Moroccan Journal of Biology
Number 15 (2018)

http://www.fst.ac.ma/mjb
e-ISSN: 2351-8456 - p-ISSN: 1114-8756

Identification and antibiotic resistance of nosocomial bacteria isolated from the hospital environment of two intensive care units

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Abstract

The hospital environment is considered as a potential reservoir of pathogenic microorganisms responsible of nosocomial infections. The identification of the main sources of contamination is necessary to overcome this major public health problem, especially in intensive care units where patients are more susceptible to nosocomial pathogens. In this context, we conducted a study, realized for the first time, in two intensive care units (adult and neonatal intensive care units) of The Regional Hospital Center of Agadir-Morocco. The first aim of this work was to isolate bacterial strains responsible of nosocomial infections from surfaces, medical equipment and healthcare workers' hands using conventional methods. The second aim was to determinate the antibiotic susceptibility of the identified bacteria by the disk diffusion test. On eighty eight collected samples, 93 bacterial strains belonging to 19 different species were isolated from the studied services. The most frequent strains were Gram negative bacilli (84.21%) with the predominance of *Acinetobacter baumannii* followed by *Klebsiella pneumoniae* in both targeted units. The antibiotic susceptibility test showed the presence of 34 multi-drug resistant bacteria. Multidrug-resistant *Acinetobacter baumannii* isolates were the most dominant in adult intensive care unit (42.8%) and Extended Spectrum Beta Lactamase Producing *Enterobacteriaceae* strains (60%) were the most common in neonatal intensive care unit of the studied hospital.

Keywords: Nosocomial infections, Adult intensive care unit, Neonatal intensive care unit, Multi-drug resistance, Hospital environment.

Introduction

Nosocomial infections (NIs) are defined by the World Health Organization (WHO) as infections occurring after 48 hours of hospitalization at a healthcare facility and that was not present or incubating at the time of admission (World Health Organization, 2002). This global phenomenon is considered as a major public health problem associated with a high morbidity and a significant lethality (Al-Tawfiq and Tambyah, 2014). Its worldwide impact has been revealed by a prevalence survey conducted by the WHO in 55 hospitals of 14 countries that showed an average of 8.7% of hospitalized patients who had a NI; which means that at any time, more than 1.4 million people all over the world suffer from it (Tikhomirov et al., 1987). In Morocco, the fight against NIs began to arouse interest in recent years (Razine et al., 2012). The first investigation at the national level has been conducted in 1994 which reported a considerable prevalence rate of 14% (Ministère de la santé, 1994).

The impact of NIs is more significant in intensive care units (ICUs) (Mora et al., 2016). Indeed, a study revealed that the incidence of NIs in those high risk units is about 2 to 5 times greater than in other hospital services (Ewans et al., 1999). The hospitalized patients in ICUs present a higher susceptibility to pathogenic microorganisms responsible for this type of infection; it's mainly due to their compromised immune system (Mora et al., 2016). The dissemination of nosocomial pathogens may be related to

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the hospital environment. In fact, the majority of NIs is believed to be transmitted directly from patient to patient (Mora et al., 2016), but increasing evidence demonstrates that also surfaces and medical equipment are often a source of infections (Salgado et al., 2013). It can harbor Multidrug resistant (MDR) bacteria such as Methicillin-Resistant *Staphylococcus aureus (MRSA)*, *Pseudomonas aeruginosa (P. aeruginosa), Clostridium difficile (C. difficile),* Extended Spectrum Beta Lactamase (ESBL) producing *Enterobacteriaceae* and *Acinetobacter* spp. (Kramer et al., 2006). Hands of Health care workers (HCWs) are also considered as a major vector for cross-contamination. It's contributing to an estimated 20% to 40 % of NIs (Weber et al., 2010).

Therefore, the monitoring of the hospital environment and ICUs in particular has become a necessity to control the main sources of NIs.In Morocco, few studies have been conducted for this purpose (Lalami et al., 2016; Bakkali et al., 2016) and none has been carried out in the studied hospital. The first aim of our work was to isolate bacterial strains responsible of NIs from surfaces, medical equipment and HCWs' hands of two ICUs (i.e the adult intensive care unit (AICU) and the neonatal intensive care unit (NICU)) of the Regional Hospital Center of Agadir-Morocco (RHCA).

The second aim was to determinate the antibiotic susceptibility of the identified bacteria. This study may help the infection control committee to implement appropriate corrective and preventive actions to master NIs in ICUs.

Materials and methods

The study was conducted over a period of 7 months (January-July 2016) where samples were carried from surfaces, medical equipment and HCWs' hands in two ICUs (the AICU and the NICU) of the RHCA -Morocco.

Sampling techniques

The samples from surfaces and medical equipment (Table 1) were collected by the method of swabbing according to the standard ISO / DIS 14698-1. Sterile cotton swabs were moistened with nutriment broth before use. For a flat surface, the swabs were applied to defined areas (10 cm²) by parallel spaced stripes with rotating them slightly and then, on the same area, other stripes perpendicular to the first ones were carried out. For irregular surfaces, the entire surface was sampled. Each swab was submitted to its case and delivered quickly to the laboratory (Norme ISO/DIS 14698-1, 1999). In total, 77 samples were obtained.

Thus, the swabs were directly seeded on four culture media (Cystine Lactose Electrolyte Deficient agar-CLED) a non selective media, Eosin Methylene Blue agar (EMB) selective for Gram negative bacteria, Mannitol Salt Agar (MSA) selective for *Staphylococcus* and Cetrimide agar for the selective isolation of *P. aeruginosa).* Streaks were carried out according to the quadrants technique. The inoculated plates were incubated at 37 °C for 24 hours.

For HCWs' hands, 11 samples were collected by using the fingerprint method. It consists on pressing gently all 5 fingertips of HCWs dominant hand against the agar (CLED, MSA, EMB, Cetrimide) for 15 seconds (Pittet et al., 1999). The agar plates were then transferred to the laboratory for incubation at 37°C for 24 hours.

After the incubation period, appearance, size, and color of the colonies were considered and the purification of each colony was performed on nutrient agar.

Identification of bacterial strains

All isolated strains were Gram stained and indentified using conventional methods. DNase culture media, coagulase (Kateete et al., 2010) and latex agglutination test (Oxoid STAPHYTECT PLUS DR0850) were used to identify *Staphylococcus aureus (S. aureus). Enterobacteriaceae* and non fermenting Gram-negative bacilli strains were subjected to an oxidase test before their identification by API 20 E and API 20 NE respectively (Biomerieux, France).

Table 1. Sampling sites in adult and neonatal intensive care units.

Surfaces	Number of samples	Medical equipment	Number of samples	Healthcare workers' Hands	Number of samples
Adult intensive care unit					
HCWs' washbasins	5	Tubing junction/jar junction	6		6
Siphon	3	Electric syringe pump	3		
Bedside tables	2	Oxygen mask	2		
Treatment trolley	3	Bubbler	3		
Taps	3				
Neonatal intensive care unit					
HCWs' washbasins	5	Incubators	7		5
Taps	5	Baby scales	7		
Changing tables	5	Reanimation tables	5		
Benches	4	Electric syringe pump	4		
Treatment trolley	5				
Total	88 samples				

Antibiotic susceptibility testing

The antibiotic susceptibility was studied by using the disk diffusion method on Muller Hinton agar medium (Balouiri, 2016). The antibiotics used for the test included Ampicillin (AMP) (10µg), Amoxicillin-clavulanat acid (AMC) (30µg) Piperacillin-Tazobactam (TZP) (30µg), Cefotaxim (CTX) (30µg), Ceftriaxon (CRO) (30µg), Ceftazidim (CAZ) (30µg), Imipenem (IMP) (10µg), Amikacin (AK) (30 µg), Gentamicin (GN) (10µg), Ciprofloxacin (CIP) (5µg), Trimethoprim-sulfamethoxazole (TS) (25µg), Colistin (COL) (25µg), Cefalotin (KF) (30µg), Meropenem (MEM) (10µg), Netilmicin (NET) (30µg), Fosfomycin (FF) (50µg), Tetracyclin (T) (30µg), Erythromycin (ERY) (15 µg), Norfloxacin (NOR) (10µg), Rifampicin (RA) (30 µg), Oxacillin (OXA) (1µg), Vancomycin (VA) (30µg), Kanamycin (K) (30 µg), Trimethoprim (TMP) (30µg), and Penicillin G (Peni G) (5µg).

The interpretation of results was performed according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CASFM, 2015).

Screening of Extended-spectrum B-lactamase production

ESBL Producing *Enterobacteriaceae* strains were detected by the double disc synergy test which combine AMC and third generation Cephalosporin discs. CAZ (30µg), CRO (30µg) and CTX (30µg) discs were placed at a distance of 30 mm from AMC disc. This test is considered as positive if a clear extension of inhibition zone's edge appeared between the Cephalosporins and AMC discs after 24 hours of incubation at 37°C (Garrec et al., 2011).

Results

Prevalence of isolated bacterial strains in adult and neonatal intensive care units

During the 7 months of study, the bacteriological analysis of 88 samples isolated from surfaces, medical equipment, and HCWs' hands of the two studied ICUs revealed 93 strains. Forty-six strains were isolated from the AICU and forty-seven from the NICU.

The Gram staining and conventionnal identification tests demonstrated that the 93 isolated strains belonged to 19 different bacterial species (Table 2). Gram-negative bacteria predominated with 84.21%, while Gram positives represented only 15.79% (Table 2).

Table 2. Prevalence of Gram negative and Gram positive bacteria isolated from different sites of adult and neonatal intensive care units.

Gram	Bacterial species	Prevalence (%)
Gram negative	*Acinetobacter baumannii* *Klebsiella pneumoniae* *Escherichia coli* *Pseudomonas aeruginosa* *Pseudomonas luteola* *Enterobacter cloacae* *Providencia rettgeri* *Stenotrophomonas maltophilia* *Serratia liquefaciens* *Citrobacter freundii* *Pseudomonas oryzihabitans* *Pantoea* spp. 2 *Klebsiella oxytoca* *Shigella* spp. *Pseudomonas fluorescens* *Chrysobacterium meningosepticum*	84.21
Gram positive	*Staphylococcus* spp. *Bacillus* spp. *Staphylococcus aureus*	15.79
Total	*19*	

Among the 46 bacterial strains isolated from the AICU, *Acinetobacter baumannii* (*A. baumannii)* was the most prevalent species (20%) (Table 3) followed by *Klebsiella pneumoniae* (*K. pneumoniae)* (17%) and *Pseudomonas fluorescens (P. fluorescens), Stenotrophomonas maltophilia (S. maltophilia), Citrobacter freundii (C. freundii), Escherichia.coli (E. coli), Pantoea* spp.2 with a proportion of 7 %. The less common isolates were *Providencia rettgeri (P. rettgeri), Staphylococcus* spp., *Shigella* spp., *Enterobacter cloacae (E. cloacae),* which represented 4 % respectively. The strains of *Klebsiella oxytoca (K. oxytoca), Serratia liquefaciens (S. liquefaciens), S. aureus, Pseudomonas oryzihabitans (P. oryzihabitans)), Pseudomonas luteola (P. luteola)* and *P. aeruginosa* species represented only 2% (Table 3).

In the NICU, and as for the AICU, the predominance of *A. baumannii* (38%) and *K. pneumoniae* (28%) was noted. For *E. coli, Staphylococcus* spp. and *E. cloacae,* they represented 11%, 9%, 4% respectively (Table 3). A prevalence of 2% was observed for *P. aeruginosa, Bacillus* spp.*, C. freundii, P. luteola*. *Chrysobacterium meningosepticum (C. meningosepticum)*, an opportunistic pathogen mainly responsible of neonatal meningitis was only detected in the NICU with a proportion of 2% (Table 3).

Bacterial strains isolated from surfaces, medical equipment and HCWs' hands

As shown in tables 4 and 5, different bacterial strains were isolated from surfaces and medical equipment of the two studied ICUs. The most contaminated sites were those that come into direct contact with patients and HCWs.

In the AICU, the most contaminated sites were: tubing suction/jar suction, HCWs' washbasins and siphon. The highest number of bacterial strains was encountered in tubing suction/jar suction (a total of 17 isolated bacterial strains) with *A. baumannii* (23%) as the most frequent species (Table 4).

As to the NICU, the contamination was observed in the majority of targeted

Table 3. Prevalence of bacterial strains isolated from adult and neonatal intensive care units.

Services	**Adult intensive care unit (AICU)**		**Neonatal intensive care unit (NICU)**	
Bacterial species	Number of strains	Prevalence (%)	Number of strains	Prevalence (%)
Acinetobacter baumannii	9	20	18	38
Klebsiella pneumoniae	8	17	13	28
Escherichia coli	3	7	5	11
Pseudomonas aeruginosa	1	2	1	2
Pseudomonas luteola	1	2	1	2
Enterobacter cloacae	2	4	2	4
Providencia rettgeri	2	4	-	-
Stenotrophomonas maltophilia	3	7	-	-
Serratia liquefaciens	1	2	-	-
Citrobacter freundii	3	7	1	2
Pseudomonas oryzihabitans	1	2	-	-
Pantoea spp.	3	7	-	-
Klebsiella oxytoca	1	2	-	-
Pseudomonas fluorescens	3	7	-	-
Staphylococcus aureus	1	2	-	-
Staphylococcus spp.	2	4	4	9
Bacillus spp.	-	-	1	2
Shigella spp.	2	4	-	-
Chrysobacterium meningosepticum	-	-	1	2
Total	46		47	

sites. It was mostly detected in HCWs' washbasins and baby scales with a total of 9 and 8 bacterial strains respectively. *A. baumannii* and *K. pneumoniae* predominated in the two contaminated sites with 33% each in HCWs' washbasins and a prevalence of 50% and 25% respectively in Baby scales (Table 5).

For HCWs' hands samples from the AICU and the NICU, the contamination was by bacterial species already encountered in the vicinity of patients. Indeed, *A. baumannii* was omnipresent with the same frequency (43%) for the two studied services. The bacteriological analysis of hands samples also revealed other bacterial species notably for the AICU: *Staphylococcus* spp., *K. pneumoniae* and *Shigella* spp. with a frequency of 29%, 14% and 14% respectively (Table 4). As for the NICU, *K. pneumoniae* (29%), *E. coli* (14%) and *P. luteola* (14%) were isolated (Table 5).

Multidrug resistant strains isolated from adult and neonatal intensive care units

The results of the in vitro susceptibility testing are shown in Tables 6 and 7. Among the 93 bacterial stains isolated from the AICU and the NICU, 34 isolates showed multiresistance to the tested antibiotics with a predominance of MDR Gram negative bacilli. These bacteria presented a great resistance to more than 3 antibiotic families especially ß-lactams and a considerable susceptibility to aminoglycosides except for *S. maltophilia*. Besides, some *A. baumannii* strains isolated from the AICU also revealed an increased resistance to carbapenems (Impenem and Meropenem) (Tables 6 and 7).

In the AICU, 14 of 34 multidrug resistant strains were indentified. MDR *A. baumannii* strains were the most isolated with a proportion of 42.8%. The second position was attributed to ESBL Producing *Enterobacteriaceae* (35.7%) followed by multiresistant strains of *S. maltophilia* (24.4%) (Table 8). The most common species within ESBL Producing *Enterobacteriaceae* included *ESBL* Producing *K. pneumoniae* with 40 % and ESBL producing *E. coli*, ESBL producing *E. cloacae*, *ESBL* Producing *K. oxytoca* with 20% each (Figure 1).

Table 4. Frequency of bacterial strains isolated from different sites of the adult intensive care unit.

Bacterial species / Sites of isolation	*S. aureus*	*A. baumannii*	*K. pneumoniae*	*k. oxytoca*	*P. aeruginosa*	*S. liquefaciens*	*P. fluorescens*	*Shigella.* spp.	*Staphylococcus* spp.	*P. rettgeri*	*E. cloacae*	*C. freundii*	*S. maltophilia*	*E. coli*	*P. luteola*	*P. oryzihabitans*	*Pantoea* spp. *2*	*Total*
Tubing Junction/jar Junction	1 **(6%)**	4 **(23%)**	3 **(17%)**	0	0	1 **(6%)**	1 **(6%)**	0	0	2 **(12%)**	0	2 **(12%)**	2 **(12%)**	0	0	1 **(6%)**	0	**17**
HCWs' washbasins	0	1 **(9%)**	2 **(19%)**	1 **(9%)**	1 **(9%)**	0	1 **(9%)**	1 **(9%)**	0	0	0	0	1 **(9%)**	1 **(9%)**	1 **(9%)**	0	1 **(9%)**	**11**
Siphon	0	0	2 **(23%)**	0	0	0	1 **(11%)**	0	0	0	1 **(11%)**	1 **(11%)**	0	2 **(22%)**	0	0	2 **(22%)**	**9**
Bedside tables	0	0	0	0	0	0	0	0	0	0	1 **(100%)**	0	0	0	0	0	0	**1**
Electric syringe pump	0	1 **(100%)**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
Treatment trolley	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Taps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Bubller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Oxygen Mask	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
HCWs' hands	0	3 **(43%)**	1 **(14%)**	0	0	0	0	1 **(14%)**	2 **(29%)**	0	0	0	0	0	0	0	0	**7**
																		46

Table 5. Frequency of bacterial strains isolated from different sites of the neonatal intensive care unit.

Bacterial species / Sites of isolation	*A. baumannii*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*Staphylococcus* spp.	*E. coli*	*P. luteola*	*Bacillus* spp.	*C. freundii*	*C. meningosepticum*	Total
Incubators	2 **(100%)**	0	0	0	0	0	0	0	0	0	**2**
HCWs' washbasins	3 **(33.33 %)**	1 **(11.11%)**	2 **(22.22%)**	3 **(33.33%)**	0	0	0	0	0	0	**9**
Baby scales	4 **(50 %)**	0	0	2 **(25%)**	2**(25%)**	0	0	0	0	0	**8**
Reanimation tables	2 **(40%)**	0	0	1 **(20%)**	0	1 **(20%)**	0	0	1 **(20%)**	0	**5**
Changing tables	2 **(33.33%)**	0	0	2 **(33.33%)**	1 **(16.66%)**	1 **(16.66%)**	0	0	0	0	**6**
Taps	1 **(14.28%)**	0	0	2 **(28.57%)**	1 **(14.28%)**	1 **(14.28%)**	0	1 **(14.28%)**	0	1 **(14.28%)**	**7**
Benches	1 **(100%)**	0	0	0	0	0	0	0	0	0	**1**
Electric syringe pump	0	0	0	1 **(50%)**	0	1 **(50%)**	0	0	0	0	**2**
Treatment trolley	0	0	0	0	0	0	0	0	0	0	**0**
HCWs' hands	3 **(43%)**	0	0	2 **(29%)**	0	1 **(14%)**	1 **(14%)**	0	0	0	**7**
											47

In regard to the NICU, 20 of 34 multidrug resistant strains were isolated. ESBL Producing *Enterobacteriaceae* and MDR *A. baumannii* were omnipresent with a prevalence of 60% and 40% respectively (Table 8). Among the 12 strains of ESBL producing *Enterobacteriaceae, ESBL K. pneumoniae* were the most encountered species (66,6 %) followed by ESBL producing *E. coli* and ESBL producing *E. cloacae* with 25% and 8.3 % respectively (Figure 1).

Table 6. Antibiotic resistance profiles of Gram negative bacterial strains isolated from the adult intensive care unit. **Legend:** AK, Amikacin; AMC, Amoxicillin-clavulanat acid; AMP, Ampicillin; ATB, Antibiotic; CAZ, Ceftazidim; CIP, Ciproflxacin; COL, Colistin; CRO, Ceftriaxon; CTX, Cefotaxim; ESBL, Extended spectrum beta lactamase; FF, Fosfomycin; GN, Gentamicin; I, Intermediate; IMP, Imipenem; KF, Cefalotin; MDR, Multidrug-resistant; MEM, Meropenem; Nd, Not determinate; NET, Netilmicin; R, Resistant; S, Sensitive; T, Tetracyclin; TS, sulfamethoxazole/trimethoprim; TZP, Piperacillin-Tazobactam; -, The test of these antibiotics is not recommended by the guidelines of the Antibiogram Committee of the French Society for Microbiology.

ATB / Strains	TZP	CTX	CIP	KF	Col	CAZ	TS	IMP	MEM	CN	AK	NET	FF	T	AMC	AMP	CRO
MDR *A. baumanniii*																	
A. baumannii 1	R	R	R	R	S	R	R	R	R	R	S	S	R	R	R	R	R
A. baumannii 2	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	R
A. baumannii 3	R	R	R	R	S	R	R	R	R	S	S	S	R	R	R	R	R
A. baumannii 4	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	R
A. baumannii 5	R	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	R
A. baumannii 6	R	R	R	R	S	R	R	R	R	S	R	S	R	I	R	R	R
ESBL Producing *Enterobacteriaceae*																	
K. pneumoniae 1	S	R	R	R	S	R	R	S	-	S	S	-	Nd	-	I	R	R
K. pneumoniae 2	S	R	R	R	S	R	R	S	-	S	S	-	Nd	-	R	R	R
K. oxytoca	S	R	R	R	S	R	R	S	-	R	S	-	Nd	-	R	R	R
E. cloacae	S	R	S	R	S	R	R	S	-	S	S	-	Nd	-	R	R	R
E. coli	S	R	S	R	S	R	R	S	-	R	S	-	Nd	-	S	R	R
MDR *S. maltophilia*																	
S. maltophilia 1	-	R	S	R	-	R	S	R	-	R	R	-	R	-	R	R	R
S. maltophilia 2	-	R	S	R	-	R	S	R	-	R	R	-	R	-	R	R	R
S. maltophilia 3	-	R	S	R	-	R	S	R	-	R	R	-	R	-	R	R	R

Discussion

In ICUs, hospitalized patients are more vulnerable to nosocomial pathogens particularly MDR bacteria (Mora et al., 2016). These microorganisms may contaminate high touched surfaces, medical equipment and HCWs' hands leading to the spread of different types of NIs by cross-contamination (Galvin et al., 2012).

In our work, we focused on the study of the main suspect sites (i.e Surfaces, medical equipment and HCWs' hands) of two ICUs (NICU and AICU) of the RHCA-Morocco. Our results showed the predominance of Gram negative bacteria belonging to *Enterobacteriaceae* and non fermenting Gram-negative bacilli with a proportion of 84.21%. Similarly, Lalami et al (2016) in a hospital in Fez city (Morocco) reported a contamination with a frequency of 73.33% by Gram negative bacteria, and only 26.67% by Gram positives. In contrast, Tajeddin et al (2016) demonstrated that the proportion of Gram positive organisms (60.7 %) was greater than for Gram negative (39.3%) pathogens in the studied hospital inanimate environment.

Table 7. Antibiotic resistance profiles of Gram negative bacterial strains isolated from the neonatal intensive care unit. **Legend:** AK, Amikacin; AMC, Amoxicillin-clavulanat acid; AMP, Ampicillin; ATB, Antibiotic; CAZ, Ceftazidim; CIP, Ciproflxacin; COL, Colistin; CRO, Ceftriaxon; CTX, Cefotaxim; ESBL, Extended spectrum beta lactamase; FF, Fosfomycin; GN, Gentamicin; I, Intermediate; IMP, Imipenem; KF, Cefalotin; MDR, Multidrug-resistant; MEM, Meropenem; Nd, Not determinate; NET, Netilmicin; R, Resistant; S, Sensitive; T, Tetracyclin; TS, sulfamethoxazole/trimethoprim; TZP, Piperacillin-Tazobactam; -, The test of these antibiotics is not recommended by the guidelines of the Antibiogram Committee of the French Society for Microbiology.

Strains \ ATB	TZP	CTX	CIP	KF	Col	CAZ	TS	IMP	MEM	CN	AK	NET	FF	T	AMC	AMP	CRO
MDR *A. baumannii*																	
A. baumannii 1	R	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	R
A. baumannii 2	R	R	R	R	S	R	R	S	R	S	S	S	R	S	R	R	R
A. baumannii 3	R	R	R	R	S	R	R	S	R	R	S	S	R	R	R	R	R
A. baumannii 4	R	R	R	R	S	R	R	S	R	R	S	S	R	R	R	R	R
A. baumannii 5	R	R	R	R	S	R	R	S	R	R	S	S	R	R	R	R	R
A. baumannii 6	R	R	R	R	S	R	R	S	R	R	S	S	R	R	R	R	R
A. baumannii 7	R	R	R	R	S	R	R	R	R	S	S	S	R	S	R	R	R
A. baumannii 8	R	R	R	R	S	R	R	R	R	S	S	S	R	S	R	R	R
ESBL Producing *Enterobacteriaceae*																	
K. pneumoniae 1	S	R	I	R	S	R	R	S	-	R	S	-	-	-	R	R	R
K. pneumoniae 2	S	R	S	R	S	R	R	S	-	R	S	-	-	-	R	R	R
K. pneumoniae 3	S	R	S	R	S	R	R	S	-	R	S	-	-	-	R	R	R
K. pneumoniae 4	S	R	S	R	S	R	R	S	-	S	S	-	-	-	R	R	R
K. pneumoniae 5	S	R	S	R	S	R	R	S	-	S	S	-	-	-	R	R	R
K. pneumoniae 6	S	R	S	R	S	R	R	S	-	S	S	-	-	-	R	R	R
K. pneumoniae 7	I	R	S	R	S	R	S	S	-	R	S	-	-	-	R	R	R
K. pneumoniae 8	I	R	S	R	S	R	R	S	-	S	S	-	-	-	R	R	R
E. coli 1	S	R	S	R	S	R	R	S	-	S	S	-	-	-	R	R	R
E. coli 2	S	R	S	R	S	I	R	S	-	R	S	-	-	-	R	R	R
E. coli 3	S	R	S	R	S	R	R	S	-	R	S	-	-	-	R	R	R
E. cloacae	S	R	S	R	S	R	R	S	-	S	S	-	-	-	R	R	R

Among the 93 identified bacteria isolated from the two targeted ICUs, *A. baumannii* (20 % for the AICU and 38 % for NICU) *and K. pneumoniae* (17% for AICU and 28% for the NICU) were the most dominant species. Other opportunistic pathogens were isolated with varying proportion according to the species. This is consistent with a previous study which reported that *A. baumannii* (30%) were the most common pathogens among ICUs environmental isolates (Mehraban et al., 2016). A study conducted in 3 high risk units of a hospital in Morocco also showed a greater prevalence rate of *A. baumannii* (38%) followed by *P. aeruginosa* (26%), coagulase negative *Staphylococci* (17%) and *Klebsiella* sp (8%) (Lalami et al., 2015). The contamination of the ICU's environment could be related to the ability of isolated pathogenic bacteria to survive in dry surfaces even for months while preserving their capacity to multiply and infect (Kramer et al., 2006). This specificity is owing to their production of adhesion molecules and biofilms formation as well as their intrinsic microbiologic characteristics which provide the resistance to disinfectants (Russotto et al., 2017). Indeed, Gram negative bacteria own an outer membrane serving as a barrier and protecting the bacterial cell from the uptake of disinfectants (Russell et al., 1999). These facts may explain the predominance of *A. baumannii* strains in the environment of the two studied high risk units. Unlike most Gram negative bacteria, this microorganism presents a great resistance to desiccation and can persist on dry surfaces for 4-5 months (Otter et al., 2011). In addition, *A. baumannii* resists the exposure to commonly used disinfectants like Chlorhexidine, gluconate, and phenols, especially if it's not used in the appropriate concentrations (Gallego et al., 2001). Indeed, it has been shown that *A. baumannii* carries efflux protein named *Acinetobacter* Chlorhexidine (AceI) which actively pump Chlorhexidine out of the cell (Hassan et al., 2013).

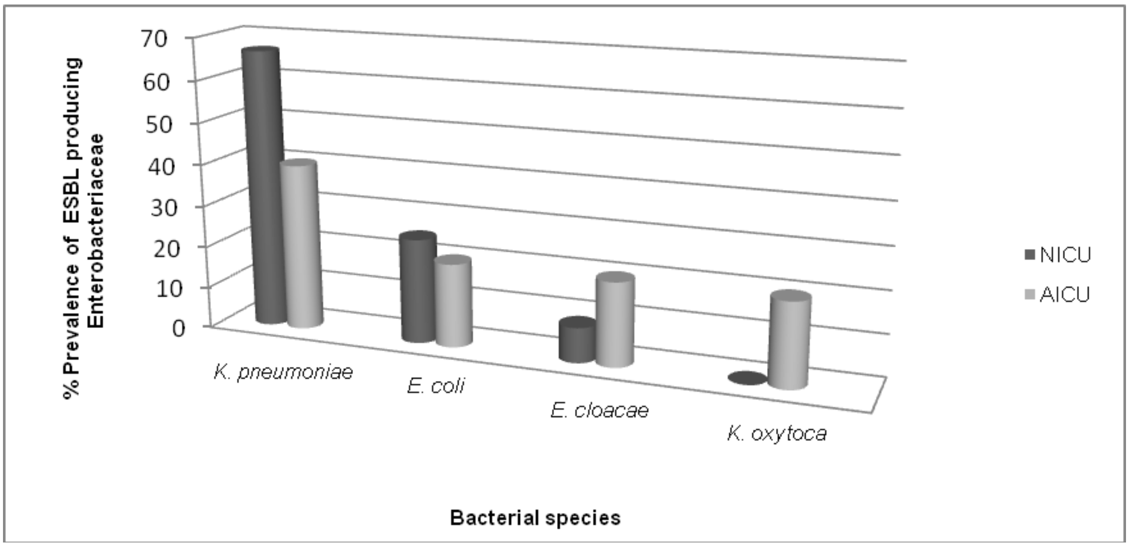

Figure 1. Prevalence of ESBL producing Enterobacteriaceae strains in adult and neonatal intensive care units. AICU: Adult intensive care unit, NICU: Neonatal intensive care unit.

Table 8. Prevalence of multidrug resistant bacteria isolated from adult and neonatal intensive care units. AICU, Adult intensive care unit; ESBL, Extended spectrum beta Lactamase; MDR, Multidrug-resistant; NICU, Neonatal intensive care unit.

MDR bacteria / Services	*ESBL producing Enterobacteriaceae*	*MDR A. baumannii*	*MDR S. maltophilia*	Total
AICU	5 (35.7%)	6 (42.8%)	3(24.4%)	14
NICU	12 (60%)	8 (40%)	0 (0%)	20

Moreover, our data demonstrated that all identified bacterial species are specially encountered in the vicinity of patients and mostly in high touched surfaces (HCWs' wash basin, reanimation table, changing table) and medical equipment (tubing junction/jar junction, baby scales). This contamination may occur as a result of direct patient shedding (infected or colonized patients) of bacteria or by HCW's hands (Russotto et al., 2017). In fact, some bacterial species were both isolated from the hospital environment (Surfaces and medical devices) and from HCW's hands of the studied ICUs. It was demonstrated that the inanimate environment could play a significant role on the contamination of HCW's hands (Bhalla et al., 2004), and increased evidence incriminated these latter in cross contamination (Longtin et al., 2011; Sax et al., 2007; Pittet et al., 2006). Besides, it should be considered that nosocomial pathogens can persist in HCWs' hands in absence of appropriate hand hygiene actions (Pittet et al., 2006), and thereby perpetuate the transmission chain (Oliveira and Damasceno, 2010).

The omnipresence of MDR bacteria is a serious problem in ICUs. Previous studies confirmed their ubiquity in the hospital environment (Bakkali et al., 2016; French et al., 2004; Dumford et al., 2009; Amiyare et al., 2015; Azizi et al., 2014). Bacterial species are exposed to an extreme selective pressure due to uncontrolled antibiotic therapies.

Therefore, they develop or acquire resistance mechanisms that allow them to survive in the presence of a wide range of antibiotics (Chemaly et al., 2014). In the two studied ICUs, 34 MDR bacteria were isolated. All of them were Gram negative bacilli and presented a great resistance to ß-lactams groups.

In the AICU, the most frequently encountered MDR species are *A. baumannii* (47%). This microorganism is considered as a major opportunistic pathogen and involved in severe NIs, especially in ventilator-associated pneumonia which is predominant in ICUs patients with mechanical ventilation (Custovic et al., 2014). This virulence is mostly due to its natural resistance to a large range of antibiotics (Howard et al., 2012). The isolated MDR strains of *A. baumannii* revealed a resistance to carbapenems (imipenem and meropenem). This resistance is, in itself, sufficient to describe *A. baumannii* strains as extremely resistant (Kluytmans-Vandenbergh et al., 2005). Parallelly, a study found a high resistance rate (94%) to imipenem among *A. baumannii* isolates from the environment samples (Tajeddin et al., 2016). In the targeted NICU, ESBL Producing *Enterobacteriaceae* were the most prevalent MDR pathogens with a frequency of 60% (12/20). A prior investigation in Algeria reported that the prevalence rate of ESBL Producing *Enterobacteriaceae* strains isolated from the hospital environment reached 21.35% (Debabza et al., 2014). In our study, the isolated ESBL Producing *Enterobacteriaceae* strains presented a high resistance to ß-lactams notably oxyimino-cephalosporins (i.e., cefotaxime, ceftriaxone, ceftazidime). These resistant bacteria remain an important reason for therapy failures with serious consequences for infection control (Paterson and Bonomo, 2005) and are recognized to be a major cause of outbreaks (Lucet et al., 1999). The most prevalent species among isolated ESBL Producing *Enterobacteriaceae* were ESBL producing *K. pneumoniae* (66.6%) followed by ESBL producing *E. coli* (25%). This is in agreement with previous studies (Guet-Revillet et al., 2012; Freeman et al., 2014; Afifi, 2013) which reported that ESBL producing *K. pneumoniae* was more incriminated in hospital environment's contamination than ESBL producing *E. coli* in an *Enterobacteriaceae* family.

Under the fight against NIs, our data emphasize the major risk of this public health problem. It reinforced the necessity of knowledge and control of the main sources of nosocomial pathogens in the hospital environment.

Conclusion

The bacteriological study conducted in adult and neonatal intensive care units of the RHCA-Morocco showed the presence of different nosocomial bacteria in surfaces, medical equipment and healthcare works' hands. It also confirmed the prominent role of hospital environment in the spread of nosocomial pathogens. Indeed, bacterial strains mainly belonging to *Enterobacteriaceae* and non fermenting Gram negative bacilli were isolated. 34 of 93 identified bacteria were multidrug resistant. The cross-transmission of these pathogens to immunocompromised patients may lead to severe nosocomial infections.

To overcome this major public health problem, it's necessary to reinforce the surveillance system applied in the studied hospital. It has to continuously report the prevalence of harmful microorganisms, especially in high risk units. A protocol of rigorous cleaning and disinfecting procedures must be established to reduce the risk of contaminations as well as the awareness of HCWs to adopt preventive measures via hospital hygiene trainings.

Acknowledgments

The authors would like to thank the Regional Hospital Center of Agadir, and all the medical staff of the adult and the neonatal intensive care units for their collaboration. Our gratitude goes to the staff of the Laboratory of Medical Analysis, and the team of the nosocomial infection control committee of the RHCA.

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