

Factors of Risk Survey of the Nosocomial Infection in the Hospital Inert Environment

Rakotozafy J. C. R.^{1,*}, Raherimandimby H.², Rasoloherimampiononiaina M. R.³, Rakotoarimanana R.²,
Randriamamonjy F.², Tanamasoandro R.³, Solofomalala G. D.³, Rabarijaona M.³

¹Laboratory of Molecular Biology, Faculty of Sciences – University of Fianarantsoa, Madagascar

²Laboratory of Biomedical Analysis, Center Hospital University of Fianarantsoa, Madagascar

³Center Hospital University of Fianarantsoa, Madagascar

Abstract In this survey, we analyzed by means of the inert environment withdrawals at the Resuscitation service, Neonatology and Operative Block in the Center Hospital University of Fianarantsoa (Madagascar). The bacteria identification has been done by biomedical laboratory usual technique. The results showed that different bacterial species contaminate the inert environment of these three services. Two bacterial species: *Staphylococcus aureus* and *Escherichia coli* are frequently isolated on these different samples. The antibiogram on disk method showed the presence of oxacillin and vancomycin-resistant *Staphylococcus aureus*; vancomycin-sensitive *Staphylococcus aureus* and oxacillin-resistant *Staphylococcus aureus*. For *Escherichia coli*, there are species cefoxitin and augmentin-sensitive *Escherichia coli* and cefoxitin and augmentin-resistant *Escherichia coli*. These results shows that the risks of nosocomial infections from the inert environment at the hospital is raised. The future perspective of this survey is to identify these two bacterial species isolated by molecular technique and to look for the new antibacterial products.

Keywords Antibiogram, Environment, *Escherichia coli*, Fianarantsoa, Madagascar, *Staphylococcus aureus*

1. Introduction

The nosocomial infections constitute a real problem of public health. 5 to 10% of the hospitalized patients incur a nosocomial infection during their hospitable stay [1].

The nosocomial infections is defined like infections appearing throughout a hospitalization [2] [3]. It can concern all types of infectious agents, but they are most frequently bacterial, and more occasionally viral, fungal or parasitic [2].

Many factors contribute to increase the risks like patient, cares, interventions, maternal-fetal infection and the inert environment contaminated [4]. These infections are the main reason of death neonatal in Africa, valued to 39% [5] [6].

The World Health Organization (WHO), published in April 30, 2014 a report on antibiotics resistance in every region of the world.

Indeed, inert environment's ecology bacterial has been done at the hospital. This survey has objective to identify the bacterial contamination to be able to take an adequate and perennial measure to the preventive and curative struggle.

2. Material and Methods

2.1. Sites of Study

The study has been achieved to the Center Hospital University of Fianarantsoa (Madagascar) in the Operative Block, Resuscitation and Neonatology Services, in 2014. The choice of these three services is based on the bacterial contamination high risk.

2.2. Withdrawal of the Environment

Different materials touched by the health staff, patients and their accompanists have been swabbed with sterile dry swabs. The withdrawals have been done between 2 to 3 pm when every service daily activity is minimal during 15 days a month.

The appropriated samples have been sent to the laboratory for bacteria isolation and identification.

2.3. Bacteria Identification

Staphylococcus aureus, *Streptococcus spp*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella spp* are the most frequently isolated and identified pathogenic bacterial to the hospitalized patients. Indeed, the study was focused on these kinds of bacteria. All withdrawals don't possess one of these bacterial species are excluded.

Bacteria identification and antibiogram have been achieved by biomedical laboratory usual technique [7] [8] [9] [10].

* Corresponding author:

dtn.claude@yahoo.fr (Rakotozafy J. C. R.)

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2.3.1. Media Culture

A preliminary identification by Gram coloration (**Kit Gram-Nicolle RAL[®]**) has been achieved for every sample, to be able to classify the bacteria in cocci and bacillus group. According to the results of Gram methods, different culture medium are used to isolate the bacteria:

- *Staphylococcus aureus*: on salty media (**Chapman, BioRad[®]**), some yellow colonies were looked for after 24 hours of 37°C incubation.
- *Streptococcus spp*: on trypticase soy media (**BioRad[®]**) to 5% of sheep blood, small colonies surrounded a halo were searched after 24 hours and 48 hours of 37°C incubation under 5% of CO₂.

Three media have been used for bacillus Gram negative:

- *Pseudomonas aeruginosa*: the presence of colony producing bluish fluorescent pigments was searched on culture medium (**KING A, BioRad[®]**) after 48 hours of 37°C incubation,
- *Escherichia coli*., the sample is sowed on media (**UriSelect, BioRad[®]**) to isolate *Escherichia coli*. Purple colonies were looked for on culture medium after 48 hours of 37°C incubation.
- *Salmonella spp*: black colonies were searched on Salmonella Shigella agar (**SS Agar, BioRad[®]**) media after 48 hours of 37°C incubation.

2.3.2. Biochemical Test (API-System)

API System (**bioMérieux[®]**) is a standardized system for genera bacteria identification, which uses miniaturized biochemical tests and a specially adapted database. The complete list of those bacteria that is possible to identify with this system can be found in the Identification Table at the end of this package insert.

The API System strip consists of 20 microtubes containing dehydrated substrates. These microtubes are inoculated with a bacterial suspension, prepared in API System Medium that reconstitutes the tests. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents.

The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index or using the identification software [11].

2.4. Antibacterial Disk Susceptibility Tests

Antibiotic susceptibility testing by a standardized single disk method have been used [7] [8] [9] [10]. Four milliliters of this bacterial suspension ($1-3 \times 10^4$ bacteria/ml) have been spread uniformly on the Mueller Hinton agar media [BioRad[®]]. Afterwards, the different antibiotics disks (**Cypress Diagnostics[®]**) have been deposited on media. After 48 hours of 37°C incubation (under an atmosphere enriched of 5% CO₂ for *Streptococcus spp.*), the inhibition area diameter has been measured (Figure 1). Every antibiotic has been tested 5 times. The results are expressed in millimeter (mm).

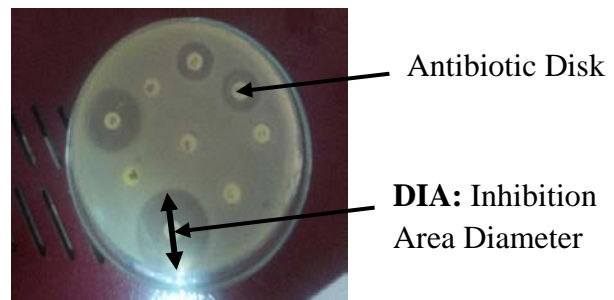


Figure 1. Inhibition Area Diameter measure technique

2.5. Statistical Analysis

The statistical analysis of the qualitative and quantitative variables was either of mono-varied type, or of multivariate type using a software Epiinfo version 6. The Student test was used for the averages and the variances comparison and the test of Chi-2 for the comparison between the rates and a significant value if $p < 0.05$.

3. Results and discussions

3.1. Operative Block

The samples appropriated results within the Operative Block show the presence of *Staphylococcus aureus* and *Escherichia coli*. The difference of antibiogram results is remarkable within *Staphylococcus aureus* compared with oxacillin and vancomycin activity and *Escherichia coli* within ceftriaxon and augmentin.

Table 1. *Staphylococcus aureus* resistant to the oxacillin and the vancomycin antibiogram results (n=5)

ATB	DIA (mm)	S	I	R
L	6			
E	8			
CN	15			
CX	6			
P	6			
VA	16			
CTR	18			
OX	6			
OFX	26			
FC	9			
C	12			
TE	20			
DO	21			
RIF	21			

ATB: Antibiotic, CN: Gentamicin, CX: Cefoxitin, CTR: Ceftriaxon, C: Chloramphenicol, TE: Tetracyclin, DO: Doxycycline, L: Lincomycin, E: Erythromycin, P: Penicillin G, VA: Vancomycin, OX: Oxacillin, OFX: Ofloxacin, FC: Fusidic acid, DIA: Inhibition Area Diameter, mm: millimeter, S: Sensitive, I: Intermediate, R: Resistant.

Table 2. *Staphylococcus aureus* resistant to the oxacillin, sensitive and the vancomycin antibiogram result (n=5)

ATB	DIA (mm)	S	I	R
L	6			
E	8			
CN	15			
CX	6			
P	6			
VA	16			
CTR	18			
OX	6			
OFX	26			
FC	9			
C	12			
TE	20			
DO	21			
RIF	21			

ATB: Antibiotic, **CN:** Gentamicin, **CX:** Cefoxitin, **CTR:** Ceftriaxon, **C:** Chloramphenicol, **TE:** Tetracyclin, **DO:** Doxycyclin, **L:** Lincomycin, **E:** Erythromycin, **P:** Penicillin G, **VA:** Vancomycin, **OX:** Oxacillin, **OFX:** Ofloxacin, **FC:** Fusidic acid, **DIA:** Inhibition Area Diameter, **mm:** millimeter, **S:** Sensitive, **I:** Intermediate, **R:** Resistant.

Table 3. *Escherichia coli* sensitive to the cefoxitin and the augmentin antibiogram result (n=5)

ATB	DIA (mm)	S	I	R
TI	6			
AMX	0			
CN	20			
CX	12			
CPM	10			
AUG	12			
CTR	8			
IPM	31			
CIP	8			
NA	8			
C	0			
TE	0			
DO	0			

ATB: Antibiotic, **TI:** Ticarcillin, **AMX:** Amoxicillin, **CN:** Gentamicin, **CX:** Cefoxitin, **CPM:** Cefepim, **AUG:** Augmentin, **CTR:** Ceftriaxon, **IPM:** Imipenem, **CIP:** Ciprofloxacin, **NA:** Nalidixic acid, **C:** Chloramphenicol, **TE:** Tetracyclin, **DO:** Doxycyclin, **DIA:** Inhibition Area Diameter, **mm:** millimeter, **S:** Sensitive, **I:** Intermediate, **R:** Resistant.

Consequently, there are oxacillin and vancomycin-resistant *Staphylococcus aureus*; vancomycin-sensitive *Staphylococcus aureus* and oxacillin-resistant *Staphylococcus aureus* (Table 1, 2). Otherwise, cefoxitin and augmentin-sensitive *Escherichia coli* and cefoxitin and augmentin-resistant *Escherichia coli* exist (Table 3, 4). Antibiogram results show that all *Staphylococcus aureus* were sensitive in doxycyclin and tetracyclin while all *Escherichia coli* are sensitive in imipenem and gentamicin.

For the other isolated and identified bacterial species, no remarkable difference is observed.

Table 4. *Escherichia coli* resistant to the cefoxitin and the augmentin antibiogram result (n=5)

ATB	DIA (mm)	S	I	R
TI	6			
AMX	0			
CN	20			
CX	12			
CPM	10			
AUG	12			
CTR	8			
IPM	31			
CIP	0			
NA	0			
C	0			
TE	0			
DO	0			

ATB: Antibiotic, **TI:** Ticarcillin, **AMX:** Amoxicillin, **CN:** Gentamicin, **CX:** Cefoxitin, **CPM:** Cefepim, **AUG:** Augmentin, **CTR:** Ceftriaxon, **IPM:** Imipenem, **CIP:** Ciprofloxacin, **NA:** Nalidixic acid, **C:** Chloramphenicol, **TE:** Tetracyclin, **DO:** Doxycyclin, **DIA:** Inhibition Area Diameter, **mm:** millimeter, **S:** Sensitive, **I:** Intermediate, **R:** Resistant.

3.2. Resuscitation Service

Four bacterial species have been isolated and identified in this service: *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermis*, *Staphylococcus saprophyticus*, *Klebsiella spp* and *Proteus spp*.

Every bacterial species antibiogram results are represented in table 5, 6, 7 and 8. For *Staphylococcus aureus* and *Escherichia coli*, the results are identical to the Operative Block. The difference is not meaningful.

Table 5. *Staphylococcus saprophyticus* antibiogram result (n=5)

ATB	DIA (mm)	S	I	R
L	6			
E	24			
CN	24			
CX	6			
P	6			
VA	6			
CTR	20			
OX	6			
OFX	26			
FC	9			
C	18			
TE	24			
DO	21			
RIF	21			

Table 6. *Staphylococcus epidermis* antibiogram result (n =5)

ATB	DIA (mm)	S	I	R
L	6			
E	18			
CN	24			
CX	6			
P	6			
VA	6			
CTR	18			
OX	6			
OFX	26			
FC	9			
C	12			
TE	20			
DO	21			
RIF	21			

ATB: Antibiotic, **CN:** Gentamicin, **CX:** Cefoxitin, **CTR:** Ceftriaxon, **C:** Chloramphenicol, **TE:** Tetracyclin, **DO:** Doxycycline, **L:** Lincomycin, **E:** Erythromycin, **P:** Penicillin G, **VA:** Vancomycin, **OX:** Oxacillin, **OFX:** Ofloxacin, **FC:** Fusidic acid, **DIA:** Inhibition Area Diameter, **mm:** millimeter, **S:** Sensitive, **I:** Intermediate, **R:** Resistant.

Table 7. *Klebsiella* spp. antibiogram result (n =5)

ATB	DIA (mm)	S	I	R
TI	24			
AMX	0			
CN	26			
CX	6			
CPM	25			
AUG	8			
CTR	26			
IPM	36			
CIP	38			
NA	26			
C	30			
TE	25			
DO	22			

3.3. Neonatology Service

All bacterial species isolated in Operative Block and Resuscitation room are also isolated to Neonatology Service except *Klebsiella* spp. No meaningful difference is observed to the antibiogram results within the Operative Block and the Resuscitation room vis-a-vis *Staphylococcus aureus* and *Escherichia coli*.

It is noted that *Enterobacter* spp. and *Pseudomonas aeruginosa* are isolated in those three services environment (Table 9, 10).

Table 8. *Proteus* spp. antibiogram result (n =5)

ATB	DIA (mm)	S	I	R
TI	8			
AMX	6			
CN	12			
CX	6			
CPM	6			
AUG	6			
CTR	11			
IPM	35			
CIP	20			
NA	8			
C	12			
TE	11			
DO	16			

ATB: Antibiotic, **TI:** Ticarcillin, **AMX:** Amoxicillin, **CN:** Gentamicin, **CX:** Cefoxitin, **CPM:** Cefepim, **AUG:** Augmentin, **CTR:** Ceftriaxon, **IPM:** Imipenem, **CIP:** Ciprofloxacin, **NA:** Nalidixic acid, **C:** Chloramphenicol, **TE:** Tetracyclin, **DO:** Doxycycline, **DIA:** Inhibition Area Diameter, **mm:** millimeter, **S:** Sensitive, **I:** Intermediate, **R:** Resistant.

Table 9. *Enterobacter* spp. antibiogram result (n =5)

ATB	ZI (mm)	S	I	R
TI	14			
AMX	0			
CN	16			
CX	0			
CPM	0			
AUG	12			
CTR	14			
IPM	27			
CIP	23			
NA	18			
C	8			
TE	24			
DO	18			

ATB: Antibiotic, **TI:** Ticarcillin, **AMX:** Amoxicillin, **CN:** Gentamicin, **CX:** Cefoxitin, **CPM:** Cefepim, **AUG:** Augmentin, **CTR:** Ceftriaxon, **IPM:** Imipenem, **CIP:** Ciprofloxacin, **NA:** Nalidixic acid, **C:** Chloramphenicol, **TE:** Tetracyclin, **DO:** Doxycycline, **DIA:** Inhibition Area Diameter, **mm:** millimeter, **S:** Sensitive, **I:** Intermediate, **R:** Resistant.

The set of results permits to say that bacterial species particularly *Staphylococcus aureus* and *Escherichia coli* known responsible of nosocomial infection circulate in Fianarantsoa hospital environment. Indeed, infection risk is especially raised to these three services where receive critical state patients. Those patients do have a raised pressure of antibiotics to avoid all bacterial infection [12], so, they are especially vulnerable to infections because of their immunodeficiency [13] or they become an easy access to bacteria due to the intensive treatment [14] [15].

The antibiogram results also permit to say that one has four *Staphylococcus aureus* varieties: oxacillin-resistant

Staphylococcus aureus; vancomycin and oxacillin-resistant *Staphylococcus aureus*; oxacillin-sensitive *Staphylococcus aureus* and vancomycin-sensitive *Staphylococcus aureus* and on the other hand two varieties of *Escherichia coli*: cefoxitin and augmentin-resistant *Escherichia coli* and *Escherichia coli* sensitive to this two antibiotics. Tetracyclin-sensitive *Staphylococcus aureus* and imipenem-sensitive *Escherichia coli* are very remarkable.

Antibiotics difference activity could be due to their mechanisms action [16] [17] [18] [19] or to the bacteria enzymes who inhibit and/or block the antibiotics action. Besides, bacteria from patients also contaminate the inert environment [20]. This acquired resistance, in general secondary, is the antibiotics abusive use consequence [21] [22].

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-sensitive *Staphylococcus aureus*, Enteropathogenic *Escherichia coli* (EPEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteroinvasion *Escherichia coli* (EIEC) and Enteroadherent *Escherichia coli* (EAEC) are frequently isolated to nosocomial infection [23] [24] [25] [26] [27]. It may be the difference activity origin of these antibiotics.

Table 10. *Pseudomonas aeruginosa* antibiogram result (n =5)

ATB	DIA (mm)	S	I	R
TI	14			
AMX	0			
CN	16			
CX	0			
CPM	0			
AUG	12			
CTR	14			
IPM	27			
CIP	23			
NA	18			
C	8			
TE	24			
DO	18			

ATB: Antibiotic, TI: Ticarcillin, AMX: Amoxicillin, CN: Gentamicin, CX: Cefoxitin, CPM: Cefepim, AUG: Augmentin, CTR: Ceftriaxon, IPM: Imipenem, CIP: Ciprofloxacin, NA: Nalidixic acid, C: Chloramphenicol, TE: Tetracyclin, DO: Doxycycline, DIA: Inhibition Area Diameter, mm: millimeter, S: Sensitive, I: Intermediate, R: Resistant.

4. Conclusions

This survey shows the bacteria multi resistant presence on the inert environment at the hospital. Indeed, nosocomial infections risk is raised. Even that the probable sources of nosocomial infection to patients in these three services are not studied, these results permit to take preventive and curative adequate measures at the hospital as creation of surveillance service assures cleanings; the cleanliness or even environment sterilization, local and materials used.

These results also allow the clinicians to choose one among the sensitive antibiotics according the laboratory analyses results. For a perennial struggle, sources infection research; the responsible agents' identification by molecular biology method (PCR) and new products antimicrobials research prove to be necessary.

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