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Hospital Acquired Infection: Bacteriological Profile of Species from Environmental Surfaces of Cotonou 5 Hospital in South Benin (West Africa)

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A B S T R A C T

Hospital environment is sometimes the reservoir of pathogens that can be passed on to patients in different ways. The emergence of multi-resistant bacteria aggravates this situation, especially in a context of very limited therapeutic options. The objective of this study was to identify the bacterial species present on the environmental surfaces of the Cotonou 5 Hospital in order to control the contamination and the spread of pathogens. This aim was achieved by examining 165 samples, 135 (81.82%) of which had positive bacterial culture. The frequency of isolation was predominant in the intensive care unit (95%), emergency department (95%) and paediatrics (85%). In addition 49.77% of the positive bacterial cultures were Gram-positive and 50.20% were Gram-negative bacteria. *Acinetobacter baumannii*, *Bacillus* spp., *Escherichia coli* and *Staphylococcus aureus* were identified in all surveyed hospital units. Among the Gram-negative isolates, Enterobacteriaceae were more abundant (31.19%), followed by Non-fermenting gram-negative bacilli (16.75%). This study revealed that different bacterial species were present on the hospital surfaces of the Cotonou 5 Hospital. It is a reflection of the level of implementation of hospital hygiene rules and the risk of the resulting healthcare-associated infections. However, the spread of pathogenic strains can be controlled by appropriate hospital hygiene measures.

Introduction

Healthcare-associated infections continue to be a major cause of morbidity and mortality for patients in hospitals (Weber *et al.,* 2010). It is a public health problem that has important economic consequences as well (Defez *et al.,* 2008; Hamza, 2010). The various pathogens of these infections sometimes have an endogenous or exogenous origin with various risk factors (Parneix, 2010; Alonso-Aguilar *et al.,* 2017). The endogenous flora of patients is a significant source of nosocomial pathogens. However, about 20−40% of healthcare-associated infections are transmitted by the hands of health personnel previously contaminated by another infected patient (Weber *et al.,* 2010). Health personnel are often a reservoir of pathogens and sometimes infect patients with Methicillin-resistant *Staphylococcus aureus* (MRSA) present in their nostrils (Ragini *et al.,* 2018; Sangeetha *et al.,* 2018). Despite advances in healthcare safety, the hospital environment contributes to the spread of pathogens (Oliveira and Damasceno, 2010). Thus, microbiological controls for the identification of germs in hospitals allow the resulting health problems to be managed and mitigated (Oliveira and Damasceno, 2010). In Benin, while healthcare-associated infections exist, they have not been extensively studied, and research on their magnitudes in hospitals is particularly limited. However, according to a study of patients admitted to a Benin hospital in 2012, 9.84% of this population acquired a nosocomial infection (Ouendo *et al.,* 2015). Of all the germs responsible for infection, bacteria play the most important role (Marty, 2010). The pathogens observed in the hospital are mainly staphylococci and gram-negative bacteria (*Enterobacteriaceae*, *Pseudomonas*, *Acinetobacter* and related bacteria) (Soussy, 2010). Non-fermenting Gram-negative bacilli (NFGNB) living in the hospital environment are also a major source of hospital infections (Siddiqui *et al.,* 2018). Other bacteria are multi-resistant and create real difficulties for health personnel, especially if their therapeutic options are limited (Cornejo-Juárez *et al.,* 2015). Thus, MRSA has a particularly important progression in hospitals (Hsu *et al.,* 1988), as these staphylococci and vancomycin-resistant enterococci (VRE) can survive for several days on hospital surfaces (Boyce, 2007).

According to Boyce *et al.,* (1997), 27% of the 350 surfaces sampled in the rooms of infected patients as a part of their investigation were contaminated with MRSA. For the safety of patient care, knowledge of the bacteriological quality of hospital surfaces is thus highly important.

This provides information on the presence of nosocomial pathogens and the quality of hygiene in the hospital environment (Oliveira and Damasceno, 2010; Galvin *et al.,* 2012; Hassan *et al.,* 2015). Thus, the aim of the present study was to determine the bacterial contamination of various surfaces of Cotonou 5 Hospital in a dynamic fight against healthcare-associated infections.

Materials and Methods

An analytical cross-sectional study was carried out from the surface samples of the Cotonou 5 hospital in South Benin (West Africa), in order to identify the presence of bacterial species.

The work spanned two months (March to April 2017). The samples were taken from various sites in the hospital environment. Services concerned were: Hospitalization adults, Maternity, Emergency services, Operating Theater, Intensive Care Unit. Bacteriological testing was carried out at the National Laboratory of Biomedical Analysis of the Ministry of Health (Benin).

Methods

Sample collection

The 165 samples were taken at different environmental surfaces according to the hospital's survey services (Table 1). Samples were taken in the morning one hour after the cleaning and disinfection of the room. For some services (surgery room), the samples were taken after disinfection of the room without its prior use according to ISO 14698-1: 2003. The sterile swabs were moistened with sterile distilled water and passed in parallel striations on the surface by turning them slightly, then in perpendicular striations on the same zones.

Subsequently, the swabs were returned to their protective cases and transmitted to the laboratory within one hour. We obtained a total of 165 samples from the 54 selected sites across the 6 investigative services. The number of samples varied between 2 and 4 per site.

Isolation

The samples were emulsified in haemolysis tubes with 5 ml of Brain Heart broth (BHB) and incubated at 37°C for 24 hours. Bacterial growth manifested in the form of turbidity. Each sample showing turbidity was streaked on Eosin Methylene-Blue, Chapman and MH medium and incubated at 37°C for 24 hours.

Identification

Gram staining was carried out directly on isolated colonies. Colonies with Gram-negative bacilli (GNB), Gram-positive bacilli (GPB) and Gram-positive cocci (GPC) were then selected. After purification, biochemical identification of GNB was carried out by seeding the API 20 E gallery. For the identification of *S. aureus*, catalase, coagulase and DNase tests were carried out on Gram-positive cocci. Catalase, oxidase, and urease tests were also performed on sporulate large Gram-positive bacilli for the identification of *Bacillus* spp.

Statistical analysis

All statistical analyses were performed using SPSS Statistics 21 software. Fisher's exact test was used to test the association between the Results of culture samples and different services at a 0.05 level of significance.

Ethics approval and consent to participate

The study was approved by the direction of Cotonou 5 Hospital (N/R N°019/CSRHI/ DIR/HM/17) dated 13 th January 2017). In the laboratory, protection and safety measures were guaranteed.

Results and Discussion

Of the total 165 samples analysed, 81.82% (n = 135) were positive for bacteria and 18.18 % (n = 30) negative (Table 1).

The distribution by services is presented in Table 1. The greatest frequency of bacterial isolation was observed in Emergency (95%) and the lowest proportion was found in the Operating Theater (65.71%). There was not a statistically significant association between the Results of culture samples and different services (F= 10.04; p=0.06).

Biochemical identification

The study of biochemical characteristics revealed 49.77% Gram-positive bacteria (GPB), and 50.20% Gram-negative bacteria (GNB) (Table 2). Gram-positive bacteria consisted of 3 different species. There were staphylococci (27.15%) and *Bacillus* (22.62%). Gram-negative bacteria had 27

different species. There were 31.19% *Enterobacteriaceae*, 16.75% Non-fermenting Gram-negative bacilli (NFGNB). Only 2.26% isolate belong to another genus of Gram Negative *Bacilli* was identified (Table 2).

Repartition of species identified according to the service

Species identified in survey services are presented in table 3. *Acinetobacter baumannii* (6.79%), *Bacillus* spp (22.62%), *Escherichia coli* (5.86%) and *Staphylococcus aureus* (18.55%) were present in all services (Table 3).

Distribution of Gram-negative *bacillus* strains in categories of bacteria

There were a total of 111 Gram-negative strains. *Enterobacteriacecae* (62.16%), NFGNB (33.33%) and 4.50% of other GNBwere identified (Figure 1).

Distribution of identified GNB strains according to genus

There were 69 genera that constituted the 111 strains of bacteria from Gram-negative bacillui group identified in the study. The most frequent were *Enterobacter* (14.41%), followed by *Acinetobacter* (13.51 %) (Figure 2).

Identified species of Gram-negative bacilli

Gram-negative bacteria species are presented in Table 4. The predominant species were *Acinetobacter baumannii* (13.51%).

Distribution of distribution of NFGNB

The NFGNB strains consisted of 18 species. *Acinetobacter baumannii* (40.54%) and *Pseudomonas oryzihabitans* (27.02%) are the most represented (Figure 3).

Inoculation of surface samples collected in Cotonou 5 Hospital yielded 81.82% positive bacterial cultures. The highest bacterial culture frequencies (95%) were recorded for the intensive care unit and the emergency department. The traffic of the nursing staff and visitors in these hospital areas increases the likelihood of pathogen dissemination. The quality of air in these units is also important, as the airborne particles settle on the surfaces more quickly because they are bulky. However, the percentage of positive bacterial cultures obtained in the present study (81.82%) is significantly higher than that (31.4%) noted in a public hospital of Boufarik, Blida-Algerie (Kais *et al.*, 2015). Overall, 18.18% of bacterial cultures were negative, with the majority of such cultures found in the operating room. The negative bacterial culture percentage obtained in this study is greater than 14% found at El Idrissi hospital in Kenitra, Morocco (Saouide *et al.*, 2014). However, it is lower than 68.60% obtained by Kais *et al.*, in their study. In Cotonou 5 Hospital, there is no statistically significant association between the culture findings pertaining to different surface samples and the hospital units in which these samples were taken ($F = 10.04$, $p = 0.06$). Microorganisms were identified in all departments and there were 49.77% Gram-positive bacteria and 50.20% Gram-negative bacteria. *Acinetobacter baumannii*, *Bacillus spp.*, *Escherichia coli* and *Staphylococcus aureus* were consistently identified in all Cotonou 5 Hospital units (Table 3). *Staphylococcus aureus* colonizes frequently used hospital surfaces (Subbalakshmi, 2018). The omnipresence of coagulase-negative *Bacillus spp.* and *Staphylococcus* was also seen in the flora of the surfaces of El Idrissi Hospital in Kenitra, Morocco (Saouide *et al.*, 2014). In the Cotonou 5 Hospital that served as the study site in this work, *Staphylococcus* (27.15%) and *Bacillus* (22.62%) were predominant.

Fig.1 Distribution of 111 Gram-negative bacilli strains according to categories of bacteria

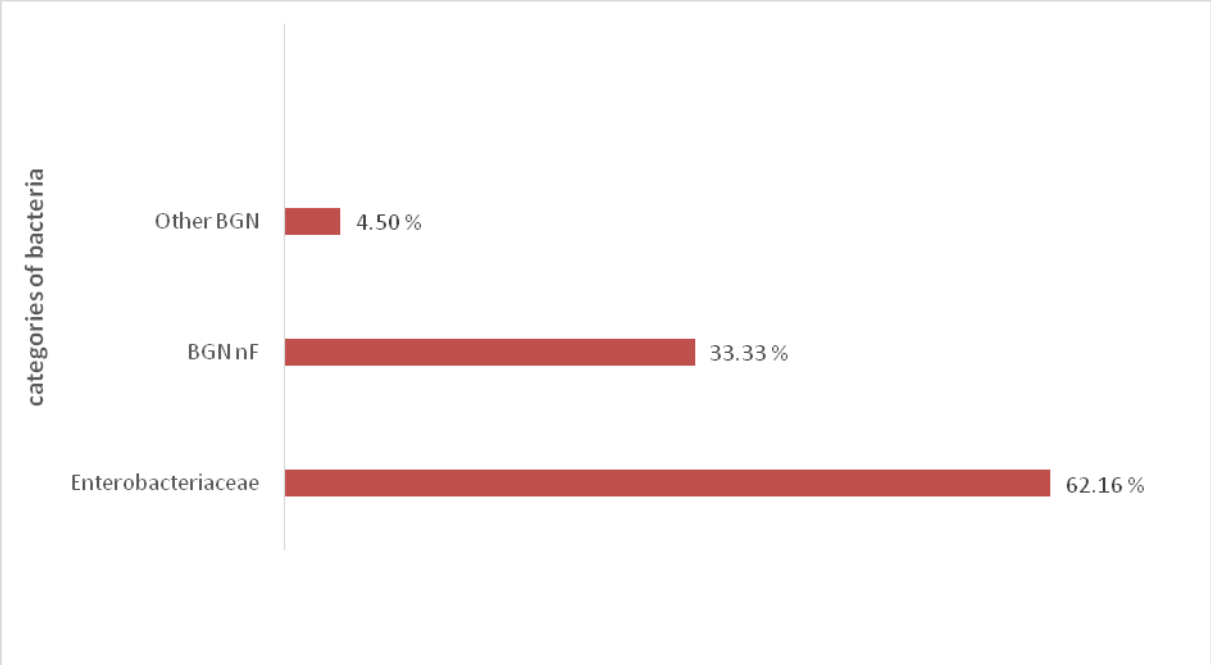

Fig.2 Distribution of Gram-negative bacilli according to genus

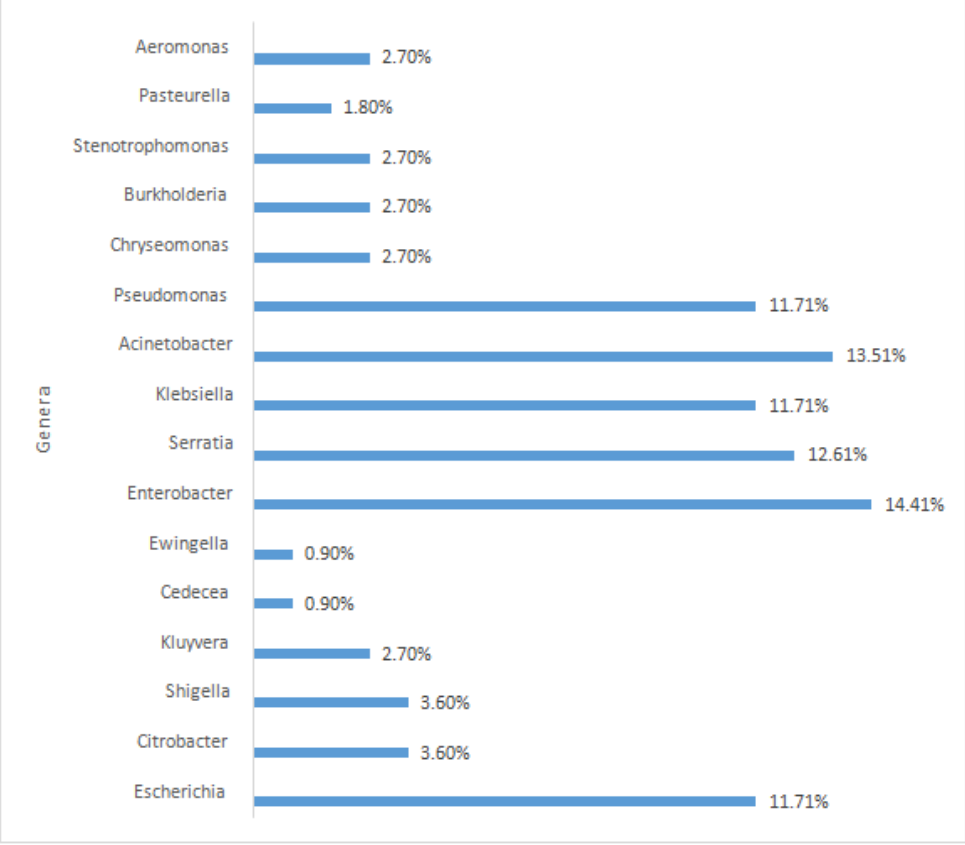

Fig.3 Distribution of the NFGNB

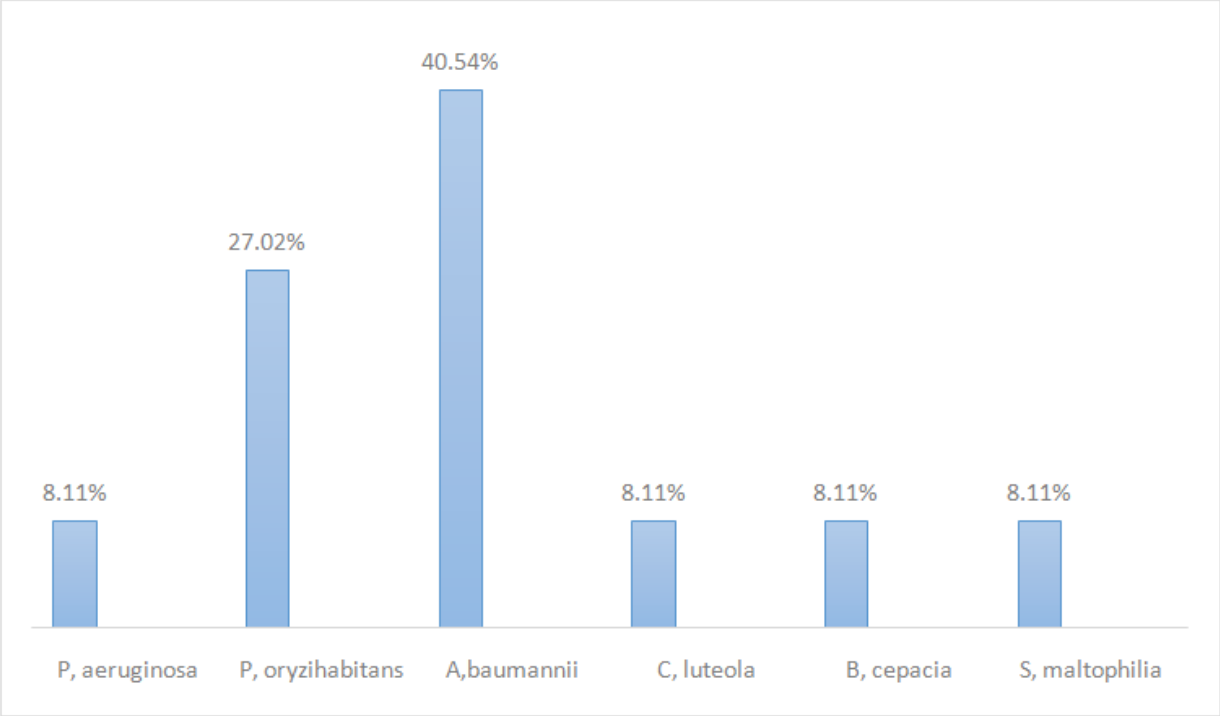

Table.1 Results of culture samples

Services	Results of culture		Numbers
	(-)	(+)	
Operating Theater	12 (34.29 %)	23 (65.71 %)	35
Intensive Care Unit	01 (5 %)	19 (95 %)	20
Maternity	05 (16.67 %)	25 (83.33%)	30
Hospitalization adults	08 (20 %)	32 (80 %)	40
Pediatrics	03 (15 %)	17 (85 %)	20
Emergency services	01 (5 %)	19 (95 %)	20
Total	30 (18.18 %)	135 (81.82 %)	165

(-) = negative environmental culture
(+) = positive environmental culture

Table.2 Repartition of species identified according to the group of bacteria

Types of bacteria	Group	Genus	Species	Number of species	% of species	Number of genus	% of genus	Number of group	% of group
BGP	-	**Bacillus**	*Bacillus spp*	50	22.62	50	22.62	50	22.62
	Staphylococcus	***Staphylococcus***	*Staphylococcus aureus*	41	18.55	60	27.15	60	27.15
			Staphylococcus spp	19	8.60				
BGN	Enterobacteriaceae	***Klebsiella***	*Klebsiella pneumoniae*	2	0.90	13	05.87	69	31.19
			Klebsiella oxytoca	4	1.81				
			Klebsiella rhinoscleromatis	2	0.90				
			Klebsiella terrigena	1	0.45				
			Klebsiella ornithinolytica	4	1.81				
		Serratia	*Serratia ficaria*	5	2.26	14	06.33		
			Serratia marcescens	4	1.81				
			Serratia odorifera 1	2	0.90				
			Serratia rubidaea	3	1.36				
		Enterobacter	*Enterobacter aerogenes*	2	0.90	16	07.23		
			Enterobacter cloacae	8	3.62				
			Enterobacter sakazakii	5	2.26				
			Enterobacter intermedius	1	0.45				
		other germs	*Escherichia coli*	13	5.88	26	11.76		
			Citrobacter freundii	4	1.81				
			Shigella spp	4	1.81				
			Kluyvera spp	3	1.36				
			Cedece alapagei	1	0.45				
			Ewingella americana	1	0.45				
	NFGNB		*Acinetobacterbaumannii*	15	6.79	37	16.75	37	16.75
			Pseudomonas aeruginosa	3	1.36				
			Pseudomonas oryzihabitans	10	4.52				
			Chryseomonas luteola	3	1.36				
			Burkholderia cepacia	3	1.36				
			Stenotrophomonas maltophilia	3	1.36				
	Other GNB		*Pasteurella pneumotropica*	2	0.90	5	02.26	5	02.26
			Aeromonas salmonicida	3	1.36				
TOTAL			**30 espèces**	**221**	**99.97**	**221**	**99.97**	**221**	**99.97**

Types and groups of bacteria: GPB: Gram-positive bacteria; GNB: Gram-negative bacteria; NFGNB: Non-fermenting gram-negative bacilli

Table.3 Repartition of species identified according to the services

species	Number of species according to the service						Number of species	% of species
	A	B	C	D	E	F		
Acinetobacter baumannii	2	2	3	1	1	6	15	6.79
Aeromonas salmonicida	1	-	1	-	1	-	3	1.36
Bacillus spp	16	11	5	3	11	4	50	22.62
Burkholderia cepacia	-	-	2	1	-	-	3	1.36
Citrobacter freundii	-	2	1	-	1	-	4	1.81
Chryseomonas luteola	1	-	-	1	1	-	3	1.36
Escherichia coli	1	5	2	1	1	3	13	5.88
Enterobacter aerogenes	-	-	-	1	1	-	2	0.90
Enterobacter cloacae	1	1	-	2	1	3	8	3.62
Enterobacter sakazakii	1	1	1	1	-	1	5	2.26
Enterobacter intermedius	-	1	-	-	-	-	1	0.45
Klebsiella pneumoniae	-	1	-	-	-	1	2	0.90
Klebsiella ornithinolytica	2	-	2	-	-	-	4	1.81
Klebsiella rhinoscleromatis	-	-	1	1	-	-	2	0.90
Klebsiella oxytoca	1	1	2	-	-	-	4	1.81
Klebsiella terrigena	1	-	-	-	-	-	1	0.45
Ewingella americana	-	-	1	-	-	-	1	0.45
Cedecealapagei	-	-	1	-	-	-	1	0.45
Kluyvera spp	-	1	-	1	1	-	3	1.36
Pasteurella pneumotropica	-	1	1	-	-	-	2	0.90
Pseudomonas oryzihabitans	2	-	2	3	1	2	10	4.52
Pseudomonas aeruginosa	2	1	-	-	-	-	3	1.36
Serratia ficaria	1	1	1	1	-	1	5	2.26
Serratia marcescens	-	2	1	1	-	-	4	1.81
Serratia odorifera 1	-	-	1	-	1	-	2	0.90
Serratia rubidaea	-	1	1	-	1	-	3	1.36
Shigella spp	2	1	1	-	-	-	4	1.81
Staphylococcus aureus	11	6	5	7	3	9	41	18.55
Staphylococcus spp	-	2	2	6	6	3	19	8.60
Stenotrophomonas maltophilia	1	1	-	-	-	1	3	1.36
Total	**46**	**42**	**37**	**31**	**31**	**34**	**221**	**99.97**

Services : A : Hospitalization adults; B : Maternity ; C : Pediatrics ; D : Emergency services; E : Operating Theater; F : Intensive Care Unit.

Table.4 Number of Gram-negative bacilli identified in the sampling of hospital surfaces

species	Number of species	% of species
Klebsiella pneumoniae	2	01.80
Klebsiella oxytoca	4	03.60
Klebsiella rhinoscleromatis	2	01.80
Klebsiella terrigena	1	00.90
Klebsiella ornithinolytica	4	03.60
Serratia ficaria	5	04.50
Serratia marcescens	4	03.60
Serratia odorifera 1	2	01.80
Serratia rubidaea	3	02.70
Enterobacter aerogenes	2	01.80
Enterobacter cloacae	8	07.21
Enterobacter sakazakii	5	04.50
Enterobacter intermedius	1	00.90
Escherichia coli	13	11.71
Citrobacter freundii	4	03.60
Shigella spp	4	03.60
Kluyvera spp	3	02.70
Cedecea lapagei	1	00.90
Ewingella americana	1	00.90
Acinetobacter baumannii	15	13.51
Pseudomonas aeruginosa	3	02.70
Pseudomonas oryzihabitans	10	09.01
Chryseomonas luteola	3	02.70
Burkholderia cepacia	3	02.70
Stenotrophomonas maltophilia	3	02.70
Pasteurella pneumotropica	2	01.80
Aeromonas salmonicida	3	02.70
Total	**111**	**99.94**

This prevalence was also recorded in 2009 in Alzahra Hospital, Isfahan, Iran (Jalalpoor *et al.*, 2009).

In the group of identified staphylococci, there is a preponderance of *Staphylococcus aureus* (18.55%) against 08.60% *Staphylococcus spp*. This proportion of *Staphylococcus aureus* is low compared to the 20% found on the surfaces of El Idrissi hospital by Saouide *et al.*, (2014). MRSA are particularly worrisome in hospitals (Hsu *et al.*, 1988). MRSA is also identified in clinical specimens, including pus, obtained from several patients (Rozina *et al.*, 2018). *Staphylococcus aureus* is a pathogen that survives for months on dry surfaces (Axel *et al.*, 2006), and methicillin-resistant Staphylococcus aureus (MRSA) survives for several days in hospital settings (Huang *et al.*, 2012).

In a study examining the relationship between environmental contamination with MRSA and its acquisition by patients, 35% of MRSA

isolated from patients and their environment was indistinguishable (Hardy *et al.,* 2006). The identification of *Staphylococcus aureus* species in the environment of Cotonou 5 Hospital indicates the existence of the infectious risk associated with this pathogen. Based on the analysis results, *enterobacteria* were predominant (62.16%) in Gram-negative bacilli, followed by 33.33% non-fermenting Gram-negative bacilli (NFGNB). Gram-negative bacilli (GNB) acquire genes that code for antibiotic resistance mechanisms and thus exacerbate the resulting infections (Ruppé *et al.,* 2015). The rate of *enterobacteria* isolated in our study is higher than the 10.70% isolated by Jalalpoor (2011) and is lower than 74.51% reported by Debabza (2015). Among the isolated GNB species, *Acinetobacter baumannii* is predominant (13.51%), followed by *Escherichia coli* at11.71% (Table 4). *Escherichia coli* is a formidable pathogen in certain infections, including those of the urinary tract (Eman *et al.,* 2018), sepsis, pneumonia, neonatal meningitis (Hassan *et al.,* 2015, Lausch *et al.,* 2013). In the group of NFGNB, *Acinetobacter baumannii* (40.54%) is predominant, followed by *Pseudomonas oryzihabitans* (27.02%) (Figure 3). These NFGNB are also found in hospital-based fluid specimens and have been identified in 121 NFGNB for 1850 clinical specimens in tertiary care hospital (Siddiqui *et al.,* 2018). NFGNB are bacteria that are generally considered opportunistic pathogens selected by repeated and prolonged antibiotic treatments (Ferroni *et al.,* 2003). *Acinetobacter baumannii* is resistant to several antibiotics (Howard *et al.,* 2012, Weber *et al.,* 2015), and its epidemic distribution is due to its manual transmission and survival in the hospital environment (Lahsoune *et al.,* 2007; Ducel *et al.,* 2002). The level of *Acinetobacter baumannii* identified in NFGNB in our study is higher than the 28.57% reported by Liazid (2016) for Tlemcen hospital in Algeria. *Acinetobacter baumannii* can persist for three days to five months and can survive on dry surfaces (Kramer *et al.,* 2006, Otter *et al.,* 2011). It can cause high mortality, up to 35% (Antunes *et al.,* 2014). The diversity of the germs identified in the analysis of hospital surfaces at Cotonou 5 Hospital provides information on its quality of hospital hygiene. The presence of *Acinetobacter baumannii, Bacillus spp., Escherichia coli* and *Staphylococcus aureus* in various units reflects hygiene inadequacies in the hospital. Thus, improvements to the cleaning practices and disinfection of hospital surfaces are essential (Weber *et al.,* 2013). Use of 1% hypochlorite is recommended for this purpose (Subbalakshmi, 2018).

Severe healthcare-associated infections constitute a challenge for patient safety in hospitals. Several germs are responsible. Our study revealed the presence of nosocomial pathogens on hospital surfaces of Cotonou 5 Hospital. In this context, the identification of a variety of germs, including *Bacillus* spp. and *Escherichia coli* in certain hospital units signifies insufficient quality of cleaning and disinfection of the patient's environment. This bacterial contamination of the hospital environment requires better management of the infectious risk and reinforcement of the rigorous hospital hygiene measures.

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