacter* sp, *Pseudomonas aeruginosa* and *Pseudomonas luteola*. Some Gram negative cocci (5%) and oxidase positive Gram-negative bacteria (6%) were also isolated but were not identified. Current hand hygiene guidelines and recommendations for surface cleaning/disinfection should be followed in managing outbreaks because of these emerging pathogens.

Keywords: Hospital Environment, Microbiological monitoring, Surface, Nosocomial Infections, Fez, Morocco.

1. Introduction

A nosocomial infection (NI) is an infection during hospitalization that was not present or not in incubation at the time of admission [1-2]. Hospital or hospital-acquired infections represent a serious public health problem in all countries [3] because of their impact on the quality of care for patients and the cost driven by their prevention

[1]. These nosocomial infections or Healthcare Associated Infections are responsible for a high morbidity but also a significant lethality [4, 2].

A study on the prevalence of nosocomial infections conducted by WHO in 2009 in four of the six WHO regions (South East Asia, Europe, Eastern Mediterranean and Western Pacific) revealed that 8.7% of hospitalized patients had acquired a nosocomial infection [5]. Nosocomial infections in developed countries affect 5% to 10% of hospitalized patients. In some developing countries, they reach 25% of hospitalized patients [5].

In Morocco, the prevalence of nosocomial infections in 2009 was approximately 8.1%: 4.1% in provincial hospitals, 7.7% in regional hospitals and between 9.5% and 11.5% in teaching hospitals [5].

In the city of Fez, a study conducted in 2007 at Hassan II University Hospital revealed that the prevalence rate of nosocomial infections was 6.7% [3].

Thus, it is clear that monitoring of the hospital environment is an essential element in the control of nosocomial infections. As possible causes of infection, contamination of surfaces may be mentioned, even if cross contamination by hands is probably the greatest risk. In fact, hospital surfaces colonized by different types of microorganisms constitute special ecological niches that require cumbersome, complex and costly procedures that are necessary for better safety of the patient [6].

Such microbiological monitoring can measure the risk level of infection through the identification of infectious germs as well as comparing local data with those of other institutions.

The objective of this work is to determine the microbial ecology at the surfaces of a public provincial hospital in the city of Fez, Morocco. No study of this type of monitoring has been conducted at the said hospital and few studies in Morocco have been achieved in this field.

2. Materials and methods

2.1. Time and place of study

This study was conducted over a period of 4 months, from 01 March to 30 June 2014, and was devoted to the evaluation of the microbiological quality of surfaces in a public provincial hospital in the city of Fez with a bed capacity of 172 beds. The hospital consists of 18 services.

2.2. Samples location

The samples were taken at surfaces of 8 services: emergency block, the central block of the neonatal unit, the trauma unit, the intensive care unit, cardio-gastrological service and the hospital kitchen. The choice of sampling points was made in consultation with the heads of departments and targeted the most critical and most representative locations in each service.

2.3. Sampling modes and Techniques

The swab technique used allows qualitative or semi-quantitative analysis. This technique is mainly of educational and epidemiological interest by seeking specific germs on a flat surface or search for germs in a difficult to access and not flat area (operating tables, equipment carts, night tables, beds, medical devices, breathing pipes, bubblers, incubators). Sterile swabs were moistened with a sterile isotonic liquid and then were applied to defined areas by parallel spaced stripes by rotating them slightly, then on the same areas in perpendicular stripes according to ISO / DIS 14698-1[7]. The samples (swabs) were delivered quickly, in a cooler kept at 5 ± 3 °C, to be analyzed at the Regional Epidemiological Diagnosis and Environmental Hygiene Laboratory (LRDEHM) under the regional health department of the city of Fez.

2.4. Samples Analysis

After the delivery of samples to the laboratory, each swab was immersed in a liquid nutrient broth (BHI) and incubated at 37 ± 1 °C for 18 h. This broth was used to streak plates, containing the culture medium (blood agar), which were incubated at 37 ± 1 °C for 48 h. The count, appearance, size and color of the colonies were scored and purification of each colony type was carried out by exhaustion PCA medium. The identification of isolated germs was performed by classical biochemical gallery and the API (Biomerieux, France). Regular

quality control of culture media, reagents, distilled water, used controls, materials and equipment, sterilization operation by autoclaving, and the ambient conditions of the laboratory (temperature and humidity); was performed according to the requirements of the NM ISO 17025 adopted by the LRDEHM since 2008.

2.5. Statistical data analysis

For data processing and achieving the figures we used the Excel 2007 version software.

3. Results

During the 4-month study, 81 samples from different surface points of the 8 hospital services were analyzed in the laboratory. Three points were negative. Seventy eight (78) spots included bacteria. A total of 15 bacterial species were isolated from different surfaces.

The distribution of strains at the surfaces in hospital services is reported in Table 1. We noted the prevalence of polymorphic flora (coagulase negative *staphylococci*, *Staphylococcus aureus*, *Bacillus* sp, *Lactobacillus*, *Aeromonas salmonicida*, *E. Coli*, *Photobacterium damsela*, *Serratia rubidaea*, *Stenotrophononas maltophilia*, *Pseudomonsa luteola*, *Pseudomonas aeruginosa*, *Acinetobacter* sp, *Pseudomonas vesicularis*, Gram negative cocci and Oxidase Positive Gram Negative Bacteria (OPGNB). The results showed a predominance of Gram negatives with 73.33%, while Gram positives represented only 26.67%.

Table 1: Bacteria isolated from different areas covered in the studied services

Gram	Bacteria
Gram +	coagulase negative <i>staphylococci</i>
	<i>S. aureus</i>
	<i>Bacillus</i> sp
	<i>Lactobacillus</i>
Gram -	<i>Aeromonas salmonicida</i>
	<i>E. coli</i>
	<i>Photobacterium damsela</i>
	<i>Serratia rubidaea</i>
	<i>Stenotrophononas maltophilia</i>
	<i>Pseudomonsa luteola</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Acinetobacter</i> sp
	<i>Pseudomonas vesicularis</i>
	Gram negative cocci
Oxidase Positive Gram Negative Bacteria	

Germ, in the number of 15, included 11 Gram-negative and 4 Gram positive bacterial species. The analysis of bacterial strains isolated from studied hospital surfaces showed a diversified microflora. The majority belongs to the species *Bacillus* sp (30%) and coagulase-negative *staphylococci* (24%). As for *Staphylococcus aureus*, *Aeromonas salmonicida* and *Lactobacillus*, they represent respectively (9%) (7%) (6%) of the entire flora analyzed (Figure 1). Less common strains of *Serratia rubidaea* and *Pseudomonas vesicularis*, were represented by 3% each, *E. coli* by 2% and a 1% was observed respectively for *Photobacterium damsela*, *Stenotromonas maltophilia*, *Acinetobacter* sp, *Pseudomonas aeruginosa* and *Pseudomonsa luteola*. Other isolated bacteria, the species of which have not yet been identified, were represented by Gram-negative cocci 6% and 5% oxidase positive Gram-negative Bacteria (OPGNB).

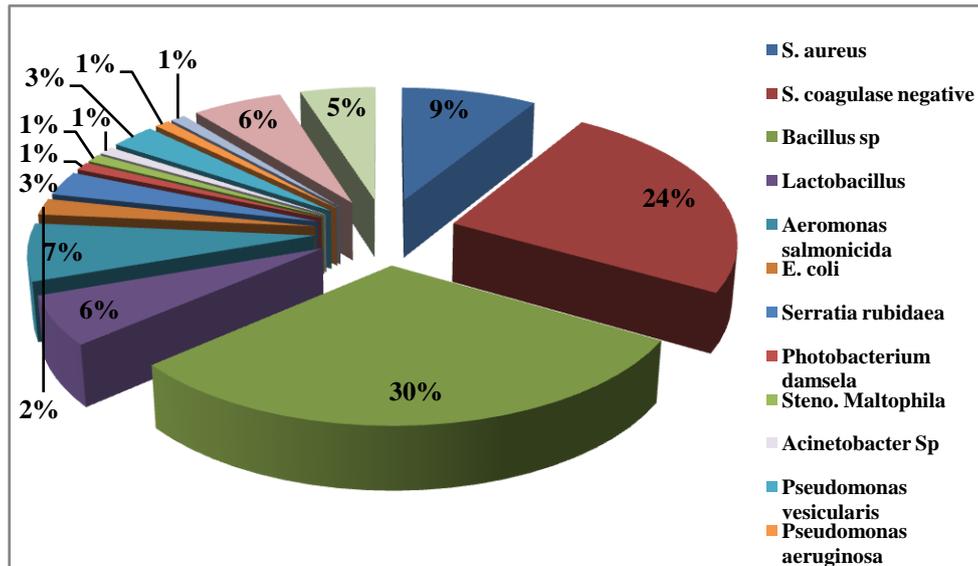


Figure 1: Nature and global distribution of isolated germs

Figure 2 shows the distribution of different types of germs in the eight services studied. We noted a predominance of bacterial strains in emergency block (19%), the central block (17%), the neonatal unit (16%) and the kitchen (14%). Trauma services, intensive care unit, surgery and cardio-gastrological unit each contained respectively 9%, 9%, 8% and 8% of identified bacterial strains.

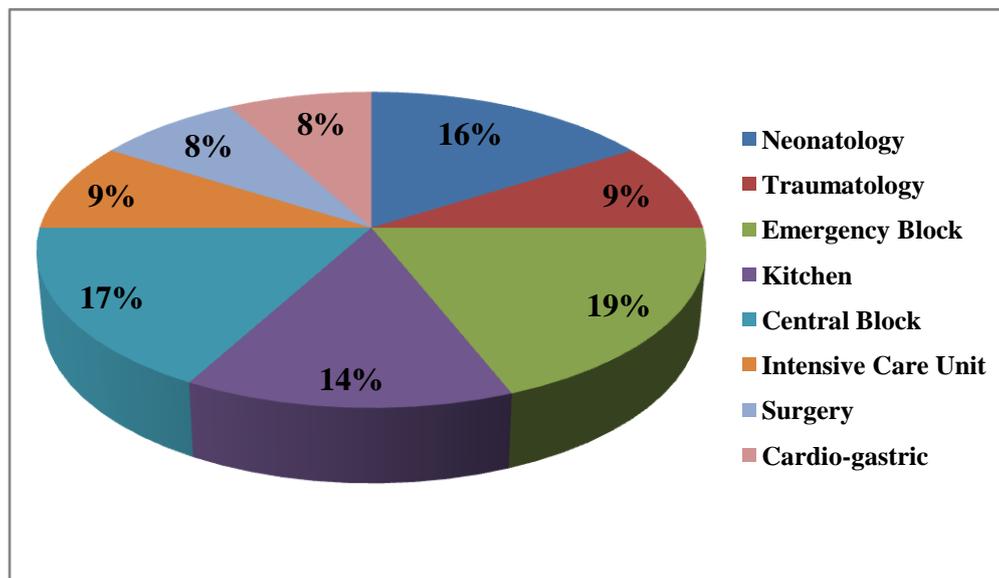


Figure 2: Distribution of isolated bacteria from surfaces in the studied services

Microbiological analysis of the different surfaces of hospital services showed a presence of coagulase negative *staphylococci* with a percentage ranging from 7% to 42.11%, the *Bacillus* sp are omnipresent in all services with the exception of the surgery unit, their percentage ranges from 11.11% to 69% (Table 2). *Staphylococcus aureus* strains were isolated in all services except from the central block and the surgery unit; the percentage varies between 6% and 15%. *Lactobacillus* was found in the trauma services 19%, Kitchen 14.5% and surgery unit 12.5%. *Aeromonas salmonicida* has been isolated in four hospital services (traumatology 6%, emergency block 15%, kitchen 14.5% and cardiovascular-gastrological unit 11.11%). *Serratia rubidaea* was isolated at the emergency unit (10%) and the kitchen (7%). *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* and *Pseudomonas luteola* were found only at the intensive care unit with a proportion of 11.11% each. *E.coli* was

found in the level of the emergency unit (10%) and *Photobacterium damsela* in the kitchen (7%). *Pseudomonas vesicularis* and *Acinetobacter sp* were isolated at the surgery unit respectively (25%) and (12.5%). Gram negative cocci, isolated in the central block, intensive and cardio-gastrological units as well as Oxidase Positive Gram Negative Bacteria isolated at the central block and surgery unit, have not been identified (Table 2).

Table 2: Percentage of different micro-organisms isolated in the different hospital services

Services Germes isolés	Neonatology	Traumatology	Emergency Block	Kitchen	Central Block	Intensive Care Unit	Surgery	Cardio-gastric
<i>S. aureus</i>	12 %	6%	15%	7%	-	11,11%	-	11,11%
<i>Coagulase negative Staphylococci</i>	19%	13%	25%	7%	42,11%	22,22%	12,5%	33,33%
<i>Bacillus sp</i>	69%	56%	25%	43%	26,29%	11,11%	-	33,33%
<i>Lactobacillus</i>	-	19%		14,5%	-	-	12,5%	-
<i>Aeromonas salmonicida</i>	-	6%	15%	14,5%	-	-	-	11,11%
<i>E. coli</i>	-	-	10%	-	-	-	-	-
<i>Serratia rubidaea</i>	-	-	10%	7%	-	-	-	-
<i>Photobacterium damsela</i>	-	-	-	7%	-	-	-	-
<i>Stenotrophomonas altophilia</i>	-	-	-	-	-	11,11%	-	-
<i>Acinetobacter sp</i>	-	-	-	-	-	-	12,5%	-
<i>Pseudomonas vesicularis</i>	-	-	-	-	-	-	25%	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	11,11%	-	-
<i>Pseudomonas luteola</i>	-	-	-	-	-	11,11%	-	-
Gram-negative cocci	-	-	-	-	15,8%	22,22%	-	11,11%
OPGNB	-	-	-	-	15,8%	-	37,5%	-

4. Discussion

Nosocomial infections are a serious public health problem with significant consequences both individually and economically [1-2]. The hospital environment is widely and naturally contaminated with microorganisms of specifically human or environmental origin [8]. This contamination varies qualitatively and quantitatively in time, from one institution to another and within the same institution, based on the services, patients, treatments and techniques used [6, 9, 10].

In this study we performed a total of 81 samples, in the frame work of microbiological monitoring of surfaces flora. Three (n = 3) samples were found negative and seventy eight (n = 78) were identified contaminated, a percentage positivity of 96%. Previous studies have shown a percentage of surface contamination of up to 50% [8]. Our results showed the presence of Gram (-) with a predominance of 73.33% and Gram (+) that represented by 26.67%. Lemmen et al. [11] reported a frequency of contamination of 24.7% by the Gram positive bacteria, and only 4.9% by Gram negative bacteria.

The identification of 89% of the isolated bacterial species showed a predominance of *Bacillus sp* (30%) and coagulase-negative *staphylococci* (24%).

Our results agree with the study by Saouide el ayne et al, [10] at the hospital El Idrissi Kenitra-Morocco, where they have found that *Bacillus* germ (27%), and predominant coagulase negative *staphylococci* (26%), followed

by *Staphylococcus aureus* (20%). These same authors have found other bacteria such as *Klebsiella pneumoniae* (16%), *Pseudomonas aeruginosa* (5%), *Enterobacter cloacae* (5%) and *Proteus vulgaris* (1%).

These results are also consistent with the work done in another hospital in the city of Fez, where bacterial species were isolated with varying proportions: *Acinetobacter baumannii* (33%), *Pseudomonas aeruginosa* (26%), coagulase negative *staphylococci* (17%) and *Klebsiella* sp 8% [12]. Previous studies [3, 4, 13, 14, 15] showed that the most frequently found germs which are candidates for causing nosocomial infections were: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli*, *Acinetobacter* (especially *A. baumannii*), *Klebsiella pneumoniae*, and *Candida albicans*.

Nosocomial strains of *Pseudomonas aeruginosa* ranked first in lung infections [15, 16]. While *Staphylococcus aureus* occurred in various infections in both community and hospital setting; especially in surgery [17]. *E. coli* was the causative agent of urinary NI and infections at surgical unit [13, 14]. Among the strains isolated from surfaces samples, we noted a dominance of *Bacillus* sp (30%) and coagulase-negative *staphylococci* (24%). In 2005, Meunier O. et al [9] reported that *Bacillus* sp bacteria are ubiquitous in the environment and seem not to be very accessible to bio cleaning, this could be explained by their ability to sporulate, and will therefore almost constantly found in the environment. The same authors [9], reported that the detection, at the analyzed surfaces, of bacteria such as coagulase-negative *Staphylococci*, *S. epidermidis*, *S. aureus*, reflect a good human activity on the premises.

The results of our study showed that the surfaces of the eight services studied are largely colonized by germs that could cause infections. Incidentally, Meunier O. et al. [9] also reported having observed severe contamination of hospital surfaces where they found that the inert surfaces and medical devices, clinical services close to patients or in care facilities, are largely covered by microorganisms such as *Bacillus* sp, *Stenotrophomonas maltophilia*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas spp*, Gram-positive cocci, coagulase negative *staphylococci*, *S. epidermidis* and *S. aureus*. All of these germs may constitute microbial reservoirs and may pose a risk of infection for people in hospital, caregivers and visitors.

The high contamination level of hospital surfaces could be related to several factors. Indeed, it has been shown for example, that surface contamination depends, besides the quality of organic cleaning, on many factors related to the microorganism: its life on an inert support (which varies depending on the material, temperature, desiccation), its adhesion to the surface, its ability to produce a biofilm usually difficult to eliminate [18], and also its ability to withstand adverse conditions [6].

The overall distribution of germs by department in two Moroccan hospitals showed the prevalence of germs especially in intensive care units, trauma and pediatrics [10, 12]. Amezian K. et al. [3], found that intensive care units were those that showed the highest prevalence rate of NI (24.8%), followed by pediatric services (11.3%). El Rhazi K. et al. [14] at the university hospital Hassan II in Fez, found that surgical site infections were the most frequent. While at the Fann Hospital in Dakar, Dia NM et al. [13], noticed that patients were hospitalized with NI in the neurology department. At the Lebanese Beirut hospital, 72% of patients with nosocomial infections were hospitalized in the intensive care unit [15].

Although the identification of various bacteria from the hospital surfaces, and also in other studies [9, 8, 19], the actual place of occurrence in the hospital environment of nosocomial infections and the transmission of pathogenic bacteria is not yet proven or established accurately [6, 20]. From this analysis we can note that the microbiological control of the hospital environment is one element of strategy against nosocomial infections. Indeed, the hospital environment is widely contaminated with microorganisms of human and environmental origin belonging to opportunistic and pathogenic species to humans [21].

The role of surface contamination in transmission of health care-associated pathogens is an important issue because transmission can be interrupted by appropriate hand hygiene [22, 23] and cleaning/disinfection of environmental surfaces [24, 25].

Some bacteria isolated from the environment could be derived from the skin of patients, which means that the colonization by this type of bacteria could play an important role in the development of nosocomial infections.

Tjoa et al. [26], have reported that some bacteria such as *Acinetobacter baumannii*, known as environmental microorganism, was found as a commensal in the human skin and digestive system in hospitalized patients.

The same authors [26] also reported that other microbes, such as *Staphylococcus aureus*, *Enterobacter asburiae*, and *Pseudomonas aeruginosa*, found on neonates' skin, plastics-covered portholes of incubator, syringe pump and humidifier liquid attached to incubator, can also give risk of infection when aseptic and antiseptic procedures are not done correctly.

In the context of fighting nosocomial infections, this work emphasizes the importance of the prevention factor in limiting the risk of infection in immunocompromised patients. The results obtained in our study impose the establishment of systematic procedures for monitoring the risk of infection in hospitals and the establishment of a comprehensive policy for the prevention of nosocomial infections.

A protocol for cleaning and disinfecting surfaces must be applied systematically to reduce the population of microorganisms. Also, hospital hygiene training and staff awareness are essential for the prevention of nosocomial infections and patients care quality.

A comparative study of the bacterial strains isolated from hospitalized patients and the surrounding surfaces must be performed using antibiogram and genotyping by pulsed-field gel electrophoresis (PFGE). The data obtained might point to the involvement of the hospital environment in nosocomial infections and may also clarify the mode of transmission of environmental microbes to patients.

To prevent the nosocomial infections related contamination of surfaces in hospitals, it would also be interesting to test the antibacterial effect of disinfectants, of synthetic or natural origin, towards the strains isolated [27-29].

Conclusion

The results of this study showed significant contamination in the different services studied. Identifying 89% of the isolated bacteria showed the presence of *Bacillus* sp, coagulase negative *staphylococci*, *Staphylococcus aureus*, *Aeromonas salmonicida*, *Lactobacillus*, *Serratia rubidaea*, *Pseudomonas vesicularis*, *E. coli*, *Photobacterium damsela*, *Stenotrophomonas maltophilia*, *Acinetobacter* sp, *Pseudomonas aeruginosa*, *Pseudomonas luteola*. Other organisms such as Gram negative cocci and oxidase positive Gram negative bacteria represented 11% of their covered germs. The frequency of germs encountered varies between species and between services.

These results will be of educational interest to prevent nosocomial infections. Hospital managers and healthcare bodies must be aware about the reality of the concept of environmental bacterial tanks and the need for respect of bio-cleaning procedures and choice of effective bio-cleaning tools.

However, we should not omitting the potential role of hands in germ transmission of the hospital environment and the occurrence of IN.

Conflict of interests: The authors declare that they have no conflict of interests.

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