

# Characterization of Bacterial Strains and their Resistance Status in Hospital Environment

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## Abstract

Drug resistant Gram-negative or positive germs are associated with increased morbidity and mortality. Little is known about the epidemiology of this group of pathogens in Moroccan hospitals. During 1-year period, contamination controls of the surfaces and hands, in Ibn Sina hospital services, were performed from 10/2009 to 06/2010. A total of 470 surface and 135 hands samples were collected in the hospital and antibacterial resistance was examined. This study highlighted the presence of *Staphylococcus aureus*, *Pseudomonas* sp, *Klebsiella* sp, which were widely disseminated in the inanimate surfaces and on the hands of health professionals and patients. An increased number of antimicrobial resistant isolates showed a reduced susceptibility to third cephalosporin's generation, quinolones and aminoglycosides. In Moroccan hospital, it is recommended to optimize antimicrobial drug use and to evaluate the microbiologic quality of environment, which it is most likely to be colonized with resistant bacteria.

**Keywords:** Bacteria; CHU in Morocco; Hospital environment; Drug resistance

## Introduction

Worldwide, the control of the hospital environment is a key of success of health care quality [1]. However, the increasing emergence and spread of pathogenic bacteria, without distinguishing between Gram-positive and Gram-negative organisms, in hospitals is of great concern and continues to challenge infection prevention and hospital epidemiology practice [2,3]. Hospital environments are responsible of the dissemination of microorganisms for different distances and progressive contamination of various supports, including surfaces, hands [4-7], air and water [8], and constitute therefore a major source of infections. Environmental microorganisms like *Legionella pneumophila*, *E. coli*, *Mycobacterium xenopi* or *acynobacter*, were the principal causes of serious recent epidemic infections, by their easier access to sterile body sites. They were the origin of increased lengths of hospital stay, severe illness, death and increased care cost [9,10]. Pathogens were found in all hospital units but the interest was usually focused on intensive care and surgery units, especially due to the vulnerability of patients in these units [11,12]. Health care professionals have established rigorous isolation guidelines; which give current recommendations comprising an infection control plan of the general measures to prevent and limit pathogen dissemination. That may provide an effective way to standardize and increase reliability the criteria of application of infection control methods in hospitals [13,14]. During the last decades, a bleaker picture has emerged with the appearance of multi-drug resistant pathogens, especially resistant strains to new generations of antibiotics, raising concerns of a future epidemic of virtually untreatable infections [9,15]. Micro-organisms in the inanimate hospital environment are said to contribute only negligibly to endemic nosocomial infections [16,17]. However, it is still difficult to document the relationship between the contamination of the hospital environment and nosocomial infections, given the difficulty of capturing such data and the limited studies made in this way, with the exception of some studies covering some co-morbidities, associated to *Legionella* sp, *Aspergillus* sp, atypical *Mycobacteria* or *Staphylococci*, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant Enterobacteria (VRE) [3,18]. Even more, nosocomial infections is the second most common cause of death

after cancer diseases, with around 1.4 million newly infection cases and over 500 000 deaths estimated to occur annually in the European Union [19]. 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. Therefore, the present study is planned to evaluate the rate of pathogen bacteria in the environment of the university hospital Ibn Sina in Rabat and to characterise their resistance status for better management of the hospital environment quality.

## Materials and Methods

### Patients

A total of 139 patients admitted for hospitalisation at the Central university hospital Ibn Sina in Rabat in January 2010 were recruited. These patients were selected among patients developing nosocomial infections occurring 48 hrs post admission, according to the definitions described by the Centers for Disease Control [20]. The specimen sources included blood, urine, cerebrospinal fluid, pleural space, respiratory tract (collected during bronchoscopy or endotracheal suction), tip of central venous catheters, bedsores and surgical incision sites. All specimens were collected at the bed site, transferred to the laboratory immediately for microbiological analysis and were inoculated on proper culture media within two hours.

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## Environmental sampling

Sampling was performed on hands of hospital personnel and admitted patients before disinfection. Environmental sampling was performed during the morning after the regular daily cleaning. The date, time, conditions and sites of sampling were noted. Different surfaces and locations were included (Bed covers, bed rails, masks, oxygen bubbler, carriage of care, laboratory bench, bedside table, surgical operation table, breathing tubes, infusion pump, aspirators, baby incubators, wash-hand basins, water taps, shower fitting, door handles, floors and others). Basically, 20 swabs were used, at least, for each sampling site, giving a total of 470 samples. For sampling, sterile polyester fibre-tipped applicator swabs (Becton Dickinson, Basel, Switzerland) were moistened in 2 ml sterile saline solution and rolled several times over a surface area of around 25 cm<sup>2</sup>.

## Bacteriological testing

Spits were inoculated onto the Chocolate enriched or bacitracin agar in 5% CO<sub>2</sub>, and for *Neisseria* sp, the 5% sheep blood agar was used. Blood was injected into two or more "blood bottles" with specific media for aerobic and anaerobic organisms and sub cultured onto 5% sheep blood agar for *Streptococcus pneumoniae*, Chapman agar plates for Gram-positive *staphylococcus*, DCL Desoxycholate citrate lactose agar plates for Gram-negative bacteria and chocolate agar for exigent bacteria. Cerebrospinal fluid was inoculated onto Chocolate enriched / bacitracin agar in 5% CO<sub>2</sub>. The urine specimens were inoculated onto phosphate buffered saline agar, Cystine Lactose Electrolyte Deficient agar and Mac Conkey agar. The other Clinical specimens were inoculated onto DCL agar, 5% sheep blood agar, Selenite broth for *Salmonella* species, Chocolate enriched / bacitracin agar in 5% CO<sub>2</sub> for *Neisseria* sp and *Haemophilus* sp. Swabs were vortexed and sub-cultured on Chapman agar plates for Gram-positive bacteria, DCL Desoxycholate citrate lactose agar plates for Gram-negative bacteria and chocolate agar for exigent bacteria. The plates were incubated for 18-24 h at 37 °C and visible colonies were further sub-cultured and incubated for 24 h at 37 °C. Isolation and identification of microorganisms were done according to standard procedures. Bacteria were identified by examination of colonial morphology, haemolytic characteristics on appropriate agar media, Gram staining, rapid tests (catalase, oxidase, coagulase/Dnase, optochin disc, bile solubility, spot indole, latex agglutination), and classic and API galleries (BioMérieux, France) [21].

## Drug susceptibility testing

Drug susceptibility testing was performed by the method of disk diffusion according to the guidelines of the National Committee for Clinical Laboratory Standards [22]. The culture of each isolate was diluted to have turbidity around 0.5 McFarland standard, then plated onto Muller-Hinton agar plate (Difco, France). Antibiotic disks (Oxoid, France) were applied to each plate. After incubation at 35°C for 24 h, the zone of inhibition diameter was measured. Data analyses were performed using the susceptibility cut points according to the Clinical and Laboratory Standards Institute guidelines [23]. Multi-resistance of Gram-negative bacteria was defined as resistance to at least three antibiotics of the following antibiotic classes: penicillins, third-generation cephalosporins, carbapenems, quinolones and aminoglycosides.

## Statistical Analysis

Statistical analysis was performed using SPSS 17.1 software that uses directly the Yates' chi-square test for small sample size. Differences were considered statistically significant for  $P \leq 0.05$ .

## Results

### Isolation of pathogen strains from patients, hospital surfaces and hands

A total of 814 sampling were made, 139 from patients, 470 from surfaces and 205 from personnel and patients' hands. Personnel and patients' hands were the most infected samples. Indeed, 98.5% of samples from personnel and patients' hands were infected with pathogen bacteria (202/205). Whereas only 46% of patients (64/139) and 26.8% of surfaces' samples (126/470) were positives. Among the 392 positive samples, 2182 pathogen strains were isolated, comprising 1112 Gram-positive and 1070 Gram-negative bacteria.

### The distribution of strains according to samples' origins

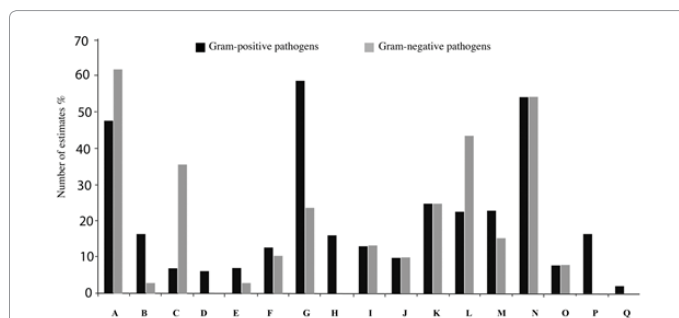
The distribution of strains isolated from patients, surfaces and hands, and their detection rates was reported in Table 1 and showed that 67 bacteria were isolated from patients, 865 from surfaces of various locations in different services and 1250 from personnel and patients' hands. Characterisation of strains isolated from patients showed clearly a high prevalence of Gram-negative bacteria. Bacterial identification showed a predominance of *Klebsiella* sp (16,42%), *E. coli* (16,42), *Enterobacter* sp (11,94%) and *Pseudomonas* sp (10,45%). Pathogen bacteria were also isolated from personnel hands Table 1. Globally, isolation of Gram-positive bacteria was higher than Gram-negative bacteria. Gram-positive bacteria were isolated from 130 of 202 (64.36%) of hands, however Gram-negative bacteria were isolated only from 35.64% of hands (72/202). Moreover, no significant difference was obtained on sampling from personnel' and patients' hands either for Gram-negative or Gram-positive bacteria ( $p=0.9656$ ). The distribution of strains isolated and their detection rates on surfaces is also reported in Table 1. Results clearly demonstrate the presence of both Gram-positive and Gram-negative bacteria with the predominance of *Pseudomonas* sp, *Klebsiella* sp, *Staphylococcus aureus* and coagulase negative *Staphylococcus*, *Pseudomonas* sp was isolated from 23.12 % and 11.60% of surfaces and hands respectively. *Klebsiella* was collected from 16.76% of surfaces and 16% of total hands. *Staphylococcus aureus* represented 12.72% and 20.8% of total isolates from surfaces and hands respectively. Interestingly, coagulase negative *Staphylococcus* were the most predominant bacteria isolated from the hospital environment and represented 30.64% and 30.80% of total strains isolated from surfaces and personnel and patients' hands, respectively. Moreover, the percentages of detection of Gram-positive and Gram-negative isolated at different locations is summarised in Figure 1. For both Gram-negative and Gram-positive bacteria, the main infected surfaces were the bed covers and bathroom representing the major reservoirs of pathogens. Moreover, Gram-negative bacteria were predominant in wash-hand basins and the patient's masks whereas Gram-positive bacteria prevail in samples taken from tables.

### Isolation and distribution of drug resistant strains

The resistance status of isolated bacteria was also evaluated. Among the 2182 isolates, 322 were drug resistant strains. Significant difference of the distribution of these strains was observed depending on the origin of sampling ( $p<0.0001$ ). Indeed, 37 strains of 67 isolates from patients were drug resistant giving the highest rate of multi drug resistance (55.22%). However, only 15.51% (150/865) and 9.53% (135/1250) strains isolated from surfaces and hands were drug resistant respectively. Table 2 illustrates the rate of multi-drug resistance of isolated strains. Drug resistant rate ranged mainly from 7.69 to 66.67%. However, all strains belonged to *Providencia* genus and only 0.31% of

	Strains	Patients	%	Surfaces	%	Hands	%
Gram-negative bacteria	<i>Pseudomonas</i> sp	7	10,45	200	23.12	145	11.60
	<i>Klebsiella</i> sp	11	16,42	145	16.76	200	16.00
	<i>Enterobacter</i> sp	8	11,94	10	1.16	65	5.20
	<i>E. coli</i>	11	16,42	60	6.94	70	5.60
	<i>Proteus</i> sp	-	-	30	3.47	45	3.60
	<i>Providencia</i> sp	1	1,49	5	0.58	0	0
	<i>Acinetobacter</i> sp	5	7,46	20	2.31	10	0.80
	<i>Citrobacter</i> sp	3	4,48	5	0.58	5	0.40
	<i>Morganella</i> sp	3	4,48	-	-	-	-
	Sub-total	55	82,09	475	54.91	540	43.20
Gram-positive bacteria	<i>Staphylococcus aureus</i>	3	4,48	110	12.72	260	20.80
	coagulase negative <i>Staphylococcus</i>	5	7,46	265	30.64	385	30.80
	<i>Streptococcus</i> sp	4	5,97	15	1.73	65	5.20
	Sub-total	12	17,91	390	45.09	710	56.80
Total		67		865		1250	

Table 1: Distribution of pathogens isolated from hospital locations and personnel and patients' hands.



A: Bed covers; B: Bed rails; C: Masks; D: Oxygen bubbler; E: Carriage of case; F: Laboratory bench; G: Bedside table; H: Surgical operation table; I: Breathing tubes; J: Infusion pump; K: Babies incubators; L: Wash-hand bassins; M: Water taps; N: Shower fitting; O: Floors; p: Doors; Q: Others.

Figure 1: Detection of multi-resistant Gram-positive and Gram-negative bacteria on different environmental items.

Isolated strains	Number of isolates	Number of multi-drug resistant isolates	Percentage
Gram-negative bacteria	<i>Klebsiella</i> sp	356	86, 24,16
	<i>Pseudomonas</i> sp	352	86, 24,43
	<i>Enterobacter</i> sp	83	38, 45,78
	<i>Citrobacter</i> sp	13	1, 7,69
	<i>E. coli</i>	141	34, 24,11
	<i>Proteus</i> sp	81	12, 14,81
	<i>Acinetobacter</i> sp	35	9, 25,71
	<i>Providencia</i> sp	6	6, 100,00
	<i>Morganella</i> sp	3	2, 66,67
	Sub-total	1070	274, 25,61
Gram-positive bacteria	<i>Staphylococcus aureus</i>	373	38, 10,19
	Coagulase negative <i>Staphylococcus</i>	655	2, 0,31
	<i>Streptococcus</i> sp	84	8, 9,52
	Sub-total	1112	48, 4,32
Total	2182	322, 14,76	

Table 2: Characterisation of multi-drug resistant strains.

coagulase negative *Staphylococcus* were drug resistant. The distribution of drug resistant strains according to the origin of sampling is reported in Table 3. Drug resistant strains belonging to *Citrobacter*, *Morganella* and coagulase negative *Staphylococcus* were isolated only from patients whereas the other drug resistant strains were isolated from patients and from surfaces and/or hands.

### Drug resistance status

The Tables 4 and 5 show the resistance profile of different drug resistant pathogens isolated from hospital during this study. The majority of gram-negative isolated strains were characterised by the high resistance to ampicillin and the association amoxicillin-clavulanic acid. *Klebsiella* sp and *Pseudomonas* sp showed high rates of resistance to 3<sup>rd</sup> generation of cephalosporins. Indeed, resistance to ceftriaxone was observed in 90.70 % of drug resistant *Klebsiella* strains whereas resistance to ceftazidime and cefotaxime was observed in all drug resistant *Klebsiella* strains. Furthermore, 71.43%, 100% and 93.51% of drug resistant *Pseudomonas* strains were resistant to ceftazidime, cefotaxime and ceftriaxone respectively. The 4 strains demonstrated a particularly high resistance to aminosidés antibiotics, especially

	Strains	Patients %	Surfaces%	Hands%
Gram-negative bacteria	<i>Pseudomonas</i> sp	16,21	30	25,93
	<i>Klebsiella</i> sp	16,21	33,33	22,22
	<i>Enterobacter</i> sp	8,11	-	25,93
	<i>E. coli</i>	10,81	20	-
	<i>Proteus</i> sp	5,41	6,67	-
	<i>Providencia</i> sp	2,7	3,33	-
	<i>Acinetobacter</i> sp	10,81	-	3,7
	<i>Citrobacter</i> sp	2,7	-	-
	<i>Morganella</i> sp	5,41	-	-
	Sub-total	78,37	93,33	77,78
Gram-positive bacteria	<i>Staphylococcus aureus</i>	8,11	6,67	18,52
	coagulase negative <i>Staphylococcus</i>	5,41	-	-
	<i>Streptococcus</i> sp	8,11	-	3,7
	Sub-total	21,63	-	22,22
Total		100	100	100

Table 3: Distribution of MDR strains isolated from patients, surfaces and hands.

Antibiotic	<i>Klebsiella sp</i>			<i>Pseudomonas sp</i>			<i>Enterobacter sp</i>			<i>E. coli</i>		
	N	R	%	N	R	%	N	R	%	N	R	%
AMP	86	86	100	82	82	100	38	38	100	27	27	100
AMC	86	86	100	82	82	100	38	38	100	27	21	77.78
IMP	73	5	6.85	77	26	33.77	38	0	-	27	0	-
ERT	46	0	-	22	22	100	5	5	100	21	0	-
CRO	86	78	90.70	77	72	93.51	38	23	60.53	22	16	72.73
CTX	68	68	100	57	57	100	33	18	54.54	17	16	94.12
CTZ	86	86	100	77	55	71.43	38	23	60.53	27	16	59.26
AK	83	10	12.05	82	30	36.58	38	2	5.26	27	1	3.70
CN	86	63	73.26	76	46	60.53	38	23	60.53	22	16	72.73
NET	86	67	77.91	72	46	63.89	38	18	47.37	27	16	59.26
CIP	86	63	73.26	72	42	58.33	38	18	47.37	27	21	77.78
OFX	53	30	56.60	56	41	73.21	23	10	43.48	16	10	62.5
SXT	80	65	81.25	82	62	75.61	30	20	66.67	26	21	80.77

N: Number of tested strains; R: number of resistant strains to the tested antibiotic; %: percentage of resistance; AMP: ampicillin; AMC: association of amoxicillin and clavulanic acid; IMP: imipenem; ERT: ertapenem; CRO: ceftriaxone; CTX: cefotaxime; CTZ: ceftazidime; AK: amikacin; CN: Gentamicin; NET: nethilmycin; CIP: ciprofloxacin; OFX: ofloxacin; SXT: Sulfamethoxazole + trimethoprim.

**Table 4:** Characterisation of antibiotic resistance of isolated gram-negative drug resistant strains

Antibiotic	<i>Staphylococcus aureus</i>		
	N	R	%
AMP	36	15	41.67
AMC	36	15	41.67
IMP	30	10	33.33
CRO	20	20	100
CTX	15	15	100
CTZ	25	20	80
AK	36	1	2.78
CN	31	11	35.48
NET	36	1	2.78
CIP	30	20	66.67
OFX	25	20	80
SXT	30	10	33.33
OX	21	10	47.62
E	36	20	55.55
AF	31	5	16.13
VA	36	0	0

N: Number of tested strains; R: number of resistant strains to the tested antibiotic; %: percentage of resistance; AMP: ampicillin; AMC: association of amoxicillin and clavulanic acid; IMP: imipenem; CRO: ceftriaxone; CTX: cefotaxime; CTZ: ceftazidime; AK: amikacin; CN: gentamicin; NET: nethilmycin; CIP: ciprofloxacin; OFX: ofloxacin; SXT: Sulfamethoxazole+ trimethoprim; OX: oxacillin; E: erythromycin; AF: fusidic acid; VA: vancomycin.

**Table 5:** Characterisation of antibiotic resistance of isolated gram-positive drug resistant strains.

gentamycin and nethilmycin, with a rate of resistance ranging from 47.37% to 76.14%. However, low rates of resistance to amikacin were registered. Infact, 36.58 % of *Pseudomonas* strains were resistant to amikacin, whereas only 12.05%, 5.26% and 3.70% of *Klebsiella*, *Enterobacter* and *E. coli* exhibited resistance to this aminoglycoside antibiotic. A relative high resistance to ciprofloxacin, ofloxacin and Sulfamethoxazole+ trimethoprim was observed for the 4 tested strains. Moreover, this study highlights the lowest frequency of resistance to imipenem. On the other hand, the gram-positive *Staphylococcus aureus* strain was characterised by the high resistance to the 3<sup>rd</sup> generation of cephalosporins (80-100%). Among the 3 tested aminoglycoside antibiotics, resistance to gentamycin was observed in 35.48% of drug resistant *Staphylococcus aureus* strains, whereas only 2.78 % of strains exhibited resistance to the other aminoglycoside antibiotics;

amikacin and nethilmycin. *S. aureus* strains showed a high resistance to quinolone drugs (>66%), including ciprofloxacin and ofloxacin, low resistance to Sulfamethoxazole + trimethoprim and fusidic acid, whereas no strain exhibited resistance to vancomycin. Moreover, more than 47% of *Staphylococcus aureus* strains were MRSA as determined by the susceptibility testing to oxacillin.

## Discussion

In this study, we have clearly demonstrated that microorganisms isolated from the hands of personnel or patients are much higher than those isolated from surfaces and patients. That ensures the possibility of disseminating microorganisms by professions who neglect to wash their hands after touching patients [24,25]. Several studies have reported the importance of frequent and adequate hand washing to reduce rates of Hospital-acquired infections [26-29], showed that hands regularly acquire bacterial pathogens, after contact with patients and the environmental surfaces near hospitalised patients [16]. Moreover, many pathogens responsible of nosocomial infections can survive on dry surfaces for several weeks [30]. Characterisation of pathogen strains showed the predominance of gram-negative bacteria from patients and surfaces and Gram-positive bacteria from hands. Previous studies have reported different rates of viable microorganisms on healthcare personnel hands, *Acinetobacter sp.* 3-15%, *Klebsiella sp.* 17%, MRSA up to 16.9%, *Pseudomonas sp.* 1.3-25% [31]. Gram-positive pathogens such as *Staphylococci* strains show much higher transmission rates compared to Gram-negatives. That could be explained by diminished survival time of Gram-negatives in the environment [32]. In fact, Gram-negative bacteria other than *Acinetobacter sp* [33], survive on dry surfaces for few hours only, while the survival time can be several days for *Staphylococci* [34,35]. In this study strains isolated from patients are basically the same as those isolated from surfaces of various hospital locations and hands, indicating the persistence of these strains in the hospital environment. However interestingly, investigations into epidemics have not confirmed that patients were infected specifically due to surrounding environmental contamination [2,36], but depend also on the patient health status. The main pathogens isolated are *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *Pseudomonas sp*, *Enterococci*, *Klebsiella sp*, *proteus sp* and *E. coli*. It's widely accepted that these pathogens are the major cause of hospital-acquired infections. Indeed, gram-positive organisms including coagulase

negative *Staphylococcus*, *Staphylococcus aureus* and *Enterococci* are responsible of nosocomial blood stream infections [37,38]. *E. coli* is a very common cause of nosocomial urinary tract infection. The other pathogens including *Pseudomonas aeruginosa*, *Klebsiella sp*, *Proteus*, *Staphylococcus epidermidis* and *Enterococci* are responsible for epidemic lower respiratory tract infection in many hospitals. Moreover, *Klebsiella sp*, *Pseudomonas sp*, *Proteus sp*, *E. coli* and *Staphylococcus aureus* are common cause of blood stream nosocomial infections in neonates [39]. Different studies have reported low bacterial counts of multi-resistant organisms in the environment of colonised patients [2,40,41]. This was confirmed by our findings reporting that only 15.51% of strains isolated from surfaces and 9.53% of strains isolated from hands were multi-drug resistant, in contrast, 55.22% of strains isolated from patients were drug resistant. Our results clearly demonstrated that the rate of drug resistant Gram-negative bacteria was much higher than Gram-positive bacteria, from both patients and the hospital environment. Previous studies showed converse results with a high degree of Gram-positive drug resistant isolates from the hospital environment [2].

Basically, resistance profiles of strains colonised hospital environment were similar to those isolated from patients; this suggests that patients could be contaminated from hospital surfaces or through healthcare workers. In this field, strain typing using molecular approaches will be of a great interest to compare bacteria from patients and the hospital environment. Thus, the implementation and/or reinforcement of effective cleaning measures is necessary to limit the dissemination of pathogens in the hospital environment and to contaminate newly admitted patients [3,42-44]. Inappropriate use of antibiotics allows bacterial pathogens, or opportunistic strains (from non-clinical environments) to acquire new resistance mechanisms [45,46].  $\beta$ -lactams are the most widely used antibiotics leading to the emergence of high number of resistant strains. Currently, the most used antibiotics in Morocco are 3<sup>rd</sup> generation of cephalosporins, carbapenems, quinolones and aminoglycosides. Unfortunately, the excessive and not efficient use of these antibiotics might be associated with increased risk of microbial resistance. In the current study, the frequency of resistance to the most used antimicrobial agents, including cephalosporins, was relatively higher. The frequency of resistance to the main antimicrobial agents was 60.53 % to 100% for ceftriaxone, 59.26% to 100% for ceftazidime, 54.54% to 100% for cefotaxime, 47.37% to 77.78% for ciprofloxacin and 6.86% to 33.77% for imipenem. Overall, our results corroborate with other studies conducted in different countries. In previous studies conducted in Iran, Oman and Turkey, the resistance to ceftriaxone ranged from 68 to 98.1% [47-49]. Conversely, the resistance to ceftriaxone was much lower in Belgian as reported by Glupczynski et al. That could be due to adequate empirical treatment using this antibiotic [50]. In the current study, the mean resistance to ceftazidime was 79.1%. It is almost similar to an Iranian study with resistance ranging from 60 to 88 % [47,49]. Once again, this resistance is higher than reported results for Belgian study [50]. In our study, resistance to cefotaxim ranged from 54.54% to 100%, this in agreement with reported data from Turkey with percentage of resistance ranging between 59.4% and 96.2% [49]. During the last decades, susceptibility to quinolones has decreased more than other antibiotics worldwide, and this might be a consequence of the wide usage of these antibiotics. Quinolone-resistant strains might therefore spread more easily than the strains resistant to other antimicrobial agents [51]. The published data on the resistance of hospital bacterial strains to ciprofloxacin are controversial. Indeed, in an Iranian study resistance to ciprofloxacin was 56% - 77% [47], 20 to 59.2% in Turkey [49]. However, it was much higher in Argentina exceeding 80% and is less than 25.5%, in a Brazilian

study [52,53]. In the present study, the mean resistance to imipenem is 16.73% (6.85% - 33.77%). The frequency of resistance to imipenem was 14% in an Iranian study, 13% in a Belgian study and 8% in a Polish study, and this is in agreement with our findings [47,50,54]. In conclusion, the increasing antimicrobial resistance rate in hospitals and the possible dissemination of resistant bacteria in the inanimate surfaces or the hands of health professionals and patients, 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 health-care associated infections is needed to collaborate with infection control committees. The establishment of a control system is also required in hospitals for the reduction of the length of stay, costs and morbidity-mortality. Such a surveillance system should continuously report the prevalence of microorganisms and their resistance pattern to hospital wards; this information will be used in defining policies for control of hospital environments, and building awareness about the diminished efficacy of antibiotics with the wide-scale use, especially in Moroccan hospitals where antimicrobial prescription is sometimes inappropriate. Thus, more attention must be given to the implementation of effective approaches to optimize antimicrobial use.

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