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# Prevalence, Circulation and Characterization of Multi-resistant Bacteria at Omar Bongo Ondimba Army Instruction Hospital in Libreville

**Thiery Ndong Mba[1,2], Anita Christel Elvire Mbongo-Kama[3], Cédric Sima Obiang[1*], Rick Léonid Ngoua Meye Misso[1], Arnault Mafoumbi[1,2], Joseph Privat Ondo[1], Louis Clément Obame Engonga[1], Patrick Mickala[2] and Edouard Nsi Emvo[1]**

*[1]Laboratory of Research in Biochemistry, University of Sciences and Technology of Masuku, P.O.Box 913, Franceville, Gabon.*
*[2]Laboratory of Molecular and cellular Biology, University of Sciences and Technology of Masuku, Franceville, Gabon.*
*[3]Omar Bongo Ondimba Army Instruction Hospital in Libreville, Libreville, Gabon.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. The data reported in the study was available to the authors and authors contributed equally to the preparation and writing of this manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

Multi-resistant bacteria (MRB) pose a global health problem. They lead to increased morbidity, mortality and hospital costs. The main objective of this study is to study the prevalence, circulation and characterization of multi-resistant bacteria.
This is a prospective cross-sectional study of 4 examinations. Bacterial identification was performed using Gram stains, oxidase, catalase, filamentation tests and the Api 20 STAPH, Api 20E systems. The assessment of sensitivity to antibiotics is based on the liquid diffusion method and on the ATB G, ATBTM-EU and ATBTM STAPH galleries previously soaked in antibiotics.

**Corresponding author: E-mail: cedricsima@gmail.com, cedricsima@univ-masuku.ga;*

The most common multidrug resistant bacteria were *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter cloacae*, MRSA and *Acinetobacter baumanii*. The number of multi-resistant bacteria from cytobacteriological urine examinations was 17, or 43.59% of all isolates. The prevalence of multi-resistant bacteria was 57.14% in blood cultures. In the collection of material from the hospital, the prevalence of multi-resistant strains was 72.73%. The prevalence of multi-resistant strains was 40% in the pus samples.
These results can help define the research perspectives and strategies to be developed to better control the emergence and spread of multi-resistant bacteria in the hospital environment.

*Keywords: Prevalence; circulation; multi-resistant bacteria; antibiotics.*

## 1. INTRODUCTION

Over the past fifty years, antibiotics have played a crucial role in the fight against many infections. Their development has revolutionized the treatment of infectious diseases. Faced with the growing and sometimes unjustified use of these molecules, bacteria have learned to defend themselves and to adapt, becoming for some of them resistant, which has led to the emergence of multi-resistant bacteria (MRB), observed around the world, including in African countries [1,2]. The selection pressure exerted by the extensive use of antibiotic therapy and the epidemic spread of resistant strains are the two main factors conditioning this development [3,4]. This situation has become particularly worrying in hospitals, where the risks of infection are multiple [5]. The implementation of rigorous hygiene practices as well as various preventive measures (including hand hygiene) are helping to combat these [6]. Thus, the increase in bacterial resistance is nowadays one of the most important major public health problems according to the World Health Organization [7]. The consequences linked to multi-drug resistant bacteria is at the root of mortality and morbidity in developing and developed countries alike. For better knowledge and management of these resistance phenomena, a global list of pathogenic bacteria resistant to antibiotics was drawn up in 2017, in order to prioritize research and development of new treatments. This list includes the following bacteria: Methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum β-lactamase-producing *Enterobacteriaceae*, Carbapenemase-producing Enterobacteriaceae and vancomycin resistant enterococci, 3rd generation cephalosporin resistant *enterobacteriaceae*, ceftazidime resistant *Pseudomonas aeruginosa*, β-lactam resistant *Acinetobacter baumanii* and *Stycoccus aurancus* [8]. In this context, this study is being carried out to carry out the characterization of multi-resistant bacteria within the Omar Bongo Ondimba Army Instruction Hospital in Libreville, Gabon.

## 2. MATERIAL AND METHODS

### 2.1 Type and Period of Study

His is a prospective and descriptive epidemiological study which was carried out over a period of approximately five months (from October 7, 2019 to March 7, 2020) in the microbiology laboratory of the Omar BONGO ONDIMBA Army Instruction Hospital from Libreville.

### 2.2 Study Population

Information about the patients was collected on anonymous questionnaire forms. They covered demographic information (surname, first name, age and sex, newborn, blood culture, children, adults and old people). Other factors relating to the study population were also noted such as hospitalization, antibiotic therapy and type of sample.

### 2.3 Inclusion Criteria

The study is carried out on all bacteriological samples for diagnostic purposes received in the laboratory from patients hospitalized in the various departments of this establishment or as an external consultant.

### 2.4 Exclusion Criteria

All redundant strains (duplicates), patients who do not comply with the conditions for urine samples and polymorphic samples in the case of cytobacteriological examination of urine.

### 2.5 Urine Collection

The sample was collected under sterile conditions. After elimination of the first draft, the

urine, preferably that of the morning, is collected in a sterile jar (about 20 to 30 ml), without touching the upper edge, the jar is then hermetically sealed and sent to the laboratory for analyzes

## 2.6 Blood Culture Samples

Blood cultures are done to detect and identify bacteria and yeasts in the blood. A volume of 15 ml of blood was collected for each blood culture, of which two equal parts were used to inoculate one flask for aerobic culture and one flask for anaerobic culture.

## 2.7 Pus Samples

The pus was collected by puncture using a sterile syringe. In the case of open abscesses, suppurative wounds or pustules and fistulas where the pus drains outside, the sample was taken with a swab after disinfection of the wound. The swabs were then sent to the laboratory for analysis.

## 2.8 Solation on Culture Media

### 2.8.1 Urine case

The urine was inoculated on Petri dishes by the streak method, the CLED and BCP (Bromocreol purple) agars were used in the absence of the urine, these dishes were incubated in an oven for 24 hours at 37 °C. BCP is a non-selective medium which is used for the isolation of many non-*Enterobacteriaceae* species.

### 2.8.2 Case of blood cultures

All the blood culture samples were inoculated only on fresh blood agar or Colombia agar + 5% sheep blood (Bio-Mérieux, Lyon, France). A needle was placed on the positive blood culture vial, then 1 to 2 drops of blood were placed on the Petri dish: thus, using a calibrated 10 µL plastic loop, the inoculation was carried out. made by the streak method, then the Petri dish was placed in the Jar, a candle was lit then the Jar was closed just until the candle went out in order to be in anaerobic conditions, then incubated for 24 hours or 48 hours in an oven.

### 2.8.3 Case of suppurations

The pus swabs were inoculated in three different media, depending on the origin of the swab. The nasal swabs were inoculated only on Chapman's medium in order to test for MRSA, swabs of pus contained in the jars; the culture was done on the poly vitex chocolate medium (PVX) to isolate the other multi-resistant bacteria. On the other hand, the anal swabs were inoculated on the BCP medium. The streak method was used for the inoculation, the appearance and color of the pus were taken into account.

### 2.8.4 Case of sampling of laboratory material

Samples were inoculated on two media namely BCP and times on Cooked Blood Agar (PVX). The streak method was used.

## 2.9 Bacterial and Biochemical Identification

### 2.9.1 Gram stain

Gram stain (fisherscientific France 2019) was performed using gentian violet, lugol and fuchsin as a stain.

### 2.9.2 Biochemical identification

For biochemical identification, oxidase, catalase and filamentation tests (Blastesis) were performed.

### 2.9.3 Antibiogram

The antibiogram is an examination which makes it possible to study the sensitivity to antibiotics of a bacterial strain isolated in a patient infected from a pathological product (blood, urine, pus, sample material, puncture fluid, cerebrospinal fluid). It is thus possible to identify possible resistance to such antibiotic.

### 2.9.4 Identifications of strains by the Api system

For staphylococci and Enterobacteriaceae, the Api20 STAPH, Api 20E systems were respectively used, for other Gram-negative organisms the Api 20E system was used.

### 2.9.5 Sensitivity to antibiotics

Antibiotic sensitivity was studied using 32-well microplates (ATB G-, ATB™-EU and ATB™ STAPH) previously soaked in antibiotics.

## 2.10 Statistical Processing of Data

All the data were collected on Excel 2016 software and analyzed by Ipi-info statistical software.

## 3. RESULTS

In this study, 443 samples were taken with 92 germs including 2 yeasts and 90 bacterial strains identified.

### 3.1 Distribution of Samples by Sex

On the 443 samples, there was a female predominance in our sample with 226 women against 217 men as shown in Fig. 1. These results show the predominance of the female sex with a rate of 51.02% compared to the male sex (48.98%).

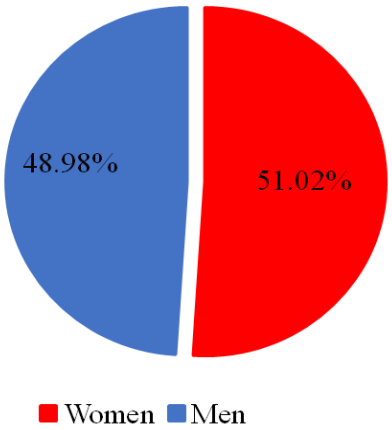


**Fig. 1. Distribution of samples by sex**

### 3.2 Prevalence of Isolated Germs Responsible for Urinary Tract Infections

Of the 332 cytobacteriological examinations of urine intended for the bacteriological laboratory during the study period, 40 met the criteria for a urinary tract infection. The prevalence of urinary tract infections was therefore 12.05% (40/332) against 87.95% (292/332) sterile cultures. Thus, several bacterial species have been implicated in urinary tract infection and a yeast that has been identified. The bacterial species identified in the 40 samples belong to three bacterial groups per family (Table 1). *Enterobacteriaceae* make up 90% of all isolated bacteria. Staphylococci represent 7.5% of the bacteria isolated. A single yeast was isolated, it is mainly represented by Candida spp.

### 3.3 Prevalence of MRB Involved in Urinary Tract Infections

Of all the bacteria isolated from cytobacteriological examinations of urine during our study, the number of MRB found was 17, or 43.59% of all isolates (Fig. 2).

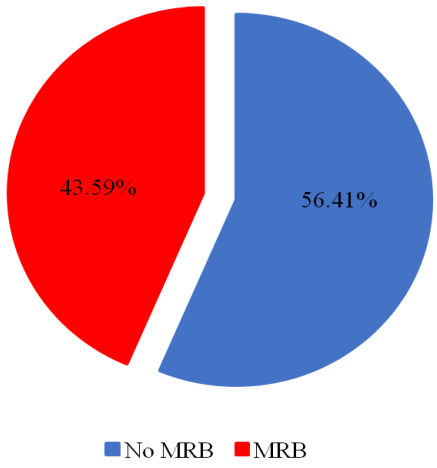


**Fig. 2. Prevalence of multi-resistant bacteria (MRB) isolated from cytobacteriological examinations of urine**

### 3.4 Distribution of MRB from Cytobacteriological Examinations of Urine

Analysis of data in Table 2 shows that extended spectrum β-lactamase-producing *Enterobacteriaceae* (n = 16) are the most frequently isolated bacteria, a single strain of *Staphylococcus aureus* resistant to methicillin (n = 1) was isolated.

### 3.5 Prevalence of Bacterial Strains Isolated in Blood Cultures

During the study period 60 blood culture samples were received at the bacteriology laboratory, 21 of which were used as witnesses of true bacteremia, giving a blood culture positivity rate of 35%. Table 3 shows the distribution of 21 isolated bacterial strains.

These results show a predominance of strains from Enterobacteria with 47.62% followed by *Staphylococcaceae*. The *Pseudomonadaceae* family had a percentage of 9.52%.

### 3.6 Prevalence of MRB in Blood Cultures

During this study, 21 bacteria were isolated, including 12 strains that were multi-resistant to 3rd generation cephalosporins and other antibiotics tested. Fig. 3 below shows the distribution of strains.

The prevalence of MRB was 57.14% in blood cultures, leading to cases of sepsis.

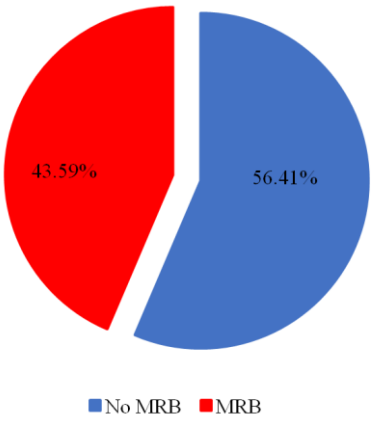


**Fig. 3. Prevalence of multi-resistant bacteria (MRB) from blood cultures**

### 3.7 Distribution of MRB in Blood Cultures

Table 4 shows the distribution of isolated MRB and their frequencies. All strains isolated were Gram negative bacilli. The germ taking first place was Klebsiella pneumoniae with 33.33% of MRB in blood cultures. In second position, Escherichia coli with a percentage of 25% of the MRB. The other germs had a very low percentage, 8.33%. The Kluyvera spp bacterium was isolated from a community sample. The Enterobacteriaceae family was the most predominant with 9 MRB or 75%.

### 3.8 Prevalence of Germs Identified in Material Samples

Of the 14 samples analyzed, 11 samples were positive, ie a prevalence of 78.57%. Table 5 shows the distribution of the germs isolated. Of the strains identified, enterobacteria represented 81.82% of germs. The other families had a very low percentage, with only one isolated strain.

The prevalence of multi-resistant strains was 72.73%. Thus, all the 8 bacterial strains of *Klebsiella pneumoniae* were multi-resistant against 2 non-multi-resistant bacterial strains and one yeast, ie 27.27% (3/10).

### 3.9 Prevalence of Bacterial Strains Isolated from Pus

A total of 37 pus samples including 20 bacterial strains could be isolated after analysis of the different samples. There are 4 bacterial families that have been identified (Table 6). The most represented was that of *Staphylococcaceae* with 50% of the isolates. The *Pseudomonaceae* family had a rate of 25%. The *Enterobacteriaceae* were represented by a species of *Klebsiella pneunomiae*, ie 15%. Finally, a single strain of *Aeromonadaceae* had been isolated (*Aeromonas hydrophilla* with 10% frequency).

### 3.10 Prevalence of MRB from Pus Isolates

The prevalence of multi-resistant strains was 40% (8/20) on the bacteria identified against 60% of non-multi-resistant strains.

Among the 8 isolated MRB, MRSA occupy the first place, ie 37.50%, followed by two species, *Pseudomonas aeruginosa* and *Pseudomonas luteola* with 25% for each of the bacterial species as shown in Table (7).

## 4. DISCUSSION

In our prospective study, the male sex was the most affected with 49 germs or 53.26% against 46.74% in the female sex.

**Table 1. Distribution of germs responsible urinary tract infection**

| Family | Germs | Gram | Effective | Percentage |
|---|---|---|---|---|
| Saccharomycetaceae | *Candida spp* | | 1 | 2.5 |
| | *Citrobacter koseri* | - | 1 | 2.5 |
| | *Enterobacter cloacae* | - | 4 | 10 |
| Enterobacteriaceae | *Enterobacter sakazakii* | - | 2 | 5 |
| (n=36) | *Escherichia coli* | - | 21 | 52.5 |
| | *Klebsiella oxytoca* | - | 1 | 2.5 |
| | *Klebsiella pneumoniae* | - | 7 | 17.5 |
| Staphylococcaceae | *Staphylococcus aureus* | + | 1 | 2.5 |
| (n=3) | *Staphylococcus spp* | + | 2 | 5 |
| Total | | | 40 | 100 |

**Table 2. MRB rate in urinary tract infections depending on the type of germs**

| Bacteria | Effective | Percentage |
|---|---|---|
| *Escherichia coli* | 9 | 52.95 |
| *Klebsiella pneumoniae* | 5 | 29.41 |
| *Enterobacter cloacae* | 1 | 5.88 |
| *Enterobacter sakazakii* | 1 | 5.88 |
| *Staphylococcus aureus* | 1 | 5.88 |
| Total | 17 | 100 |

**Table 3. Distribution of bacterial strains in blood cultures**

| Family | Germs | Gram | Effective | Percentage |
|---|---|---|---|---|
| Enterobacteriaceae | *Klebsiella pneumoniae* | - | 4 | 19.05 |
| | *Escherichia coli* | - | 3 | 14.29 |
| | *Enterobacter cloacae* | - | 1 | 4.76 |
| | *Kluyvera spp* | - | 1 | 4.76 |
| | *Providencia rettgeri* | - | 1 | 4.76 |
| Pseudomonadaceae | *Pseudomonas aeruginosa* | - | 1 | 4.76 |
| | *Pseudomonas luteola* | - | 1 | 4.76 |
| Moraxellaceae | *Acinetobacter baumanii* | | 1 | 4.76 |
| Staphylococcaceae | *Staphyloccocus spp* | + | 8 | 38.10 |
| Total | | | 21 | 100 |

**Table 4. MRB frequency of blood cultures**

| Bacteria | Effective | Percentage |
|---|---|---|
| *Acinetobacter baumanii* | 1 | 8.33 |
| *Enterobacter cloacae* | 1 | 8.33 |
| *Escherichia coli* | 3 | 25 |
| *Klebsiella pneumoniae* | 4 | 33.33 |
| *Kluyvera spp* | 1 | 8.33 |
| *Pseudomonas aeruginosa* | 1 | 8.33 |
| *Pseudomonas luteola* | 1 | 8.33 |
| Total | 12 | 100 |

**Table 5. Prevalence of microorganisms from samples**

| Family | Germs | Gram | Effective | Percentage |
|---|---|---|---|---|
| Saccharomycetaceae | *Candida spp* | | 1 | 9.09 |
| Enterobacteriaceae | *Klebsiella pneumoniae* | - | 8 | 72.73 |
| | *Klebsiella oxytoca* | - | 1 | 9.09 |
| Staphylococcaceae | *Staphylococcus aureus* | + | 1 | 9.09 |
| Total | | | 11 | 100 |

**Table 6. Distribution of bacteria isoled from pus**

| Family | Germs | Gram | Effective | Percentage |
|---|---|---|---|---|
| Aeromonadaceae (n=2) | *Aeromonas hydrophila* | - | 2 | 10 |
| Enterobacteriaceae (n=3) | *Klebsiella pneumoniae* | - | 3 | 15 |
| Pseudomonadaceae | *Pseudomonas aeruginosa* | - | 3 | 15 |
| (n=5) | *Pseudomonas luteola* | - | 2 | 10 |
| Staphylococcaceae | *Staphyloccoccus aureus* | + | 6 | 30 |
| (n=10) | *Staphylococcus spp* | + | 4 | 20 |
| Total | | | 20 | 100 |

**Table 7. Distribution of MRB isolated from pus**

| Bacteria | Effective | Percentage |
|---|---|---|
| *Aeromonas hydrophila* | 1 | 12.50 |
| *Pseudomonas aeruginosa* | 2 | 25 |
| *Pseudomonas luteola* | 2 | 25 |
| *Staphylococcus aureus* | 3 | 37.50 |
| Total | 8 | 100 |

Urinary tract infection maintains a high frequency, which is 12.5%, this frequency is lower than that found at the level of the Bacteriology-Serology laboratory Ibn Sina Rabat during two years which was 16.4% [9]. During this prospective study, there were 17 multi-resistant bacteria among the 40 isolates, so the prevalence of MRB was 43.59%. These so-called extended spectrum β-lactamase strains were mainly represented by Gram-negative bacilli, namely: *Escherichia coli* with 52.95%, followed by *Klebsiella pneumoniae* (29.41%), *Enterobacter cloacae* and *Enterobacter sakazakii* (5.88 %) with a single isolated strain. A single strain of *Staphylococcus aureus* was multi-resistant to methicillin, ie 5.88% of all the strains isolated. Our results are very close to those reported in the Moroccan study carried out at the Avicenne Military Hospital in Marrakech [10] which observed rates of 52% for *Escherichia coli*, 22% for *Klebsiella pneumoniae*, *Enterobacter cloacae* with 16% and 5 % for *Pseudomonas aeruginosa*.

Regarding blood cultures, a prevalence of 35% was recorded. Our results are different from those carried out in a study in Sweden by Appelgren et al. [11] and those carried out in Greece by Michalopoulos et al. [12] who observed sepsis with 22.4% and 17% of blood cultures of nosocomial origin. All of our multidrug resistant strains were Gram negative bacilli. The prevalence of MRB was 57.14%. The main MRBs are represented by *Klebsiella pneumoniae* with 33.33% of MRB including blood cultures, followed by *Escherichia coli* (25%), *Acinetobacter baumanii*, *Enterobacter cloacae*, *Kluyvera spp*, *Pseudomonas aeruginosa*, *Pseudomonas luteola* with percentages of 8, 33% each. These results are opposed to those of a Tunisian study [13] which found a prevalence of 36% of MRB and the responsible germs were as follows: *Klebsiella pneumoniae* (69.84%), *Staphylococcus aureus* (12.70%), *Escherichia coli* (11.11%), *Acinetobacter baumanii* (4.76%) and *Pseudomonas aeruginosa* with 1.59%. The frequencies recorded in this study could be explained by the bacterial ecology specific to each department.

In terms of material sampling, an overall prevalence of 78.57%, including 72.73% of MRB, was recorded for all germs. All strains of *Klebsiella pneumoniae* were MRB with a percentage of 100%. These results are opposed to the Tunisian study which reports a rate of 31.77% for *Klebsiella pneumoniae* at the level of material samples [13]. Two other studies reported lower prevalence of MRB than that of our study, that of Rabat in Morocco in 2013 [14] with a rate of 45% for the species *Klebsiella pneumoniae* and that carried out in France in 2014, with 59, 2% for the species *Klebsiella pneumonia* [15]. In fact, the *Klebseilla pneumoniae* strain has been described as a typical and major agent responsible for numerous nosocomial epidemics [16].

As regards the pus on the 20 bacterial strains isolated, 8 strains were multi-resistant, ie a prevalence of 40%. The bacteria incriminated were MRSA which occupied the first place, i.e. 37.50%, *Pseudomonas aeruginosa* (25%), *Pseudomonas luteola* (25%) and *Aeromonas hydrophila* (12.50%). Our results are comparable to the Moroccan study carried out at the Mohammed V Military Hospital which recorded a prevalence of 40.4% of MRB in pus [17]. In pus, *Pseudomonas aeruginosa* or *pyocyanic bacillus* is endemic with sometimes epidemic outbreaks, according to some authors, the isolation of this pathogen is a predictor of a poor prognosis. Thus, in the Moroccan study [17], we observed a higher frequency of *Pseudomonas aeruginosa*, ie 70.8%, and the rate of MRSA in pus was 37%, which is similar to our results. For methicillin-resistant Staphylococcus aureus, the delay and difficulty in initiating effective treatment due to the multi-resistance of this pathogen are factors that worsen the often precarious situation of patients hospitalized in some units.

## 5. CONCLUSION

The problem of resistance is a phenomenon that affects all hospital departments but to varying degrees, depending on whether or not the hospital implements surveillance, hygiene and antibiotic prescription measures. It is therefore necessary for each service to define a strategy based on local epidemiological data. The fairly high rate of BMRs and their ever-increasing evolution show that the efforts made to fight against multi-resistant strains in our hospitals must be stepped up.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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