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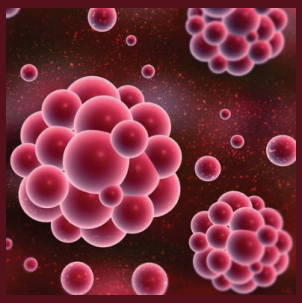
RAS MICROBIOLOGY AND INFECTIOUS DISEASES

Research Article: Contamination of hospital environment with multi drug resistant (MDR) bacteria responsible for healthcare acquired infections:Case of Universitary Hospital Center of Marrakesh-Morocco



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Abstract:
The hospital environment plays a significant role in the transmission of healthcare acquired multidrug resistant bacteria among hospitalized patients, especially in high-risk infectious units. Therefore, the present study aims to describe the distribution of microorganisms present on inanimate surfaces, hospital equipment and healthcare workers hands of the hospital units in Marrakech's Mohammed VI University Hospital (UHC).

Material and Methods: This is a prospective study carried out in nine hospital units (two operating units, two adult intensive care units ICUs, four surgical units and one medical unit) of Mohammed VI UHC of Marrakesh, during a period of eight months (March to October, 2021). The samples concerned inanimate surfaces and the hands of different medical staff. The level of antibiotic resistance was studied by the diffusion method in agar medium. The choice of antibiotics and the criteria for interpretation of the antibiogram were made according to the standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020).

Results: Among 220 samples carried out from the hospital environmental, 200 bacterial strains were isolated. This study highlighted the presence of methicillin-resistant *Stapylococcus aureus* (MRSA) 70,37%, the *enterobacteriacae* producing extended spectrum beta-lactamases (ESBLE) 22,22% and *Acinetobacter baumannii* resistant to imipenem (ABRI) 7,41%. The ABRI was mainly found in the inanimate surfaces of intensive care units (ICU), the EBLSE were predominant in the surfaces of the operating units. However, the MRSA was isolated mostly from the staff hands and the surfaces of the four studied units.

Conclusion: The significant presence of MDR bacteria in the hospital environment, specifically SARM, must therefore be more than ever a priority for all healthcare professional and urges the hospital structures to double their efforts to master these harmful bacteria.

Key words: Multidrug resistant bacteria, epidemiology, hospital environment, healthcare workers' hands, high-risk infectious unit, UHC Marrakesh.

Introduction

The role of the hospital environment as a reservoir and a potential source of nosocomial infections is now widely recognized. However, it is the responsibility of all healthcare workers to protect the patients from microorganisms, that are frequently present in the healthcare environment and carried by their hands [2]. The hospital environmental and healthcare workers' hands are among the nosocomial bacterial transmission sources [3]. On the other hand, the investigation of the rate of bacterial contamination of healthcare workers' hands and the hospital environmental surfaces could provide recommendations for preventing transmission of pathogenic bacteria to patients and medical staff in healthcare settings [4]. Although most enteric Gram-negative bacilli cannot remain viable on the dry surfaces of medical equipment or in the hospital environment, because of their sensitivity to common hospital used disinfectants and are biofilm-forming bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. They are strongly associated with healthcare acquired infections through contaminated medical devices and other environmental equipment in hospitals [5]. The bacterial

strains from patients, the hands of HCWs, and the Intensive care unit (ICU) environment have been demonstrated to be associated with hospital-acquired outbreaks by several studies [5-9].

In Morocco, as other developing countries, data is limited to the number of cases registered in some medical centers, like Ibn Sina hospital in Rabat, which highlighted the presence of pathogens in distinct areas of the hospital environment like *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella sp* and various Gram-negative bacilli [10]. However, few data exist on the microbial contamination of HCWs' hands and the hospital environment in the studied hospitals [11].

The study aims to detect quantitatively and qualitatively the bacterial contamination from hands of healthcare workers and surfaces in the units of University Hospital Center (UHC) Mohammed VI Marrakesh, Morocco, by studying their diversity, frequency and their resistance profile.

Material and methods:

Design and environmental sampling setting

The study was conducted in nine units of University Hospital Center Mohammed VI in Marrakesh, Morocco as follows: two intensive care units, two operating rooms for six months and five surgery units for two months, from 1st March to 30th October 2019. For microbiological sampling, all hospital environment samples were taken from the surfaces (floor, door handles, linen, bed rail, patient's table and oxygen mask) and hands of healthcare workers (Doctors, nurses, caregivers and cleaning agents). In order to do this, sterile polyester fibre-tipped applicator swabs were moistened in 2 ml sterile saline solution and rolled several times over a surface area of around 25 cm^2 [12]. Accordingly, based on the types of surfaces that had the greatest and lowest contact with patients, defined numbers of samples were randomly selected for microbial examination [11]. Regarding the surfaces of the ICU environment, the sampling was performed the morning after the regular daily cleaning. While medical devices, healthcare workers hands and admitted patients were swiped by sterile cotton swabs before disinfection. The sampling on the hands of the hospital staff (doctors, nurses and caregivers) was carried out by the fingerprint method [13]. All of the HCWs and equipment were sampled several times during the study period.

Bacterial isolation and identification

The swabs were immediately streaked onto blood agar, Chap Man and MacConkey agar media for the initial screening of suspected pathogens and an estimation of the total colony counts. The inoculated plates were transported to the laboratory at ambient temperatures and were incubated at 37◦C for 24 h under aerobic conditions. The grown colonies were identified by Gram staining and standard biochemical tests. For the biochemical identification of the Gram-positive isolates, catalase, oxidase, nitrate reduction, hydrolysis of esculin, fermentation of mannitol and glycerol, and coagulase tests were used [14]. The media employed foraerobic specimens were Mueller-Hinton agar. These agar plates were incubated for 48 h at 37 ° C. In case of positivity, the colonies were identified and tested according to the usual laboratory methods.

Drug susceptibility testing

The culture of each isolate was diluted to have turbidity around 0.5 McFarland standards. Then plated onto Muller-Hinton agar plate and antibiotic disks were applied to each plate. After incubation at 37°C for 24 hours, the zone of inhibition diameter was measured and the study of antibiotic resistance level was performed by the agar diffusion method in Muller Hinton agar as recommended by the French Society for Microbiology and European committee on antimicrobial susceptibility (EUCAST, 2019).

Results

Bacteriological findings

A total of 220 surfaces and healthcare workers' hands samples were taken from nine units (two intensive care units, two operating rooms and five surgery units). In this work, all the cultures were positive and 200 bacterial strains were isolated from all samples. The isolated bacteria were both Gram-positive (74%) and Gram-negative bacteria (26%).The most common isolates are *Staphylococcus spp.* (60%), *Enterobactereacae spp.* (17%), followed by *Bacillus spp.* (14%) and *Acinetobacter spp.* (5,50%) (Figure1). The dominance of *Staphylococcus spp.* and *Enterobactereacae spp.* was determinedto be the most common multi-bacterial contamination among the studied environmental samples.

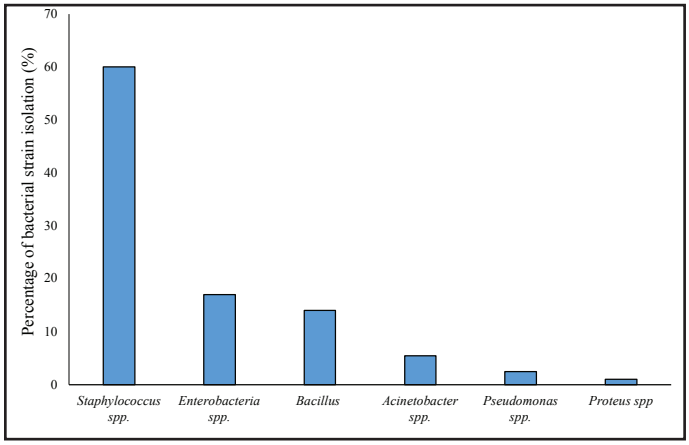

Figure 1: Types and frequency of bacteria isolated from hands and inanimate surfaces of University Hospital Center Mohammed VI in Marrakesh, Morocco.

The most important bacterial species isolated arecoagulase-negative *staphylococcus* (34%), *staphylococcus aureus* (26%), *Bacillus subtilis* (14%) and *Klebseilla pneumonae* (7,5%) (Figure 2).

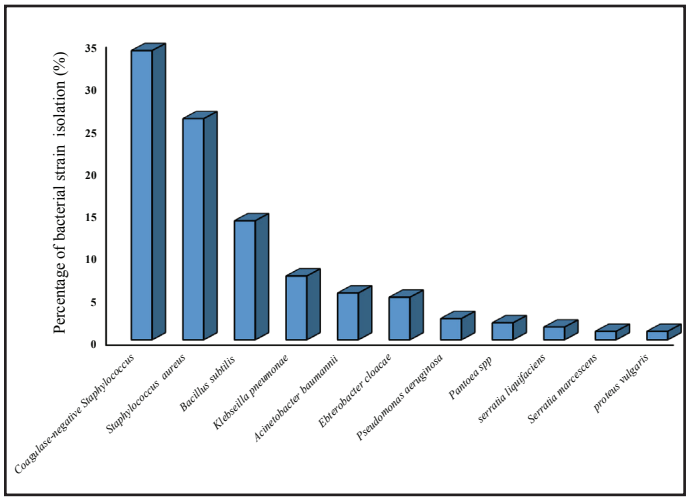

Figure 2: Distribution of bacterial species isolated from the hospital equipment and environment.

The figure 3 shows the bacterial strains distribution in every studied unit. We found that *Staphylococcus spp.* are mostly isolated from intensive care unit of emergencies (11%) and trauma unit (10,5%), the Enterobactereacae were more isolated in intensive care unit (4%) and maxillofacial surgery (3%), *Bacillus subtilis* is more found in trauma unit (5%) and *Acinetobacter baumannii* was isolated in intensive care unit, visceral surgery and neurosurgery unit.

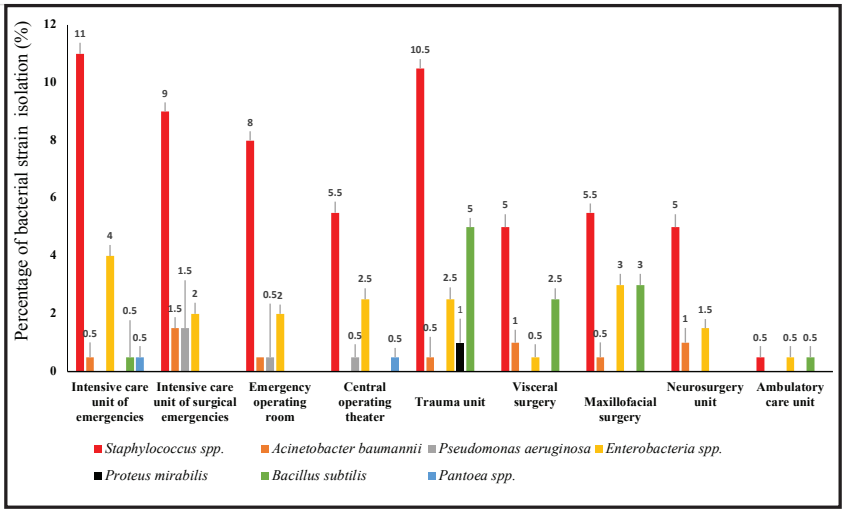

Figure 3: Distribution of isolated bacterial species among the hospital environment of studied unit.

Antimicrobial resistance patterns

Isolation and distribution of drug resistant strains

The **figure 4** reveals the distribution of the most important multidrug-resistant bacteria in the units of University Hospital Center Mohammed VI in Marrakesh, Morocco, according to antibiotic resistance tests that were conducted during the study. The antibiotic resistance tests revealed that 26% of the isolated strains were MDR bacteria. The most common was *S. aureus* resistant to methicillin (SARM) 70,37%, followed by multi-resistant Enterobactereacae (22,22%) and *A. baumannii* resistant to imipenem (ABRI) 7,31%. In our situation, no *P. aeruginosa* resistant to ceftazidime (PARC) were isolated from hospital environment surfaces and healthcare workers' hands.

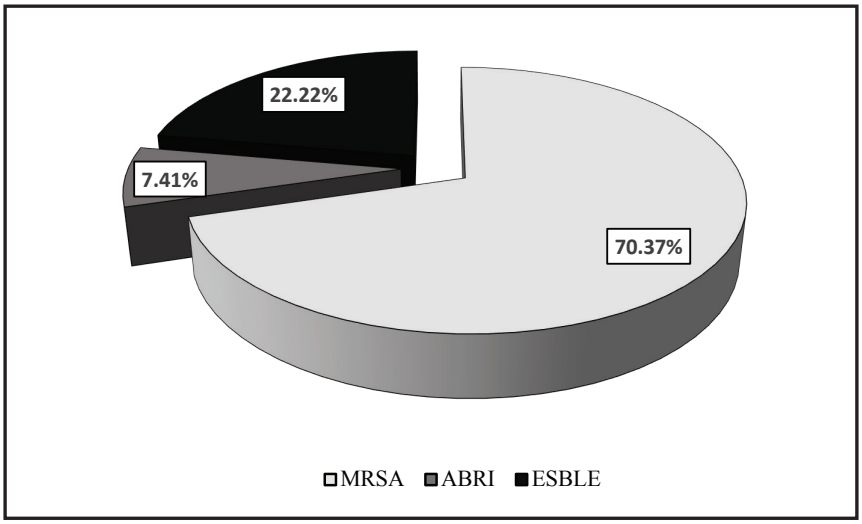

Figure 4: Distribution of MDR bacteria isolated during the period study from hospital environment surfaces and healthcare workers hands.

NOTE: ABRI, *Acinetobacter baumannii* resistant to imipenem; **ESBLE,** Extended-spectrum β-lactamase–producing Enterobacteriaceae; **MRSA,** *Staphylococcus aureus* resistant to methicillin.

Distribution of the MDR bacteria among surfaces and healthcare workers' hands

*** Surfaces contamination with MDR resistant bacteria:**

The **table 1** presents the repartition of surfaces contamination with MDR bacteria, the analysis demonstrated that MRSA were isolated from intensive care unit of emergencies (16%), emergency and central operating room and intensive care

unit of surgical emergencies (12%) respectively. However, ABRI strains were mainly isolated from intensive care unit of surgical emergencies surfaces (12%). Finally, ESBL producing Enterobacteriaceae were isolated from intensive care unit of emergencies samples (12%) **(Table 1).**

*** Healthcare workers' hands contamination with MDR resistant bacteria:**

On the other side, the prevalence of the healthcare workers' hands contamination with MDR bacteria in the intensive care unit of surgical emergencies and emergency operating room were mostly contaminated with MRSA (25,63%) and (22,22%), correspondingly. While, ABRI was found on the workers' hands of only trauma and maxillofacial surgery units with the same rate (3,70 %). Finally, we found only (7,41%) of this ESBLE bacteria in the intensive care unit of surgical emergencies and trauma unit workers' hands, followed by neurosurgery unit (3,70%).In total, the frequency of isolated MDR bacteria was similar among the hospital unit environments and workers' hands **(Table 1).**

Table 1: Distribution of MDR bacteria among the surfaces of hospital environment and healthcare workers' hands of investigated units.

Samples	Units	MRSA	ABRI	ESBLE	Total MDR bacteria
Surfaces	Intensive care unit of surgical emergencies	3 (12%)	3(12%)	0	6(24%)
	Intensive care unit of emergencies	4(16%)	1(4%)	3(12%)	8 (32%)
	Emergency operating room	3(12%)	0	1(4%)	4(16%)
	Central operating theater	3(12%)	0	1(4%)	4(16%)
	Trauma unit	1(4%)	0	0	1(4%)
	Visceral surgery	0	1(4%)	0	1(4%)
	Maxillofacial surgery	0	0	0	0
	Neurosurgery unit	0	0	0	0
	Ambulatory care unit	0	0	1(4%)	1(4%)
Total		14 (56%)	5(20%)	6(24,55%)	25 (100%)
Hands	Intensive care unit of surgical emergencies	6(22,22%)	-	2 (7,41%)	8 (29,63%)
	Intensive care unit of emergencies	4 (14,81%)	-	0	4(14,81%)
	Emergency operating room	7(25,63%)	-	1(4%)	8(29,63%)
	Central operating theater	2(7,41%)	-	0	2(7,41%)
	Trauma unit		1(3,70%)	2(7,41%)	3(11,11%)
	Visceral surgery				0
	Maxillofacial surgery		1(3,70%)		1(3,70%)
	Neurosurgery unit			1(3,70%)	1(3,70%)
	Ambulatory care unit				0
Total		19(70,73%)	2(7,41%)	6(22,22%)	27 (100%)
Total					52

NOTE: ABRI, *Acinetobacter baumannii* resistant to imipenem; **ESBLE,** Extended-spectrum β-lactamase–producing Enterobacteriaceae; **MRSA,** *Staphylococcus aureus* resistant to methicillin; **MDR,** multi-drug resistant bacteria.

Discussion:

The hospital environment is largely contaminated by bacteria of human origin [15]. In the current study, we have clearly demonstrated that bacteria isolated from the hands of medical staff or surfaces are represented by the dominance of the gram-positive bacteria 74%, mainly 60% of the contamination was by *staphylococcus spp*. While, 26% were gram-negative bacteria, which is represented by 12% of Enterobactereacae. In the same sense, *Staphylococcus spp.* was represented by 26% of *Staphylococcus aureus* and 34% of coagulase-negative Staphylococcus. However, the Enterobactereacae were mainly represented by 15% of *Klebseillapneumonae* and 10% of *Enterobactercloacae*. Elahe T, et al. demonstrated that the isolated bacteria from the environment surfaces and healthcare workers' hands of a hospital in Tehran, Iran were both Gram-positive with 60.7% and Gram-negative bacteria 39.3%, where the multi-bacterial contamination of the environmental samples was estimated to be 11% and was mainly found on surfaces (40.4%) represented by *K. pneumoniae, S. aureus,* and *S. epidermidis)*

[4]. In fact, recent studies showed the predominance of gram-negative bacteria from surfaces and Gram-positive bacteria from hands[16]. Different rates of viable microorganisms on healthcare staff hands have represented by *Acinetobacterspp.* (3-15%), *Klebsiella spp.* (17%), MRSA up to 16.9%, *Pseudomonas spp.* With 1.3-25% [16]. Furthermore, an Indian work reported that among the bacteria isolated from surfaces, Gram-negative bacteria constituted the dominant colonized bacteria compared with Gram-positive cocci (68.85% versus 31.14%) [17]. These results could be explained by diminished survival time of Gram-negative in the environment. In fact, Gram-negative bacteria other than *Acinetobacter spp*, survive on dry surfaces for only few hours, while the survival time can be several days for Staphylococci [16-19].

In a Moroccan study, the most frequently isolated predominant germs were *Bacillus* (27%) and *Staphylococcus* negative coagulases (26%), followed by *staphylococcus aureus* (20%) and *Klebsiela pneumoniae* (16%) [10]. Previous study showed similar results in comparing type of bacterial gram and proved that contamination of the inanimate environments by diverse groups of bacteria, including both Gram-positive (60%) and Gram-negative (39.3%) types [13]. Similar rates of contamination with both Gram-positive and Gram-negative bacteria 50.4% and 49.5%, respectively [11].

Antimicrobial resistance patterns

Surfaces and hands of healthcare workers contamination with MDR resistant bacteria:

The obtained data showed clearly that the rate of drug resistant Gram-positive bacteria (71,37%) was much higher than Gram-negative bacteria (29,63%) in the hospital equipment and surfaces environment. These results are similar to those found on other studies, in which the rate of drug resistant Gram-positive bacteria was much higher than Gram-negative bacteria in the equipment and surfaces of hospital environment [10]. Similarly, the antimicrobial susceptibility profile of the isolates showed that about 31.7% (32/101) of the Gram-negative isolates were found to be MDR bacteria, and the percentage of resistance is also high among isolates of *S. aureus* of which 44.7% (20/47) MRSA [20]. In this respect, 70,73% of Gram-positive strains (MRSA) and 29,37% of strains isolated Gram-negative from healthcare hands were multidrug resistant bacteria [10]. However, previous studies showed same results with a high degree of Gram-positive drug resistant isolates from the hospital environment [21]. In fact, Gram-negative bacteria other than *Acinetobacter spp.* [22], survive on dry surfaces for few hours only, while the survival time can be several days for *Staphylococcus spp.* [23,24]. Different studies have reported low bacterial counts of multi-resistant organisms in the hospital environment surfaces[21, 25,26]. These results could be considered as possible answers for the higher contamination rates with Gram-positive pathogens such as *Staphylococcus spp.* strains compared to Gram-negatives and could be explained by diminished survival time of Gram-negatives in the different equipment and surfaces of hospital environment [27].

The results of our study showed contamination of the inanimate environments with MDR bacteria. This contamination is dominated by the presence of MRSA on intensive care unit of emergencies surfaces with a percentage of 16%, emergency and central operating room and intensive care unit of surgical emergencies surfaces with 12%. While, ABRI strains were mainly isolated from intensive care unit of surgical emergencies surfaces (12%). Finally, ESBL producing Enterobacteriaceae were isolated from intensive care unit of emergencies samples (12%). correspondingly, some reports in the literature showed that hospital environment surfaces (ventilators, patients' oxygen masks and bed linens) were among the most frequently contaminated environmental samples, which was particularly contaminated by *S. aureus* and *A. baumannii*, that are the most resistant bacteria[11]. The results reported in another investigation support the findings of our surveillance and showed that bacterial contamination of hospital surfaces (beds and bed linen samples) for at least one bacterial species was 45.3%. The common bacterial species in these samples included *S. aureus* (10.9%), coagulase negative *staphylococcus* (19.5%) and *Acinetobacter spp.* (12.5%). In France, the estimated contamination rate of hospital environment, in the case of bed linen was mainly including *Staphylococci* (93%) and *Pseudomonas spp.* (23%) [28]. To our knowledge, Saudi Arabia, has come up with a total contamination of patient files in ICU, estimated to be 85.2% and was higher than our rates. They reported *P. aeruginosa* to be the most commonly isolated bacteria 32.3%, but MRSA was similarly isolated from 6.8% of their ICU patient files [29]. In the same sense, other authors demonstrated that bacterial contamination was found in 25% of phone handsets *(S. aureus)*, which was lower than what was reported in Italy by Messina et al. (89.2%, including *Staphylococci, Enterococci,* and *E. coli*) and Brady et al.in the UK 95.7%, including *S. aureus*, coagulase negative staphylococcus, and *E. coli* [22, 23]. In contrast, an epidemiological survey shows that the multidrug resistance phenotypes were detected among 79.38% (127/160) of the environmental samples. In the case of samples from the ICU environments, the MDR phenotype was most common in *A. baumannii* (94%), followed by MRSA (22/33, 66.6%), *K. pneumoniae* (6/11, 54.5%), *Enterococcus spp.* (4/8 50%), and *P. aeruginosa* (1/8, 12.5%) [4,5,9].

Hands contamination with MDR bacteria:

On the other side, the rate and prevalence of the healthcare workers' hands contamination with 70,37% of Gam-positive MDR bacteria and 29,63% of Gram-negative MDR bacteria. Also, the healthcare workers hands in the intensive care unit of surgical emergencies and emergency operating room were mostly contaminated with MRSA 25,63% and 22,22%, correspondingly. On the contrary, ABRI was found on the workers hands of only trauma unit and maxillofacial surgery unit with the same rate 3,70%. Finally, we found relatively low rate 7,41% of the ESBLE bacteria on intensive care unit of surgical emergencies and trauma unit workers hands, followed by neurosurgery unit 3,70%. These results are similar to those found in other studies where the scientists showed the predominance of Gram-positive bacteria from hands in the characterization of pathogen strains [10]. In addition, the results obtained by Sepehri et al. suggest that the colonization of the heathcare workers' hands with Gram-positive bacteria was nearly 40% of heathcare workers' hands had bacterial contamination, mostly with *S. epidermidis*, while contamination with nosocominal pathogens was observed among 6% of heathcare workers'hands [30]. In the other hand, a survey study show that the multidrug resistance phenotypes were detected among 35.89% (14/39) of the heathcare workers' hands samples. Especially, *A. baumannii* (7/11, 63.6%) and *S. aureus* (6/53, 11.3%)[4,5]. Also, Larson et al. and Waters et al.

reported that 21% and 30% of hospital employees' hands were persistently colonized by Gram-negative bacilli, respectively, including *Acinetobacter, Klebsiella, Enterobacter, S. marcescens,* and *E. coli* [31]. Besides, a report found by Ulger et al. has demonstrateda prevalence of 37.7% of MRSA strains among the hands of heathcare workers [32]. While, other studies have reported different rates of viable microorganisms on healthcare personnel hands, *Acinetobacter spp.* (3-15%), *Klebsiella spp.* (17%), MRSA up to 16.9%, *Pseudomonas spp.* with 1.3-25% [33]. In our work, the most of *Staphylococci* and *Acinetobacter* isolates were among the bacteria commonly found in the hospital environment surfaces or on the heathcare workers' skin. This finding suggests the occurrence of cross contamination and the heathcare workers' hands could be the major sources of transmission of nosocomial pathogens. In order to solve the hospital environmental contamination with MDR bacteria, more emphasis needs to be placed on the cleaning and disinfection of the ICU, operating rooms and surgical units'environment. Also, education of environmental services and heathcare workers' hands, the surfaces contamination could be reduced with the act of washing hands before and after being in contact with the patients and the various surfaces. The clarification of the role that surfaces have in the dissemination of healthcare acquired infections could provide support to increase adherence to control measures and reviewing policies, besides notifying about the means of dissemination that are still underestimated [34]. Basically, the resistance profiles of strains colonized hospital environment and healthcare workers' hands at the same Universitary Hospital Center, particulary Ibn Tofail Hospital were similar to those isolated from patients, this suggests that patients could be contaminated from hospital surfaces or through healthcare workers [35].

Conclusion

The increasing antimicrobial resistance rate in hospitals and the possible dissemination of resistant bacteria to the inanimate surfaces, equipment and hands of healthcare professionals, reinforce the need for knowledge and control of the sources of pathogens in the hospital environment. The evaluation of the environmental role in the acquisition of healthcare associated infections is needed to collaborate with infection control committees. It should also be highlighted that it is important to look at the quality of environment cleaning, execution methods, products, the workers' degree of knowledge about the important of those aspects and the relation with the reduction of healthcare acquired infections dissemination.

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