

## Antibiotic Resistance of Bacterial Strains Isolated in Kisangani, Democratic Republic of Congo: A Retrospective Study

Jean-Marie Liesse Iyamba<sup>1,4</sup>, Salomon Batina Agasa<sup>3</sup>, Lukonga Hatibu<sup>2</sup>, Cyprien Mbundu Lukukula<sup>1,4</sup>, Gabriel Mongulu Monatu<sup>2</sup>, Grégoire Mbusa Vihembo<sup>1,4</sup>, Joseph Welo Unya<sup>1,4</sup>, Benjamin Kodondi Ngbandani<sup>1,4</sup>, Junior Disashi Tshimpangila<sup>1,4</sup>, and Takaisi Kikuni NB<sup>1,4</sup>

<sup>1</sup>Laboratory of Experimental and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Kinshasa, Democratic Republic of Congo.

<sup>2</sup>Laboratoire de Santé Publique, Faculté de Médecine et de Pharmacie, University of Kisangani, Kisangani, Democratic Republic of Congo.

<sup>3</sup>Département de Médecine Interne, Cliniques Universitaires de Kisangani, Faculté de Médecine et de Pharmacie, Université de Kisangani, Kisangani, Democratic Republic of Congo.

<sup>4</sup>Centre Universitaire de Référence de Surveillance de la Résistance aux Antimicrobiens, Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo.

### \*Correspondence:

Jean Marie Liesse Iyamba, Centre Universitaire de Référence de Surveillance de la Résistance aux Antimicrobiens, Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo.

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### ABSTRACT

Investigation reports or data on the profiles of microorganisms causing infections, as well as on their respective antibiotic resistance patterns are lacking in the health care institutions present in Kisangani Region. In this purpose, the present retrospective study was carried out, in order to determine the frequency of relevant pathogens and their resistance patterns to commonly used antibiotics in the Laboratoire de Santé Publique (Public Health Laboratory) of Kisangani.

**Settings and Design:** Retrospective study for a period of 5 years, from January 2013 to December 2017, in the Laboratoire de Santé Publique of Kisangani.

**Materials and Methods:** The culture and sensitivity data of the pathogens from different clinical samples were collected from the records of Laboratoire de Santé Publique of Kisangani for study period. Samples were cultured on blood agar and MacConkey media and incubated at 37°C overnight, and organisms were identified by standard methods. Antibiotic susceptibility was carried out by Kirby-Bauer disk diffusion method, according to the Clinical and Laboratory Standards Institute guidelines.

**Results:** From a total of 1530 strains of pathogenic bacteria isolated from patient samples, 823 (53.8%) of them were *Staphylococcus aureus*, 240 (15.7%) *Enterobacter* sp., 218 (14.2%) *Escherichia coli*, 113 (7.4%), 44 (3%) *Pseudomonas aeruginosa*, 34 (2.2%) *Streptococcus pyogenes*, 30 (2%,0) *Proteus mirabilis*, 13 (0.8%) *Salmonella* Typhi, 8 (0.51%) *Enterococcus faecalis*, 4 (0.2%) *Neisseria gonorrhea*, and 3 (0.19%) were *Klebsiella pneumoniae*.

The majority of these bacteria pathogens displayed very high multidrug-resistance, particularly to ampicillin and amoxicillin (> 80 %) and to other tested antibiotics.

These observations necessitate improving of a rational antibiotic use and call for the surveillance of antimicrobial resistance, in order to reduce the spread of drug-resistant pathogenic bacteria involved in common infections.

## Keywords

Common infections, Pathogenic bacteria, Antibiotic resistance, Kisangani, Democratic Republic of Congo.

## Introduction

The emergence of bacteria resistant to multiple antibiotics used in the treatment of infections has become an important health problem worldwide, particularly in African countries [1]. Antibacterial susceptibility patterns and bacterial spectra vary geographically, highlighting the importance of local surveillance data. In industrialized countries, these data are available at regional, national and international levels; for instance, as provided by the European Antimicrobial Resistance Surveillance System (EARSS) database [2]. However, current surveillance data are scarce in Africa [3,4]. In developing countries, the cost of health care is a matter of major concern [5]. This is especially true for Democratic Republic of Congo (DRC), where reports on antibiotic resistance are mainly from hospital and health care settings of Kinshasa [6]. In contrast, investigation reports or data on the profiles of microorganisms causing infections, as well as on their respective antibiotic resistance patterns are lacking in the health care institutions present in the Kisangani Region. In this purpose, the present retrospective study was conducted, in order to determine the frequency of relevant bacteria pathogens and their resistance patterns to commonly used antibiotics in the Laboratoire de Santé Publique (Public Health Laboratory) of Kisangani. Results of antibiogram tests performed in the public laboratories should be used to show trends in the evolution of the antibiotic resistance.

## Materials and Methods

A retrospective study was undertaken in a period of 5 years (from 1<sup>st</sup> January 2013 to 31 December 2017), in order to record the antibiotic resistance profiles of relevant bacterial pathogens isolated from samples of urine, stool, sputum, blood, surgical site infections (SSIs), ear and nasal swabs which were analyzed in the Laboratoire de Santé Publique (Public Health Laboratory) of Kisangani, the DRC. These samples were obtained from in and out patients of the Cliniques Universitaires and the Hôpital Provincial Général de Référence de Kisangani. The culture and sensitivity reports were collected from the records of this Laboratory.

Approval from the responsible of Laboratory was obtained prior to start the study.

The clinical samples were cultured on blood agar and MacConkey media and incubated overnight at 37°C. The bacterial isolates were identified by Gram-staining and conventional biochemical tests [7]. Antimicrobial susceptibility test was done by using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar and the interpretations of the results were carried out according to the Clinical and Laboratory Standards Institute guidelines [8]. The following antibiotic disks were tested: amoxicillin, ampicillin, cefotaxime, ceftriaxone, ciprofloxacin, and gentamicin. All data were tabulated and analyzed. Descriptive statistics were used for analysis, and the results were expressed as frequency and percentage. Microsoft Excel 2016 software was used to analyze the data.

## Results

### Bacteria spectrum

The spectrum of bacterial pathogens was highly diverse for the majority of infections. A total of 1530 strains of clinically relevant pathogenic bacteria were isolated from different patient samples collected in this period of study. The prevalence of bacterial species was reported in Table 1. The most frequent bacteria isolated from urine were *S. aureus* (59.7 %, n = 664), followed by *E. coli* (16 %, n = 177), *Enterobacter sp.* (14.7 %, n = 163), *Citrobacter sp.* (8.8%, n = 98), *Neisseria gonorrhea* (0.3%, n = 4), *Enterococcus faecalis* (0.35%, n = 4), and *Pseudomonas aeruginosa* (0.1%, n = 2). *S. aureus* was also the most important pathogen isolated from SSIs (43.9%, n = 115), followed by *Enterobacter sp.* (16.8%, n = 44), *E. coli* (13.7%, n = 36), *P. aeruginosa* (11.5%, n = 30), *Proteus mirabilis* (11.5%, n = 30), *Citrobacter* (1.5%, n = 4), and *Klebsiella pneumoniae* (1.1%, n = 3). The two most pathogens isolated from tissue and soft tissue infections were *S. aureus* (78,6%, n = 22) and *P. aeruginosa* (21.4%, n = 6). The most important pathogens isolated from ear, nose and throat infections were respectively *S. pyogenes* (32.7%, n = 34), followed by *Enterobacter sp.* (31.7%, n = 33), *S. aureus* (17.3%, n = 18), *Enterobacter sp.* (10.6%, n = 11), *P. aeruginosa* (5.8%, n = 6), and *E. coli* (1.9%, n = 2). *Salmonella* Typhi (81.25%, n = 13) was the sole and the most important pathogen isolated from the digestive infections.

**Table 1:** Distribution of isolates from various infections.

Pathogens	Urinary tract infections	Surgical site infections	Bloodstream infections	Skin and soft tissue infections	Ear, nose, throat infections	Digestive infections	Total
<i>S. aureus</i>	664	115	4	22	18	-	823(53.8%)
<i>Enterobacter sp.</i>	163	44	-	-	33	-	240 (15.7%)
<i>Escherichia coli</i>	177	36	-	-	2	3	218(14.2%)
<i>Citrobacter sp.</i>	98	4	-	-	11	-	113 (7.4%)
<i>Pseudomonas aeruginosa</i>	2	30	-	6	6	-	44 (3%)
<i>Proteus mirabilis</i>	-	30	-	-	-	-	30 (2 %)
<i>Salmonella</i> Typhi	-	-	-	-	-	13	13 (0.8%)
<i>Klebsiella pneumoniae</i>	-	3	-	-	-	-	3 (0.19%)
<i>Neisseria gonorrhea</i>	4	-	-	-	-	-	4 (0.2 %)
<i>Streptococcus pyogenes</i>	-	-	-	-	34	-	34 (2.2%)
<i>Enterococcus faecalis</i>	4	-	4	-	-	-	8 (0.51%)
<b>Total</b>	<b>1112 (72.7%)</b>	<b>262(17.12 %)</b>	<b>8 (0.5 %)</b>	<b>28 (1.83%)</b>	<b>104 (6.8%)</b>	<b>16 (1.05%)</b>	<b>1530 (100%)</b>

## Antibiotic resistance

We observed in the present study that the antibiotic sensibility test was not systematically performed for each strain isolated in this laboratory during five past years. The resulting resistance patterns of the isolated strains of different bacterial species are reported on Table 2.

### *Staphylococcus aureus*

The highest rates of acquired resistance were observed with ampicillin (98.8%), amoxicillin (67.4%), ceftriaxone (59.1%), and gentamicin (56.7%). This resistance rate reached 48.2% for ciprofloxacin and 32.4% for cefotaxime.

### *Escherichia coli*

*E. coli* strains were 95.7% resistant to ampicillin, 83.9 % to amoxicillin 68.1 % to gentamicin, and 40.6% and 36.6% of the strains were resistant to cefotaxime and ciprofloxacin, respectively.

### *Enterobacter sp.*

About 93.8 % of strains were resistant to ceftriaxone, 87.8% to ampicillin, 82.6% to amoxicillin, 69.1% to gentamicin, 38.8% to ciprofloxacin, and 36.4 % to cefotaxime.

### *Citrobacter sp.*

*Citrobacter* strains showed high resistance to ceftriaxone (89.9%), ampicillin (88.9%), amoxicillin (87.1%), and gentamicin (70.9%), moderate and less resistance to cefotaxime (32.3%) and to ciprofloxacin (16.0%), respectively.

### *Proteus mirabilis*

This bacterium showed high resistance rates to ampicillin (100.0%) and amoxicillin (80.0%), and moderate rates to cefotaxime (33.3%), ciprofloxacin (47.05%), and gentamicin (47.6%).

### *Pseudomonas aeruginosa*

*P. aeruginosa* strains were moderate resistant to ciprofloxacin (36.7%) and gentamicin (38.9%), and highly resistant to amoxicillin (100.0%), ampicillin (100.0%), as well as to ceftriaxone (60.0%).

### Other pathogens

*Salmonella typhi* were 100% resistant to ampicillin. *Neisseria gonorrhoeae* were 100 % sensitive to ciprofloxacin and gentamicin,

but 100% resistant to cefotaxime and ampicillin. *Enterococcus faecalis* isolates were highly resistant to ampicillin (100.0%), amoxicillin (80.0%), as well as to gentamicin (62.5%).

## Discussion

In the present study, we observed an important increase in the prevalence of clinically relevant pathogenic bacteria in patient infections, as well as of their resistance against the most commonly used antibiotics. In all cases of infections, in exception of the digestive infections, *S. aureus*, followed by *Enterobacter sp.*, *E. coli*, *Citrobacter*, *P. aeruginosa*, *S. pyogenes*, *P. mirabilis*, and *E. faecalis* were the bacterial species mostly isolated during the last five years in the Laboratoire de Santé Publique (Public Health Laboratory) of Kisangani. Selective pressure by antimicrobial drugs would be by far the most important driving force for the development of such resistance [9].

During the 5-year period included in the present study (2013-2017), the Microbiology Unit of the Laboratoire de Santé Publique (Public Health Laboratory) of Kisangani reported 1530 bacterial strains isolated from positive samples, of which 823 (53.8%) were *S. aureus*. This gram-positive bacterium was predominant in the majority of infections. A high prevalence of enterobacteria, in particular *E. coli*, *Enterobacter* and *Citrobacter*, were also observed. The spectrum of isolated pathogenic bacteria observed in our study was very similar to the bacterial profile obtained in a study done in Gabon [10]. *S. aureus* was the major cause of UTI accounting for 59.7%, followed by *Enterobacteriaceae* (*E. coli* (16.0%), *Enterobacter sp.* (14.7%) and *Citrobacter sp.* (8.8%)). However, our results were in contrast with data reported in the literature, in which *E. coli* is generally the most common uropathogen [11,12].

The predominance of gram-negative bacteria in SSIs observed in the present study was in agreement with findings from Uganda, Tanzania, and Ethiopia [13-15]. But, in contrast, *S. aureus* was the dominant pathogen among all bacteria species isolated from SSIs.

In this retrospective study, in vitro antibiotic susceptibility testing to the commonly used drugs showed that the isolated bacterial strains were multidrug resistant and presented different resistance profiles

**Table 2:** Antibiotic resistance pattern of prevalent pathogens

Pathogens	Antibiotics					
	Cefotaxime	Ceftriaxone	Ciprofloxacin	Amoxicillin	Ampicillin	Gentamicin
<i>S. aureus</i>	227/701 (32.4%)	13/22(59.1%)	290/601(48.2%)	289/429(67.4%)	526/532(98.8%)	353/623(56.7%)
<i>Enterobacter sp.</i>	72/126 (36.4%)	15/16 (93.8%)	59/156(38.8%)	95/115(82.6%)	126/144 (87.50%)	121/175 (69.1%)
<i>Escherichia coli</i>	71/175 (40.6%)	0/3 (0.0%)	56/153(36.6%)	94/112(83.9%)	135/141 (95.7%)	111/163 (68.1%)
<i>Citrobacter sp.</i>	31/96 ( 32.3 )	16/18 (88.9%)	12/75 (16.0%)	54/62 (87.1%)	56/63 (88.9%)	63/89 (70.8%)
<i>Pseudomonas aeruginosa</i>	21/35 ( 60.0%)	ND	11/30 (36.7%)	24/24 (100%)	26/26 (100%)	14/32 (38.9%)
<i>Proteus mirabilis</i>	9/27(33.3%)	ND	8/17(47.05%)	12/15 (80.0%)	17/17 (100%)	10/21 (47.6%)
<i>Salmonella Typhi</i>	3/6 (33.3%)	4/4 (100%)	2/13 (15.4%)	3/3 (100%)	10/10 (100%)	2/4(50.0%)
<i>Klebsiella pneumoniae</i>	0/3 (0.0%)	2/2 (100%)	ND	ND	3/3 (100%)	3/3 (100%)
<i>Neisseria gonorrhoea</i>	4/4 (100%)	ND	0/4 (0.0%)	ND	4/4 (100%)	0/4 (0.0%)
<i>Streptococcus pyogenes</i>	ND	ND	ND	ND	ND	ND
<i>Enterococcus faecalis</i>	0/9 (0.0%)	ND	3/12 (25.0%)	12/15 (80.0%)	8/8 (100%)	5/8 (62.5%)

to the tested antibiotics, in exception to ampicillin and amoxicillin. All gram-positive cocci and gram-negative rods showed a very high resistance to ampicillin and amoxicillin, as well as a high or a moderate resistance to other antibiotics tested. Similar results were observed elsewhere [13,15-19]. The reason behind this could be, among others, the irrational use of antibiotics, in particular of ampicillin and amoxicillin, which are the most commonly used antibiotics in empiric prophylaxis and therapy, as well as in self-medication in the community. Furthermore, the excessive and inappropriate use of antibiotics in hospitals, health care facilities and in the community contribute to the development of bacterial resistance.

In the Democratic Republic of Congo (DRC) as well as in the Sub-Sahara countries, antimicrobials can be purchased from pharmacies without a prescription. This situation has led to the rapid emergence of many resistant bacteria [20]. There is now evidence that the use of poor-quality antibiotics, including both sub-standard and falsified products, and the absence of microbiological report before antibiotic use have undoubtedly exacerbated the problem of antimicrobial resistance in Africa [20,21]. As the use of under-dosed or poorly bioavailable antimalarials contributed to the rise of resistance to traditional antimalarials [22,23], there is now evidence that the same may apply to poor-quality antibiotics [24,25], including both sub-standard and falsified products.

Despite some limitations of this study, it alerts to the development of resistance of all isolated microbes to commonly used antibiotic in a context of limited resources.

## Conclusion

The retrospective study furnished the details about the profiles of common pathogenic bacteria isolated in the Laboratoire de Santé Publique of Kisangani and their respective antibiotic resistance patterns. It appeared that *S. aureus* and *Enterobacteriaceae* were the predominant bacteria isolated from different clinical samples and involved in different kinds of infections in this city during the period included in this study. Bacteria strains were highly resistant to ampicillin and amoxicillin. The higher resistance rates of strains against the most commonly used antibiotics observed in the present study suggest the necessity of the implementation and development of national policies and plans to prevent and combat the spread of resistant bacteria in the community and in the health care institutions.

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