

Multidrug Resistant Bacteria in the Hospital Environment: Threats for Health Professionals and Patients

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Abstract:

Introduction: The contamination of the environment by a micro-organism differs according to the establishment, the services and the care practiced. The main objective of this study is to identify bacteria in the hospital environment of the University Hospital Center of Anosiala that may be responsible of nosocomial infections.

Methods: This is a prospective study realized at the Anosiala Teaching Hospital over a period of 2 weeks from 14 to 25 February 2018. The duration of the study was 4 months from February to May 2018.

Results: 69 samples were taken from the 4 departments including the intensive care unit (ICU), the Pediatric Surgery Department, the emergency and the operating room. Of these samples, 48 (69,6%) samples were positive to the groups of germs studied (non *aureus* *Staphylococcus*, *Staphylococcus aureus*, enterobacteria, as well as non-fermentative bacteria from the environment) and 21 (30,43%) samples were negative. The service most concerned by the contamination was pediatric surgery followed by ICU and the operating room.

Conclusion: Bacteria isolated from the environment of Anosiala show the importance of hygiene. Reinforced hygiene and cleaning measures will have to follow in order to reduce and eliminate these microorganisms in the context of hospital hygiene and the protection of patients or the staff against the acquisition of an infection hospital.

Keywords - Bacteriology, Environment, Hospital Hygiene, Nosocomial Infections, Surface Sampling.

I. INTRODUCTION

The hospital environment is largely contaminated by micro-organisms of human or specifically environmental origin [1, 2]. This contamination varies qualitatively and quantitatively over time, from one institution to another and, within the same establishment, according to the services, patients care and techniques practiced. The contamination of the environment by microorganisms raises the question of their responsibility in the genesis of nosocomial infections (NCIs) [3]. In this study, the target germs will be the germs found in NCIs and resistant to antibiotics, such as Methicillin resistant *Staphylococcus aureus* (MRSA),

Coagulase negative *Staphylococcus* (CNS) resistant to methicillin, extended spectrum beta-lactamase producing Enterobacteria (ESBL-E), Imipenem-resistant *Acinetobacter baumannii* (*A. baumannii*), and multidrug-resistant (MDR) *Pseudomonas aeruginosa*.

Bacteria in the *patient's* environment can be classified into two groups, including human bacteria (from the skin and mucous membranes), including MRSA, ESBL-E or Enterococci resistant to vancomycin (ERV) [4] and environmental bacteria some of which have frequent natural resistance to antibiotics, including Gram-negative bacilli (GNB) such as *P. aeruginosa*, *A. baumannii*,

Stenotrophomonas maltophilia (*S. maltophilia*), *Burkholderia cepacia* (*B. cepacia*), *Legionella pneumophila* or atypical mycobacteria.

No bacteriological studies of the hospital environment were made in the University Hospital Center (UHC) Anosiala. According to our knowledge, no other studies have been carried out in the other Antananarivo UHCs.

The aims of this study is to identify MDR bacteria that may be at the origin of an NCIs in the hospital environment of the University Hospital Center of Anosial and to submit suggestions for hygiene measures, cleaning and disinfection of materials according to the results found.

II. MATERIAL ET METHODS

This is a descriptive prospective study conducted at the UHC Anosiala over a 2-week period from 14 to 25 February 2018. The total duration of the study was 4 months from February to May 2018.

A sampling plan was done before sampling. The places chosen were the dirtiest, the most manipulated, the closest to the patients. In the meantime, a list of places and sampling sites has been drawn up. Among the places, the Emergency, the reanimation services, the Pediatric surgery and the operating room were chosen. In these places, the door handles, the feet of the bed, the switches, the keys, and the oxygen regulators, the tables of office, the materials used by the doctors such as the stethoscope, the staplers and the cabinets were sampling. In the operating room, operating tables, anesthesia equipment, feet and armrests, sterile and soiled instrument tables were sampling.

All sampling site were classified in sector according to the risk of contamination: the sector 1 was sector with minimal risks like the offices, the sector 2 with medium risks like the external consultation represented by Triage, the sector 3 with severe risks such ICU, Emergency, septic and obstetric operating room, bathroom and the sector 4 with very high risk such as neonatology and oncology represented in this study by pediatric surgery.

During this study, surface samples were taken at the Anosiala. These samples are taken by biologists and are carried out using a sterile dry swab (COPAN) moistened with sterile physiological water. For flat surfaces, swab approximately 16 cm². The sampling area is measured and then delimited with a masking tape. The swabbing will be done inside the sampling area. Collect tight streaks and swab perpendicular to previous streaks.

Ninety six samples were collected. The number of samples per site was defined before the start of the study. In the ICU, nineteen swabs were performed. They are divided into four different rooms including a doctor's office (three samples), a common room for medical reanimation (six samples), a common room for surgical reanimation (six samples) and a fee room (four samples). In the ATU service, twelve samples were taken in three locations, including a doctor's office (five samples), a decoating room (four samples) and a public toilet (three samples). In the operating room department, twenty samples, distributed between the septic surgical ward of visceral surgery (ten samples), the Traumatology-Orthopedic operating room (ten samples) and the outside of the block (Three samples) were performed. In the pediatric surgery department, fifteen samples were collected, including three samples taken from the doctors' office, four samples taken from the special category room, three samples taken from a common room (4th category), three samples taken from a paying room and two samples taken from the ward public toilets.

The sample is sent quickly to the laboratory to be discharged successively on selective media. These media are ChromID MRSA (Biomérieux, France) for the detection of MRSA that are green on the medium and white coagulase-negative staphylococci (CNS), ChromID ESBL (Biomérieux, France) for the detection of ESBL-E and ChromID CARBA/OXA (Biomérieux, France) for the detection of Carbapenemase producing enterobacteria (CPE) and oxacillinase producing enterobacteria. All the agar culture media are incubated at 37°C for 24-48 hours.

Bacteria growing on ChromID MRSA media were identified using the catalase and coagulase assays in which positive catalase and coagulase positive strains are affiliated with *S. aureus* while catalase positive but Coagulase negative test are defined as NCS.

The bacteria growing on ESBL and CARBA / OXA media are identified by seeding Api 20E test (Biomérieux, France). The API 20E gallery is reserved for the negative oxidase strains for enterobacteria, *Acinetobacter spp* and *P. aeruginosa*. API 20NE has been used for positive oxidase strains for non-fermentative NGB including *Acinetobacter spp* and *P. aeruginosa*.

For Enterobacteria, in the interpretation of the results, they are grouped into opportunistic Enterobacteriaceae frequently responsible for NCIs such as *E. coli*, *Enterobacter aerogenes*, *E. cloacae*, *K. pneumoniae*, *K. oxytoca*, *Citrobacter freundii* (*C. freundii*), *Proteus mirabilis* (*P. mirabilis*), and will separate "environmental"

enterobacteria rarely isolated in human clinical *E. sakazakii*, *Pantoea agglomerans*, *Leclercia adecarboxylata*, *Enterobacter vulneris*, *Enterobacter amnigenus*, *Enterobacter intermedius*. An antibiotic susceptibility test was then performed on the identified strains.

For NCS and *S. aureus*, 7 antibiotics were tested including Penicillin G (PIU), Cefoxitin (FOX30), Kanamycin (K30), Tobramycin (TOB30), Gentamicin (CN30), Erythromycin (E15), and Clindamycin (DA2).

For enterobacteria and non-fermentative BGNs, 14 antibiotics were tested including amoxicillin (AML25), clavulanic acid amoxicillin (AMC30), piperacillin tazobactam (TZP36), cefoxitin (FOX30), Cefotaxime (CTX30), Ceftazidime (CAZ10), Cefepime (FEP30), Imipenem (IMI), Ertapenem (ETP10), Aztreonam (ATP30), Ciprofloxacin (CIP5), Amikacin (AK30), Tobramycin (TOB30), Gentamicin (CN30). A synergy test, characterized by approximation of the AMC disks with the C₃G spaced 3 mm apart, was performed for confirmation of ESBL.

With the result of this antibiotic susceptibility test, the search for resistance to the usual antibiotics was carried out. Staphylococci were evaluated according to their resistance to methicillin via the cefoxitin disk according to the recommendations of the 2017 CA-SFM (strains < 22 mm in diameter are defined as resistant to methicillin), enterobacteria by the production of ESBL or not here. *A. baumannii* by its resistance to Imipenem and finally *P. aeruginosa* by resistance to ceftazidime or multidrug resistance.

III. RESULTS

During the study period, 69 samples were taken from the four above-mentioned services. Among these samples, 48 (69,6%) samples were positive to the groups of bacteria research on the selective media (NCS, *S. aureus*, human and environmental enterobacteria, as well as non-fermentative bacteria from the environment) and 21 (30,43%) samples were negative.

Among the 48 positive samples, 40 were from ChromID MRSA medium and 8 from ChromID ESBL and ChromID CARBA / OXA media.

On ChromID MRSA, 23 NCS and 17 *S. aureus* were identified and on ChromID ESBL and ChromID CARBA / OXA, 6 enterobacteria and 6 non-fermentative bacteria.

Enterobacteria of human origin were found in 68% of cases which are *Raoultella terrigena* 17% (n = 1), *Citrobacter freundii* 17% (n = 1), *E. cloacae* 17% (n = 1),

Cedecea lapagei 17% (n = 1). Enterobacteria of environmental origin were isolated such as *E. sakazakii* observed in 32% (n = 2). The non-fermentative NGB found were *A. baumannii* (67%, n = 4) and *Pseudomonas oryzibitans* (33%, n = 2).

About the susceptibility test, among the 23 NCS, 7 (30,5%) were methicillin-sensitive (methi-S) and 16 (69,5%) methicillin-resistant (methi-R). Resistance to all tested aminoglycosides was found in 74% of isolated NCS (n = 17). Erythromycin was sensitive only on 4 strains (18%).

Among the 17 strains of *S. aureus*, 8 (47%) were methi-S and 9 (53%) were methi-R. Resistance to all aminoglycosides tested was found in 6 strains (35%). For Erythromycin, 9 strains (53%) were sensitive and 8 (47%) were resistant.

Among the enterobacteria strains isolated, six were all ESBL producers. Five enterobacterial strains were resistant to all aminoglycosides tested. Resistance to ciprofloxacin was found among the four enterobacterial strains.

Of the 4 strains of *A. baumannii* isolated, half (n = 2) were resistant to Imipenem.

Two *P. aeruginosa* were isolated and there were no multi-drug resistant after the susceptibility test.

According to the department, in ICU, out of 19 samples, 14 were positive for Staphylococci (9 NCS and 5 *S. aureus*), one with enterobacteria (*Raoultella terrigena*), one with non-fermentative NGB (*A. baumannii*). In pediatric surgery, out of 15 samples, 10 were positive for Staphylococci (2 NCS and 8 *S. aureus*) and 2 for NGB, including one enterobacteria (*C. freundii*) and two non-fermentative bacteria (*A. baumannii*).

In the emergency department, 12 samples were collected, 9 returned positive for Staphylococci (5 NCS and 4 *S. aureus*), but no enterobacteria were isolated.

In the operating room, 23 sampling were collected, 7 were positive for Staphylococci, all NCS and 5 were positive for NGB whose five were isolated in the trauma block (*E. sakazakii*, *Cedecea lapagei*, *Pseudomonas oryzibitans*) and two in the visceral block (*E. cloacae*, *Pseudomonas oryzibitans*).

The doctors' offices of the ICU, Pediatric Surgery and emergency were analyzed. In the ICU, NCS were found on the table and foot of the guard bed. In the emergency service, no NCS was found but MRSA were isolated on the stapler and the edge of the table. The stethoscope that was

swabbed was contaminated with NCS methi-R. In the pediatric surgery department, the office key, the wrist and the table were contaminated with MRSA.

NCS contamination was found in 43% (n = 3/7) on the switch. These contaminations were found in a common

ward of ICU and in the pediatric surgery ward. The switch in the charge room of the ICU was contaminated with MSSA but they produced a penicillinase.

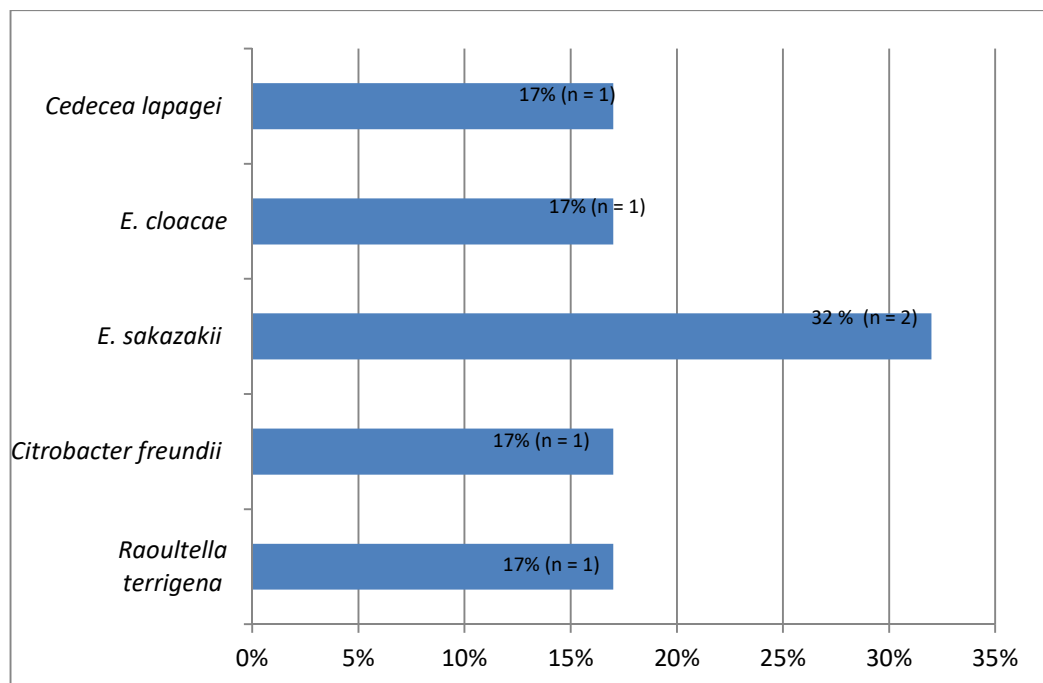


Figure 1. Isolated human and environmental enterobacteria

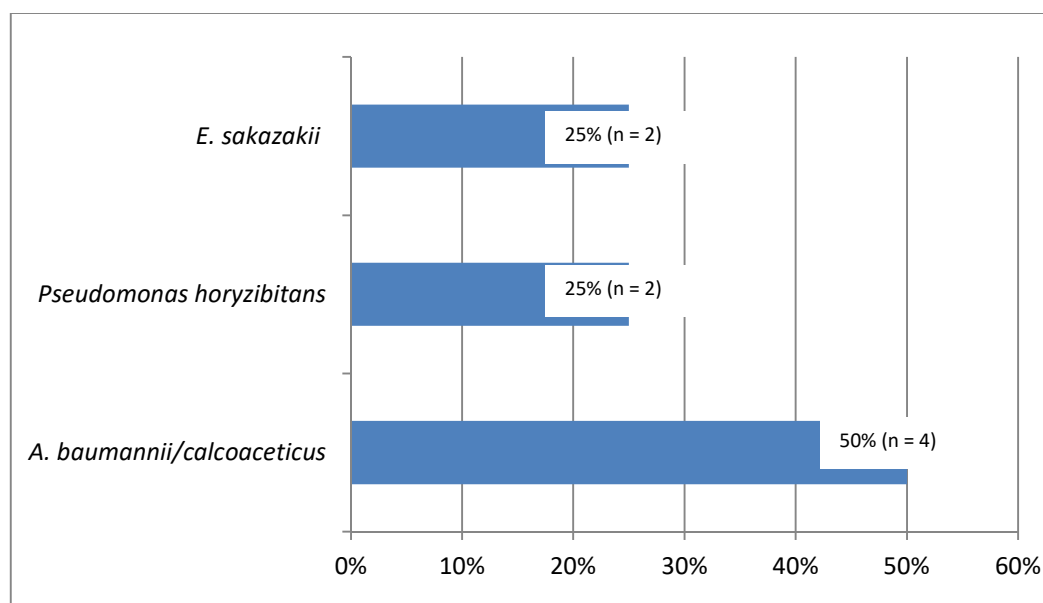


Figure 2. Environmental enterobacteria

For the various keys studied, the key of the toilets of the medical resuscitation service was contaminated by NCS and a MRSA was found on the key of the office of doctor of guard.

Beds in the doctor's ward were contaminated by NCS methi-R. The different beds coming from the common room of the surgical resuscitation, from the emergency room, from the common and paid rooms of pediatric surgery are contaminated by *S. aureus*, three of which were MRSA and two of the MSSA. In addition to MRSA, the paid room of pediatric surgery contained ABRI.

NCS resistant to methicillin were found on the wrists of door of the common room of the surgical reanimation and the pediatric surgery, offices outside the blocks (office of the anesthetists and the doctors) and of the public toilet. NCS sensitive to methicillin were identified on the wrist of door of a paid room in the reanimation department, the room of care and the office of the pediatric surgery physicians. MRSA strains have been found on the wrist of the public toilet of orthopedic surgery. An ESBL-producing enterobacteria was found on the wrist of the Surgical Reanimation of the common room.

The public toilets of emergency and pediatric surgery were studied. Contamination by MRSA strains was observed in 40% (n = 2/5) of which a wrist and a mop wash-off valve and by NCS in 40% (n = 2/5) including a wrist and a water tap reserved for hand washing.

Two mouths of aspirators from each operating room (traumatology and visceral) were studied. They were contaminated with ESBL-producing enterobacteria in 75% of cases. The Enterobacteria found were *Cedecea lapagei*, *E. sakazakii* and *E. cloacae*. The tables for soiled instruments in these operating room were studied. They were contaminated by NCS resistant to methicillin. A table in the trauma block were contaminated with an ESBL-producing enterobacteria (*E. sakazakii*). A table for sterile instrument of the visceral operating room was contaminated by NCS resistant to methicillin. On the anesthesia mask and the image intensifier sleeve of the traumatology operating room, a NCS resistant to methicillin was isolated.

IV. DISCUSSION

Current indications for microbiological sampling of the environment are now well defined in the field of hospital hygiene [5, 6]. They have an undeniable educational interest to raise awareness of bacterial or fungal contamination of surfaces. They make it possible to search for a microbial reservoir, although it is often difficult to conclude on the

role of the environment in the genesis of an infection. They can be used to evaluate the impact of new cleaning, disinfection or hand hygiene measures [5].

Several sampling techniques are proposed in the literature [7,8] such as direct fingerprints using agar plate or "tact tact" or "Rodac" contact plates, transfer on velvet, "brushing-washing-recovery" ", Spraying-recovery "and swabbing with a wet swab pressed in sterile water or simply exhausted in culture medium. The yield of each of these methods is variable, depending on a coefficient "tearing" of the microorganisms at the sampled surface, which depends in particular on the nature of this surface and the bacterial species present. In this study, agar seeding from a moistened swab was adopted. The choice of this method was guided by the fact that it is a simple method, applicable to all types of surfaces and culture media that we had. In addition, all the other methods mentioned above require expensive equipment.

This study is limited by the use of agar media without prior enrichment. In the literature, prior enrichment is carried out before seeding on agar. The results obtained by the techniques without enrichment could be compared with Rodac samples since in both cases, a direct application on the agar medium of the bacteria coming from the surface is carried out. A very low efficiency of this method without enrichment was noted to isolate the bacteria of interest in terms of hospital hygiene and NIs. Even the ubiquitous species in an environment frequented by humans (*Bacillus spp*, *Staphylococcus spp*...) are not systematically highlighted. In this study, many environmental microorganisms were isolated within 24-48 hours of incubation on the selective media used at 37°C.

In France, a strong contamination of the surfaces was noted in the litterature. Only 13% of samples do not show any bacteria and 43% reveal *S. aureus*, *P. aeruginosa* or enterobacteria "pathogens", the three bacterial species or families most often responsible for NIs [9].

In this study conducted at the UHC Anosiala, 30.43% (n=21/69) of the samples were negative to all the germs sought and 69.6% (n=48) were positive for *S. aureus*, enterobacteria with pathogenic potential and *A. baumannii* resistant to imipenem. The results obtained in the operating room by a team from Tours [10] out of the 5 384 analyzes carried out, 10 germs announced as pathogens are isolated: *P. fluorescens*, *P. putida* and *S. aureus*. The technique wasn't detailed but this is certainly very different and does not allow the same yield of extraction or culture of bacteria. Indeed, in better experience, even in the operating room, the

isolation on almost all smears made from environmental germs such as *Bacillus spp*, NCS such as *S. epidermidis* or non-epidermidis. Thus, the presence of a large number of NCS (n = 23) and *S. aureus* (n = 17) out of 40 positive samples on ChromID MRSA in this study proves the good performance of sampling methods and media. About our finding, GNB such as *Bacillus spp* present on 67% of samples could be a marker of effectiveness of the technique to highlight bacteria from surfaces [11].

Studies [12, 13, 14, 15] have investigated the immediate environmental contamination of MDR-infected patients with antibiotics and investigated these particular pathogens. They do not give results of the "basic" contamination of the establishment outside the epidemic. In our study, germs with high pathogenic potential were targeted. Indeed, it is an inventory of possible contamination of the environment outside the epidemic in order to implement hygiene measures and cleaning and disinfection method corresponding to the found germs.

The presence of a large number of *Staphylococcus spp* at the end of this work should promote the use of hydro-alcoholic solution as recommended. Hand washing with a hydro-alcoholic solution will reduce the transmission of bacteria and avoid NCIs. This must be done at the entrance to the service, before a care, after a care, between two care, between two patients and at the exit service because an unsoiled hand can be contaminated community.

The cleaning of the floor and horizontal surfaces alone reduces the germs by 80% and cleaning with a disinfectant allows a reduction of up to 95 to 99%. Cleaning and hygiene measures must take into account that germs survive for a long time in the environment. For example *Staphylococcus aureus* including MRSA can survive for more than 6 months after drying. To reduce the risk of contamination, it would be necessary to reduce the number of occupants in the room or increase the frequency of cleaning.

Depending on the different areas of the hospital, the choice between cleaning alone and the associated use of disinfectants is necessary. In sectors 1 and 2 at minimal or medium risk (offices and outpatient) not contaminated by pathogenic germs, the use of disinfectants is useless. In sectors 3 with severe risks (the areas of accommodation of the patients of the common and paying rooms type) for which one can wonder about the advisability of using a disinfectant, except in particular cases of infection by MDR germs. In sector 4 at very high risk (operating room, intensive care, reanimation...) where a detergent-disinfectant must be used. In this case, it is a biocleaning where the

cleaning is combined with rinsing and disinfection using detergent-disinfectant products. The objective of this biocleaning is to reduce the quantity or eliminate the microorganisms present on the surfaces. The periodicity will depend on the level of contamination and attendance of the various premises. In this study, the level of contamination of the premises did not make it possible to differentiate the risk of contamination of a frequented place compared to another less frequented. The Reanimation service, Pediatric Surgery and Operating room departments were the most contaminated premises therefore requiring much more cleaning-disinfection than the other premises. For all sectors, soil cleaning must be done daily. Cleaning floors and furniture several times a day would be essential for high-risk areas and the walls should be cleaned weekly.

V. CONCLUSION

This study made it possible to make an inventory of the bacteria likely to cause a nosocomial infection in the hospital environment of the University Hospital Center of Anosiala. Out of 69 samples taken in the environment of four services, negative coagulase *Staphylococcus* predominated, followed by *Staphylococcus aureus* and pathogenic enterobacteria. Negative coagulase *Staphylococcus* although they are environmental germs, can become pathogenic for vulnerable patients and postoperatively. Methicillin resistant *Staphylococcus aureus* and extended spectrum betalactamase-producing enterobacteria have been found in pediatric intensive care and surgery departments. This situation poses an increased risk of acquiring nosocomial infections due to multidrug resistant bacteria. Reinforcement of the hygiene and cleaning measures according to the recommendations will have to be realized in order to reduce and eliminate these microorganisms within the framework of the hospital hygiene and the protection of the patients against the acquisition of a hospital.

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