ORIGINAL ARTICLE

IDENTIFICATION AND RESISTANCE TO ANTIBIOTICS OF BACTERIA ISOLATED FROM THE ENVIRONMENT OF A HOSPITAL SERVICE IN NORTHERN MOROCCO

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ABSTRACT

Context: The hospital environment is a microbial reservoir capable of contaminating people hospitalized which can cause nosocomial infections. Controlling one's infectious risk is an important element to overcome this major public health problem, especially in units where patients are more susceptible to nosocomial germs. Due to their immunodeficiency, circumstances of exposure to blood, infections in patients treated with dialysis are 100 times more common than in the general population. **Objective:** The aim of this study was to assess the percentage of contamination air's patient treatment room, identify isolated germs and determinate their antibiotic susceptibility by the disk diffusion test. **Method:** We conducted microbiological monitoring of the air patient's treatment room in the Al Ghassani Hospital's Hemodialysis Center from March 2015 to June 2015. **Result:** The results of this control varied from 12 to 52 CFU / m^3 and were all in accordance with standard NF S 90-351. The isolated germs revealed the absence of molds and the presence only of bacteria. The most frequent strains were Gram-positive bacteria, included various microorganisms such as *Staphylococcus* negative coagulase, *Bacillus sp* and Gram-negative bacilli non-fermentative positive oxidase. Concerning the antibiotic sensitivity, we noted differences depending on the type of bacteria and the antibiotic tested. All isolates showed significant resistance to at least three antibiotics. All strains remain sensitive to Imipenem. **Conclusion:** Our microbiological monitoring environment study testifies a bacterial contamination and the presence of different nosocomial bacteria in the air. It also confirmed the protuberant role of hospital environment in the spread of nosocomial pathogens. Indeed, bacterial strains mainly belonging to positive Gram were isolated. Among bacterial strains isolated, *Staphylococcus* negative coagulase was the most prevalent species (66.67%), followed by *Bacillus sp* and Gram-negative bacilli non-fermentative positive oxidase. All isolates were resistant to at least three antibiotics. The cross-transmission of these bacteria to hemodialysis patients may lead to severe nosocomial infections**.** In order to avoid nosocomial infections that may result, it is required to support the surveillance system applied in the studied center, support standard precautions' agreement, in particular hand hygiene, as well as contact precautions. It would also be essential to strengthen staff training in hospital hygiene, to elaborate and optimize the environment's disinfection protocol.

Keywords: *Nosocomial infections, Hemodialysis center, Multi-drug resistance, Hospital environment, air.*

1. INTRODUCTION

Infection is the most common cause of hospitalization and the second most common cause of mortality among hemodialysis (HD) patients, after cardiovascular disease. HD patients as well as the dialysis staff are vulnerable to contracting health-care-associated infections (HAIs) due to frequent and prolonged exposure of many possible contaminants in the dialysis' environment [1]. The direct involvement of the hospital environment in this infections' occurrence, was discussed and remains difficult to assess [2]. Some authors consider its role to be negligible, while for others, it serves as a relay in cross-transmission, in particular for Gram-positive bacteria [3]. In fact, the air quality's hospital is influenced by several factors such as seasons, ventilation systems, visitors, human activities and cleaning in hospitals. These factors may be associated with microbial growth conditions, which may cause serious infections [4-6]. The environmental risk of infection depends on the nature of the microorganism, its survival on an inert surface and the use made of the premises [7]. In addition, the indoor air quality in healthcare establishments has also become a growing concern, resulting from the evolution of healthcare practices and the presence of fragile people [8]. Thus, periodic checks, mainly microbiological, must be carried out systematically in order to identify any contamination, to determine microbial ecology's hospital, to carry out preventive actions, to evaluate corrective measures, disinfection protocol and to proceed for controlling infectious risks in the system hospital [8-13].

The Al Ghassani Hospital's Hemodialysis Center, created in Fez city in 1996, was renovated in 2012 according to national and international recommendations [14, 15], which have enabled a general improvement and consistency of premises with the activities taking place there [16].

As part of the quality approach's implementation, and following previous work done at this center in order to reduce the risk of HAIs [16-18], we conducted microbiological monitoring of the air in the center after its renovation. The aim of this study was to assess the percentage of contamination air's patient treatment room, identify isolated germs and determinate their antibiotic susceptibility by the disk diffusion test.

2. MATERIALS AND METHODS

2.1. Study design and sampling

Our prospective study was carried out from March 2015 to June 2015, concerned the air of the patient's treatment room, and was taken at the Al Ghassani Hospital's Hemodialysis Center. For the unavailability of bio-collector, the sampling technique used consisted in exposing two Petri dishes containing respectively Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA). These petri dishes have been placed in certain places within the test zone with their lid removed for 16 min. The total number of samples taken was twelve.

2.2. Analysis of samples

The TSA dishes, previously opened and exposed, were incubated at 37 ± 1° C for 48 hours. As for the petri dishes containing the SDA, we incubated them at 20 ± 2 ° C for 5 days. After incubation, we enumerated all positive cultures that have grown in TSA / SDA medium and determined the morphological characters of the colonies. The bacteria's identification was based on classical, cultural, morphological and biochemical characters, as for the mold identification, it was founded on its appearance, size and shape.

The culture media used were prepared according to standard NM 08.0.155 [19], and checked before use (pH, sterility). Moreover, quality control of the distilled water, materials and equipment (incubator, autoclave, etc.), as well as ambient conditions of the analysis environment were carried out daily according to the internal laboratory procedure, and written according to Canadian guidelines [20].

2.3. Antibiotic susceptibility testing

Antimicrobial susceptibilities were determined by disk diffusion method on Mueller Hinton agar (BD Microbiology Systems, USA) according to the Committee of Antimicrobial Testing of the French Society of Microbiology [21]. The antibiotics tested by the disc diffusion method included Penicillin G (PG-10UI), Ampicillin (AMP-10µg), Amoxicillin + Clavulanic Acid (AMC- 20/10µg), Tetracyclin (TET-30µg), Ciprofloxacin (CIP-5µg), Cefotaxim (CTX-30µg), Gentamycin (GM-10 µg), Fusidic Acid (FD-10 µg), Cefaclor (CEC-30µg), Trimethoprim + sulfamethoxazole (SXT- 1,25/23,75 µg) and Imipenem (IPM-10 µg).

2.4. Ethics and confidentiality

Before initiating the study, an authorization from the Ethical Institution was obtained.

3. RESULTS

3.1. Overall contamination

Based on its appearance, size and shape, all samples taken in SDA medium contained no mold. Nevertheless, all the samples taken in TSA medium were positive. The contamination rate was 100%. However, the bacteriological enumeration shows that the average level of total aerobic mesophilic bacteria (AMC) was 26CUF/m^3. The Minimum bacterial load was 12CUF/m^3, while the Maximum was 52UFC/m^3 (Fig. 1).

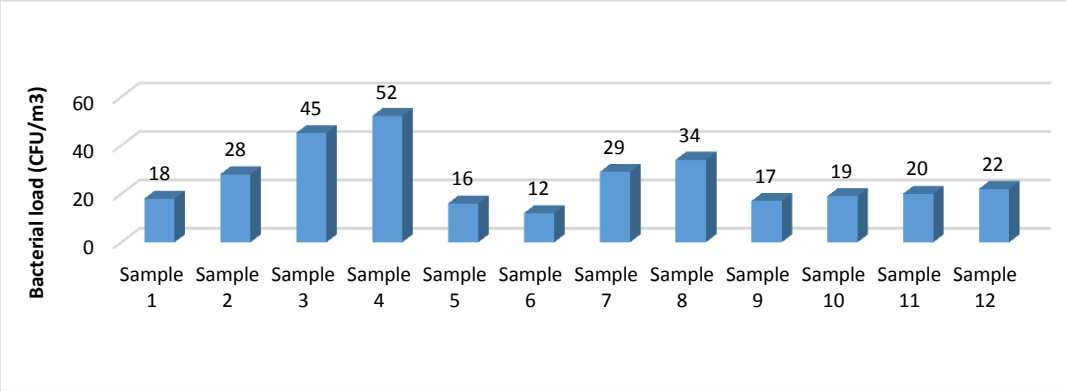

Figure 1: The figure presents the bacterial load of samples analyzed.

3.2. Distribution of microbial isolates

The identification of isolated germs revealed the absence of mold in the patient treatment rooms and presence only of bacteria. The distribution of bacteria by Gram staining showed the predominance of Gram-positive bacteria at a rate of 80%. Among the 20 bacterial strains isolated, *Staphylococcus* negative coagulase (St NC) was the most prevalent species at a rate of 60%, followed by *Bacillus sp* and Gram-negative bacilli non-fermentative positive oxidase (GNB NF POx) (Fig.2).

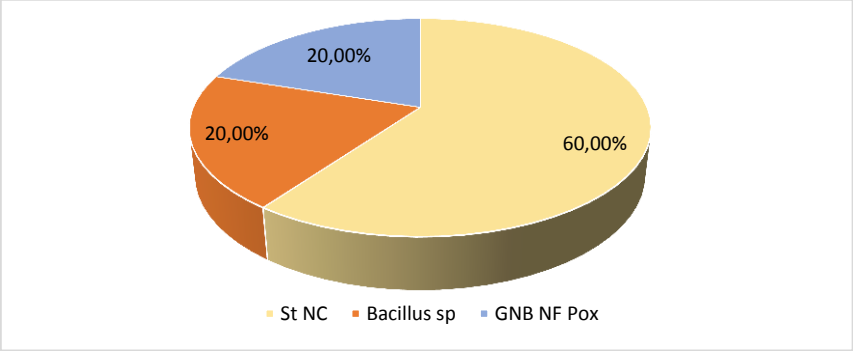

Figure 2: The figure presents the distribution of microbial isolates.

3.3. Antibiotic susceptibility

The results of the in vitro susceptibility testing are shown in Table 1. All bacterial strains isolated from the air patient's treatment room hemodialysis center presented significant resistance to at least three antibiotics.
Staphylococcus negative coagulase showed maximum resistance to Penicillin G and high resistance to Ampicillin, Trimethoprim combined with sulfamethoxazole, Gentamycin, and Tetracyclin. In addition, moderate resistance to the combination of Amoxicillin/Clavulanic Acid, Cefaclor, and Fusidic acid have been found. However, this genus remains susceptible to Cefotaxim, Ciprofloxacin and Imipenem.
As presented in the table 1, the resistance rate of GNB NF POx to Penicillin G and Ampicillin was maximum. Besides, these isolates showed a high sensitivity to Tetracyclin and Ciprofloxacin (75%), and remain vulnerable to Imipenem.
Bacillus sp isolates tested retained subtiscibilty to Imipenem, high sensitivity to Fusidic Acid, Cefotaxim and Gentamycin.

Table 1: Rate of antibiotic resistance of germs isolated from the hemodialysis environment (expressed in %)

Antibiotic	*Staphylococcus* negative coagulase	GNB NF POx	*Bacillus sp*
Penicillin G	100	100	100
Ampicillin	83,33	100	100
Tetracyclin	66,66	25	50
Ciprofloxacin	8,3	25	50
Cefotoxim	8,3	75	25
Trimethoprim + sulfamethoxazole	83,33	75	75
Gentamycin	75	75	25
Fusidic Acid	40	75	25
Amoxicillin+ Clavulanic Acid	41,66	75	75
Cefaclor	41,66	75	75
Imipenem	0	0	0

4. DISCUSSION

If the share of nosocomial infections linked to environmental contamination was difficult to assess, and the importance of microbiological control was controversial [22, 23], international recommendations currently indicate the multiple interests of microbiological environmental monitoring in the prevention of nosocomial infections [24, 25].

On the other hand, prevalence surveys, considered as a basic recommended tool for surveillance of nosocomial infections [26], have been the subject of various studies carried out both in Morocco and in other countries such as Algeria, Tunisia, Egypt, Cameroon and Italy [27-31]. The results of these studies, although variable from one country to another, underline the importance of setting up a global policy for the prevention of nosocomial infections and the need to reinforce the necessary means allowing continuity and care services. The quality of indoor air in healthcare establishments has become a growing concern, resulting from the evolution of healthcare practices and the presence of fragile people [8].

In this context, we conducted this study, which represents one of the first to nationwide and which is interested to environmental control in terms of management and prevention of infectious risk on hemodialysis. The results of this control varied from 12 to 52 CFU / m^3 and were all in accordance with standard NF S 90-351 [32]. Besides, this

contamination would not present a problem in itself if the contaminants remained permanently in the air, and would be a real risk if the contaminants were deposited on surfaces [33]. In addition, the risk would be higher for patients at risk, such as the immunosuppressed and hemodialysis patients [34].

Examination of the isolated germs revealed the absence of molds. Twenty bacteria, contaminants have been isolated. Their morphological identification showed the predominance of cocci over bacilli. Indeed, the hospital environment is a reservoir for Gram-positive bacteria, which are widely dispersed by human activity [35].

The flora included various microorganisms such as *Staphylococcus* negative coagulase, *Bacillus sp* and Gram-negative bacilli non-fermentative positive oxidase. A predominance of *Staphylococcus* negative coagulase was found. This result recalls that of other studies [36, 37], but does not agree with the one found by Thilbaut (2011), who noted the predominance of *Staphylococcus aureus* compared to other isolated bacteria [38].Indeed, the variability of contamination could be due to hospital activities [39].

Concerning the antibiotic sensitivity of the bacteria found, we noted differences depending on the type of bacteria and the antibiotic tested. All isolates showed significant resistance to at least three antibiotics. All *Staphylococcus* isolates were resistant to penicillin G, approximately 80% resistant to ampicillin and sulfonamides. The rate of resistance to aminoglycosides and cyclins exceeded 60%. In addition, More than 40% of the strains were resistant to the amoxicillin / acid combination, Cefaclor, and Fusidic Acid. However, this genus remains sensitive to quinolones, carbapenems and lincosamides. These results are similar to those of other authors [31, 40 and 41]. The high rate of resistance to penicillin G could result from the production of a penicillinase, which could also cause resistance to other antibiotics such as aminoglycosides and macrolides [42].

As for Gram-negative bacilli non-fermentative positive oxidase, 66.66% were multi-resistant to most of the antibiotics tested. Only Imipenem had shown complete inhibition of these isolates. These results agree with those noted by Njall & *al* (2013), who found resistance patterns of *Pseudomonas aeruginosa* and *Provindencia stuartti*, similar to those found in our study. The resistance rates observed could be linked to the ability of these bacteria to persist and resist in the hospital environment, and to accumulate resistance factors quickly leading to a therapeutic impasse [43].
Bacillus sp isolates showed maximum susceptibility to Imipenem, and high susceptibility to Fusidic Acid. Cefotaxim and gentamycin revealed efficacy on 66.66% of the strains tested. The high resistance rates of bacteria to antibiotics indicate that the hospital environment is largely contaminated with multi-resistant bacteria [44].

Comparisons to previous studies may explain the concern and the magnitude of severity reported by the majority of researchers, and may reflect the rapid development of bacterial resistance to antibiotics. Antimicrobial resistance is a global problem and is worrying in both developed and developing countries [45-47]. In addition, the emergence of multi-resistant bacteria observed, could result from self-medication, moderate costs, and abuse of antibiotics on both quantity and quality [48-51]. This phenomenon could result also to the cross transmission of the strains, favored by the lack of hygiene, the promiscuity of the patients, the sharing of possibly contaminated objects, the immune status of the people in contact and the characteristics of the strains [52].

Prevent therapeutic failures in infections requires immediate awareness such:

- Early detection of patients with multi-resistant bacteria (BMR) and the rapid implementation of specific isolation precautions;
- The design of an architectural and material environment facilitating the geographic and the technical isolation of patients with BMR;
- The implementation and execution of a strategy regarding screening, treatment of the carriers and monitoring of the actions implemented;
- Maintaining an antibiotic management policy aimed at prudent and controlled use in all sectors, the development of new antibiotics and bioactive molecules, research into procedures to combat certain bacterial species, education and training of professionals and users, as well as the promotion of basic research and applied research of alternative antibiotic molecules [53, 54].

5. CONCLUSION

Our microbiological monitoring environment study conducted in Al Ghassani Hospital's Hemodialysis Center testifies a bacterial contamination and the presence of different nosocomial bacteria in the air. It also confirmed the protuberant role of hospital environment in the spread of nosocomial pathogens. Indeed, bacterial strains mainly belonging to positive Gram were isolated. Among the 20 bacterial strains isolated, *Staphylococcus* negative coagulase was the most prevalent species (66.67%), followed by *Bacillus sp* and Gram-negative bacilli non-fermentative positive oxidase. All isolates were resistant to at least three antibiotics. The cross-transmission of these bacteria to hemodialysis patients may lead to severe nosocomial infections. In order to avoid health-care-associated infections that may result, it is necessary to support the surveillance system applied in the studied center, reinforce compliance with standard precautions, in particular hand hygiene, as well as contact precautions. It would also be essential to strengthen staff training in hospital hygiene, to elaborate and optimize the environment's disinfection protocol.

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