Antibiotic Resistance Pattern and Biofilm Formation of *Staphylococcus* and *Enterobacteriaceae* Isolates from Clinical Samples of Patients with Urinary Tract and Surgical Site Infections in Kinshasa, Democratic Republic of Congo

Jean-Marie Liesse Iyamba\(^1,2,6\), Cyprien Mbundu Lukukula\(^1,2\), Joseph Welo Unya\(^1,2\), Benjamin Kodondi Ngbandani\(^1,2\), Edouard Bissingou\(^1\), Musomoni Mabankama\(^2\), Nelson Nsiata Ngoma\(^2\), Thierry Mukendi Kajinga\(^1\), Blaise Mabamu Maya\(^1\), Aline Diza Lubonga\(^1\), NB Takaisi-Kikuni\(^1,2\)

**Abstract**

**Background:** Gram-negative and Gram-positive microorganisms are responsible for both community and hospital acquired infections. The increase, emergence, and spread of antimicrobial resistance among bacteria are the most important health problems worldwide. One of the mechanisms of resistance used by bacteria is biofilm formation. The aim of this study was to investigate the antibiotic resistance pattern and the biofilm formation ability of *Staphylococcus aureus* and *Enterobacteriaceae* isolates.

**Methods:** A total of 18 *Staphylococcus aureus* and 60 *Enterobacteriaceae* clinical isolates were collected from patients with urinary and surgical site infections in Hôpital Biamba Marie Mutombo and Saint Joseph Hospital. The antibiotic susceptibility profile of the isolates were determined by disk-diffusion method. Microtiter plate method was used to assess the ability of bacteria strains to produce and to form un biofilm.

**Results:** The majority of *S. aureus* and *Enterobacteriaceae* clinical isolates were highly resistant to the majority of antibiotics and biofilm producers. *S. aureus* strains were 100 % resistant to ampicillin-sulbactam, piperacillin-tazobactam, vancomycin, amoxicillin-clavulanic acid, levofoxacin, and aztreonam. *E. coli*, *Enterobacter*, *Citrobacter*, and *Serratia* strains were 100 % resistant to third generation cephalosporins, imipenem, and amoxicillin-clavulanic acid. Non-relationships were found between the ability to form biofilm and antimicrobial resistance.

**Conclusion:** The results of the present study demonstrate the emergence of multidrug resistant organisms and suggest the implementation of antimicrobial resistant monitoring program.

**Keywords:** Antibiotic resistance, *Staphylococcus aureus*, *Enterobacteriaceae*, Biofilm

**Introduction**

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria [1]. Gram-positive and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance [1]. Treatment of infections is compromised worldwide by the emergence of bacteria that are resistant to multiple antibiotics [2]. Infections caused by multidrug-resistant organisms (MDROs) are associated with increased mortality,
morbidity, length of hospitalization, cost of health care, and the cost-effectiveness of antibiotics with different degrees of resistance [3, 4]. MDROs include vancomycin-resistant enterococci (VRE), methicillin-resistant Staphylococcus aureus (MRSA), and resistant gram-negative bacilli (RGNB) [1]. Bacterial resistance to antibiotics is primarily the consequence of a variety of phenomena such as alteration of the target of the drug, impermeability of the bacteria to the antibiotic, destruction of the antibiotic molecule, efflux system able to pump antibiotic out of the cytoplasm of bacteria, and genetically associated changes (mutational events, genetic transfer of resistance genes through plasmids, and mutations of target genes) [5]. Enterobacteriaceae had become resistant to β-lactam antibiotics and carbapenems due to the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases enzymes such as oxacillinase (OXA)-48-like β-lactamases respectively [6, 7]. However, this is not the only reason for antimicrobial treatment failure. Bacteria are able to colonize host tissues or medical devices and to form a biofilm. By definition, biofilms are microbially derived sessile communities characterized by cells that are irreversibly attached to a substratum or interface or each other, are embedded in a matrix of extracellular polymeric substances that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription [8]. Growth in biofilms enhances the survival of bacterial populations in hospital settings and inside patients, increasing the probability of causing nosocomial infections. Biofilm formation confers pathogenic bacteria increased resistance to convectional antibiotics and host defenses mechanisms [9]). Previous studies showed a correlation between biofilm production and multiple drug resistance in clinical isolates [10, 11].

Information concerning the true extent of the problem of AMR in the African Region is limited because surveillance of drug resistance is carried out in only a few countries. In order to provide data on antibiotic resistance, our laboratory collects bacterial strains from hospitals in order to monitor the resistance of certain important pathogens. The purpose of the work reported here was to evaluate the antibiotic resistance of S. aureus and Enterobacteriaceae strains from patients with urinary tract and surgical site infection respectively at Biamba Marie Mutombo Hospital and Saint Joseph Hospital located in Eastern Kinshasa city, to determine the prevalence OXA-48-producing Enterobacteriaceae, and to study the formation of the biofilm by clinical strains isolated.

Material and Methods

Bacteria isolates

From Biamba Marie Mutombo Hospital, a total of 13 clinical isolates of S. aureus isolates (from urines, vaginal smears, prostatic fluid, infected devices and from surgical site infections[SSI]), and 19 clinical isolates of Enterobacteriaceae (10 Escherichia coli and 9 Enterobacter sp.) from urinary tract samples (UTI) were investigated. From Saint Joseph Hospital, 5 S. aureus and 41 Enterobacteriaceae (19 E. coli, 8 Enterobacter sp., 9 Citrobacter sp. and 5 Serratia sp.) isolates from SSI were tested. The clinical samples were collected for diagnostic purposes by the bacteriology laboratories of these hospitals, and were from hospitalized and non-hospitalized patients.

All Staphylococcus sp. were initially identified by standard microbiological methods including Gram stain, catalase and coagulase tests. In the microbiology laboratory of the Faculty of Pharmaceutical Sciences, University of Kinshasa, the identification of Staphylococcus aureus strains was performed with latex agglutination test (Pastorex Staph-Plus, BioRad, Marnes-la-Coquette, France) and DNase test. All staphylococcal strains, negative for latex agglutination and DNase tests, were considered as coagulase negative staphylococci.

Isolated strains of Gram negative bacilli were identified using microbiological conventional methods including Gram staining, oxidase tests, indole and urease production, citrate utilization, hydrogen sulphide, gas production and fermentation of sugars, phenylalanine deaminase, lysine decarboxylase (L.D.C.), ornithine decarboxylase (O.D.C.), arginine dihydrolase (A.D.H.) tests, and methyl red reaction. In our laboratory Gram negative bacilli were confirmed as Enterobacteriaceae species using the same tests. All cultures were maintained on trypticase soy agar (Liofilchen, Roseto degli Abruzzi, Italy).

Antibiotic susceptibility tests

Antibiograms of each isolated Staphylococcus spp strains using the diffusion method on Mueller Hinton Agar were realized with the following antibiotic disks (Liofilchen, Roseto degli Abruzzi, Italy): amikacin (30 µg), amoxicillin + clavulanic acid (30 µg), ampicillin (30µg), ampicillin-sulbactam (30/20 µg), azithromycin (15 µg), aztreonam (30 µg), ceftazidime (30 µg), cefxime (5 µg), ciprofloxacin (5µg),clarithromycin(15µg),erythromycin(15µg),fosfomycin (200 µg), kanamycin (30 µg), levofloxacin (5 µg), netilimicin (30 µg), piperacillin - tazobactam (100/10 µg), teicoplanin (30 µg), temocillin (30 µg), tobramycin (10 µg), trimethoprim (5 µg), and vancomycin (30 µg). Test for methicillin resistance was performed with diffusion method using oxacillin (1 µg) on Mueller Hinton agar with 4 % NaCl.

Enterobacteriaceae were tested against the following antibiotic disks (Liofilchen, Roseto degli Abruzzi, Italy): ampicillin (30 µg), amikacin (10 µg), amoxicillin (10 µg), ampicillin (30 µg), ampicillin-sulbactam (20 µg), aztreonam
(30 µg), cefixime (5 µg), cefotaxime (5 µg), cefuroxime (30 µg), ceftazidime (30 µg), fosfomycin (200 µg), imipenem (10 µg), norfloxacin (5 µg), levofloxacin (5 µg), tobramycin (10 µg), temocillin (30 µg), and piperacillin-tazobactam (100/10 µg). After incubation of plates at 37°C for 24 hours, diameters of zone of inhibition were measured. Evaluation of the results was done according to the criteria of Clinical Laboratory Standards Institute (CLSI) [12]. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control.

**Detection of OXA-48 producers**

OXA-48-producing *Enterobacteraeae* were detected on Chromatic™ OXA-48 chromogenic medium (Liofilchem, Roseto degli Abruzzi, Italy). After incubation at 37°C/24-48 hours, the color and the morphology of the colonies were observed and the results interpreted as follow: red colony (*E. coli*-producing OXA-48), blue-violet colony (*Klebsiella* sp. producing OXA-48), blue-green (*Enterobacter* sp. producing OXA-48), blue colony with red halo (*Citrobacter* sp. producing OXA-48). *E. coli* ATCC 25922 was used for quality control.

**Biofilm formation assay**

In present study, we screened all isolates for their ability to form biofilm by Crystal Violet Staining method as previously described [13], with modifications. A suspension equivalent to the McFarland 0.5 turbidity standard was prepared in Trypticase Soy broth (Becton Dickinson, Franklin Lake) for each strain. Accuracy of bacterial counts in the suspension was confirmed by serial dilution in log steps. Polystyrene sterile strips were inoculated with 200 µL of each calibrated bacterial suspension and incubated for 24 hours at 35°C in a humid atmosphere. A control well was inoculated with sterile medium. Each strain was evaluated in triplicate. Medium was removed from the wells which were washed 3 times with 200 µL sterile distilled water. The strips were air-dried for 45 min and the adherent cells were stained with 200 µL of 0.1% Crystal violet solution. After 45 min, the dye was eliminated and the wells were washed 5 times with 300 µL of sterile distilled water to remove excess stain. The dye incorporated by the cells forming a biofilm was dissolved with 200 µL of 33% (v/v) glacial acetic acid and the absorbance of the well was obtained by means of enzyme-linked immunosorbent assay (ELISA) reader, at the wavelength of 540 nm. The results were expressed as variation of Optical density (OD)540 nm (OD540 nm sample - OD540 nm control). These OD values were considered as an index of bacteria adhering to surface and forming biofilms. For interpretation of biofilm production, the average of the three wells was calculated, and the criterion proposed by Stepanovic et al. [14] was adopted: non-adherent (OD < 0.12), moderate producer (0.12 < OD < 0.24) and strong producer (OD > 0.24).

**Results**

**Antibiotic susceptibility**

The *S. aureus* isolates in Biamba Marie Mutombo Hospital and from UTI were 100 % resistant to ampicillin-sulbactam, piperacillin-tazobactam, levofloxacin, and amoxicillin-clavulanic acid. With the exception for fosfomycin, netilmicyn and amikacin, the resistance rates of clarithromycin, azithromycin, cefixime, ceftazidime, tobramycin, trimethoprim, and aztreonam to *S. aureus* was within the range 69 - 92 %. All *Staphylococcus* studied were MRSA and resistant to glycopeptide antibiotics, vancomycin and teicoplanin (Table 1).

**Table 1: Antibiotic susceptibility pattern of *S. aureus* isolates from UTI and SSI**

| S. aureus isolates from UTI (Biamba Marie Mutombo Hospital) | Antibiotics | Resistance pattern |
|-------------------------------------------------------------|-------------|--------------------|
|                                                             |             | Resistant | Sensitive |
| Oxacillin                                                   | 13 (100.0%) | 0 (0.0%)  |
| Clarithromycin                                              | 9 (69.2%)   | 4 (30.8%)  |
| Fosfomycin                                                  | 4 (30.8%)   | 9 (69.2%)  |
| Levofloxacin                                                | 13 (100.0%) | 0 (0.0%)   |
| Ampicillin-sulbactam                                        | 13 (100.0%) | 0 (0.0%)   |
| Azithromycin                                                | 10 (77.0%)  | 3 (23.0%)  |
| Teicoplanin                                                 | 13 (100.0%) | 0 (0.0%)   |
| Cefixime                                                    | 11 (84.6%)  | 2 (15.4%)  |
| Ceftazidime                                                 | 12 (92.3%)  | 1 (7.7%)   |
| Tobramycin                                                  | 12 (92.3%)  | 1 (7.7%)   |
| Vancomycin                                                  | 13 (100.0%) | 0 (0.0%)   |
| Amikacin                                                    | 2 (15.4%)   | 11 (84.6%) |
| Trimethoprim                                                | 12 (92.3%)  | 1 (7.7%)   |
| Piperacillin-tazobactam                                     | 13 (100.0%) | 0 (0.0%)   |
| Aztreonam                                                   | 12 (92.3%)  | 1 (7.7%)   |
| Netilmicyn                                                  | 4 (30.8%)   | 9 (69.2%)  |
| Amoxicillin-clavulanic acid                                 | 13 (100.0%) | 0 (0.0%)   |

| S. aureus isolates from SSI (Saint Joseph Hospital)        | Antibiotics | Resistance pattern |
|-------------------------------------------------------------|-------------|--------------------|
|                                                             |             | Resistant | Sensitive |
| Oxacillin                                                   | 5 (100.0%)  | 0 (0.0%)   |
| Ampicillin                                                  | 5 (100%)    | 0 (100%)   |
| Fosfomycin                                                  | 5 (100%)    | 0 (0.0%)   |
| Levofloxacin                                                | 4 (80.0%)   | 1 (20.0%)  |
| Ciprofloxacin                                               | 4 (80.0%)   | 1 (20.0%)  |
| Trimethoprim                                                | 2 (40.0%)   | 3 (60.0%)  |
| Teicoplanin                                                 | 5 (100.0%)  | 0 (0.0%)   |
| Ceftazidime                                                 | 4 (80.0%)   | 1 (20.0%)  |
| Vancomycin                                                  | 5 (100.0%)  | 0 (0.0%)   |
| Amikacin                                                    | 2 (40.0%)   | 3 (60.0%)  |
| Erythromycin                                                | 5 (100%)    | 0 (0.0%)   |
| Aztreonam                                                   | 4 (80.0%)   | 1 (20.0%)  |
| Temocillin                                                  | 4 (80%)     | 1 (20.0%)  |
| Amoxicillin-clavulanic acid                                 | 5 (100.0%)  | 0 (0.0%)   |
The 5 *S. aureus* strains isolated in Saint Joseph Hospital (Kinshasa) from SSI were highly resistant to ampicillin (100%), ceftazidime (80%), fosfomycin (100%), amoxicillin + clavulanic acid (100%), aztreonam (100%), temocillin (80%), and erythromycin (100%). All strains were MRSA. All MRSA strains were fully resistant to vancomycin and teicoplanin (Table 1).

In *E. coli* isolates, imipenem, cefixime, cefotaxime, ceftazidime, aztreonam, norfloxacin, temocillin, amoxicillin, ampicillin-sulbactam, and piperacillin-tazobactam resistance was observed in 100% of cases. All *Enterobacter* sp. strains were fully resistant to imipenem, cefixime, temocillin, cefotaxime, aztreonam, amoxicillin, ampicillin-sulbactam, and piperacillin-tazobactam. *E. coli* and *Enterobacter* sp. strains demonstrated good sensitivity to fosfomycin. For other antibiotics, resistance was over 70%, with the exception of amikacin (Table 2).

The *E. coli*, *Citrobacter* sp., *Enterobacter* sp., *Serratia* sp. strains from SSI isolated in Biamba Marie Mutombo Hospital were highly resistant to the majority of antibiotics tested. *E. coli* isolates were particularly 100% resistant to ampicillin, temocillin, kanamycin, amoxicillin – clavulanic acid, cefotaxime, and imipenem (Table 3).

Multidrug resistance (MDR) was observed in *Staphylococcus* and *Enterobacteriaceae* isolated from UTI and SSI.

### Detection of OXA-48-producing Enterobacteriaceae

Cultures in Chromatic™ OXA-48 chromogenic medium revealed 48 (87.2%) OXA-48 producers in general. All *Enterobacteriaceae* strains from SSI were OXA-48 producers (Table 4).

### Biofilm formation

The results of biofilm formation of different clinical isolates studied are presented in Table 5.

#### Enterobacteriaceae and *S. aureus* isolates from UTI

From the total number of 13 *S. aureus* isolates from Biamba Marie Mutombo Hospital and tested for biofilm formation, strong biofilm producers (SBP) were 4 (30.8%), 7 (53.8%) were moderate producers (MBP), and 2 (15.4%) were non-biofilm producers (NBP). Out of 10 *E. coli* tested for biofilm formation, 2 (20.0%) were SBP, 4 (40.0%) MBP, and 4 (40.0%) NBP. In *E. cloacae* strains, 3 (33.3%) were SBP, 4 (44.5%) MBP, and 2 (22.2%) NBP (Table 5).

#### Enterobacteriaceae and *S. aureus* isolates from SSI

Among 5 *S. aureus* strains isolated from SSI in Saint Joseph Hospital, Kinshasa, 3 (60%) were resistant to amoxicillin, 2 (40%) were resistant to amikacin, and 1 (20%) were resistant to kanamycin.
Joseph Hospital and tested for biofilm formation, 4 (80.0%) were SBP, and 1 (20.0%) was NBP. Ten (52.6%), 9 (47.4%) of E. coli strains were SBP and MBP respectively. For a total of 9 Enterobacter sp. studied for biofilm formation, 6 (62.5%) were SBP and 3 (33.5%) were MBP. Five (66.7%) of Citrobacter strains have formed a strong biofilm and 3 (33.3%) have produced moderate biofilm. Out of 5 Serratia sp. strains, 3 (60.0%) were SBP and 2 (40.0%) were MBP (Table 5).

Resistant pattern of S. aureus and Enterobacteriaceae isolates among biofilm producers and non-biofilm producers

To determine whether biofilm formation was correlated with resistance to any particular antibiotic(s), we compared the biofilm forming capacities among isolates from UTI and SSI with different resistance profiles for all antibiotics (Table 6 and 7).

Enterobacteriaceae and S. aureus from UTI

For S. aureus isolates, resistance to oxacillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, cefixime, aztreonam, vancomycin, teicoplanin, levofloxacin, tobramycin, trimethoprim, clarithromycin, and azithromycin were higher in MBP and SBP than in NBP. Resistance to ampicillin-sulbactam; cefotaxime, cefuroxime, amoxicillin, piperacillin-tazobactam, ceftazidime, cefixime, imipenem, aztreonam, levofloxacin, norfloxacin, and tobramycin were higher in MBP and NBP than in SBP in E. coli isolates. Among Enterobacter cloacae, resistance to ampicillin-sulbactam; cefotaxime, cefuroxime, amoxicillin, piperacillin-tazobactam, cefazidime, cefixime, imipenem, aztreonam, levofloxacin, norfloxacin, and tobramycin were higher in MBP and SBP than in NBP (Table 6).

Enterobacteriaceae and S. aureus from SSI

Among S. aureus isolates, resistance to oxacillin, ampicillin, amoxicillin-clavulanic acid, ceftazidime, aztreonam, vancomycin, teicoplanin, amikacin, levofloxacin, ciprofloxacin, trimethoprim, fosfomycin, erythromycin, and temocillin were notably high in SBP than in NBP. Resistance to ampicillin, amoxicillin-clavulanic acid, cefotaxime, amikacin, kanamycin, norfloxacin, and imipenem were higher

| Organisms          | N°(%)OXA-48 type carbapenemase (Enterobacteriaceae isolates from UTI) | N° (%) OXA-48 type carbapenemase (Enterobacteriaceae isolates from SSI) | Total | Typical color colony |
|--------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------|-------|---------------------|
| Escherichia coli   | 3/10 (30%)                                                             | 19/19 (100%)                                                          | 22/29 | (75.8%) Red         |
| Enterobacter sp.   | 9/9 (100%)                                                             | 8/8 (100%)                                                           | 17/17 | (100%) Blue-green   |
| Citrobacter sp.    | -                                                                     | 9/9 (100%)                                                           | 9/9   | (100%) Blue with red halo |
| Serratia sp.       | -                                                                     | ND                                                                   |       |                     |
| Total              | 48/55 (87.2%)                                                          |                                                                      |       |                     |

| Classification according to bacterial biofilm production | E. coli | Enterobacter sp | Citrobacter sp | Serratia sp | S. aureus |
|----------------------------------------------------------|---------|----------------|----------------|-------------|-----------|
| Adherent (strong biofilm producer) (OD > 0.24)            | 10(52.6)| 5(62.5)        | 6(66.7)        | 3(60.0)     | 4(80.0)   |
| Moderate biofilm producer (0.12 < OD < 0.24)              | 9(47.4)| 3(37.5)        | 3(33.3)        | 2(40.0)     | 0(0.0)    |
| Non-adherent (non-biofilm producer) (OD < 0.12)           | 0(0.0)| 0(0.0)         | 0(0.0)         | 0(0.0)      | 1(20.0)   |
| TOTAL                                                     | 19(100.0)| 8(100.0)   | 9(100.0)      | 5(100.0)    | 5(100.0)  |

Citation: Jean-Marie Liesse Iyamba, Cyprien Mbundu Lukukula, Joseph Welo Unya, Benjamin Kodondi Ngbandani, Edouard Bissingou, Musomoni Mabankama, Nelson Nsiata Ngoma, Thierry Mukendi Kajinga, Blaise Mabamu Maya, Aline Diza Lubonga, NB Takaisi-Kikuni. Antibiotic Resistance Pattern and Biofilm Formation of Staphylococcus and Enterobacteriaceae Isolates from Clinical Samples of Patients with Urinary Tract and Surgical Site Infections in Kinshasa, Democratic Republic of Congo. Journal of Pharmacy and Pharmacology Research 6 (2022): 159-168.
Table 6: Biofilm formation and antibiotic resistance pattern *Enterobacteriaceae* and *S. aureus* isolates from UTI (Biamba Marie Mutombo Hospital)

| Antibiotic agent            | Percentage of antibiotic-resistant strains in different biofilm phenotype | S. aureus | E. coli | E. cloaceae |
|-----------------------------|---------------------------------------------------------------------------|-----------|---------|-------------|
|                             | SBP | MBP | NBP | SBP | MBP | NBP | SBP | MBP | NBP |
| Oxacillin                   | 100%(4/4) | 100%(7/7) | 100%(2/2) | ND | ND | ND | ND | ND | ND |
| Ampicillin-sulbactam        | 100%(4/4) | 100%(7/7) | 100%(2/2) | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 100%(2/2) |
| Amoxicillin-clavulanic acid | 100%(4/4) | 100%(7/7) | 100%(2/2) | ND | ND | ND | ND | ND | ND |
| Cefotaxime                  | ND | ND | ND | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 100%(2/2) |
| Cefuroxime                  | ND | ND | ND | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 75%(3/4) | 50%(1/2) |
| Amoxicillin                 | ND | ND | ND | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 100%(2/2) |
| Piperacillin-tazobactam     | 100%(4/4) | 100%(7/7) | 100%(2/2) | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 100%(2/2) |
| Ceftazidime                 | 75%(3/4) | 100%(7/7) | 100%(2/2) | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 50%(1/2) |
| Cefixime                    | 50%(2/4) | 100%(7/7) | 100%(2/2) | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 100%(2/2) |
| Imipenem                    | ND | ND | ND | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 100%(2/2) |
| Aztreonam                   | 75%(3/4) | 100%(7/7) | 100%(2/2) | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 100%(2/2) |
| Vancomycin                  | 100%(4/4) | 100%(7/7) | 100%(2/2) | ND | ND | ND | ND | ND | ND |
| Teicoplanin                 | 100%(4/4) | 100%(7/7) | 100%(2/2) | ND | ND | ND | ND | ND | ND |

SBP: strong biofilm producers; MBP: moderate producers; NBP: non-biofilm producers; ND: not determined

Table 6 Continued: Biofilm formation and antibiotic resistance pattern of *Enterobacteriaceae* and *S. aureus* isolates from UTI (Biamba Marie Mutombo Hospital)

| Antibiotic agent            | Percentage of antibiotic-resistant strains in different biofilm phenotype | S. aureus | E. coli | E. cloaceae |
|-----------------------------|---------------------------------------------------------------------------|-----------|---------|-------------|
|                             | SBP | MBP | NBP | SBP | MBP | NBP | SBP | MBP | NBP |
| Amikacin                    | 25%(1/4) | 14.2%(1/7) | 0%(0/2) | 50%(1/2) | 75%(3/4) | 25%(1/4) | 66.7%(2/3) | 50%(2/4) | 0%(0/2) |
| Netilmicin                  | 75%(3/4) | 14.2%(1/7) | 0%(0/2) | ND | ND | ND | ND | ND | ND |
| Levofloxacin                | 100%(4/4) | 100%(7/7) | 100%(2/2) | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 75%(3/4) | 50%(1/2) |
| Norfloxacin                 | ND | ND | ND | 100%(2/2) | 100%(4/4) | 100%(4/4) | 100%(3/3) | 100%(4/4) | 50%(1/2) |
| Tobramycin                  | 100%(4/4) | 85.7%(6/7) | 100%(2/2) | 50%(1/2) | 100%(4/4) | 50%(2/4) | 100%(3/3) | 75%(3/4) | 100%(2/2) |
| Trimethoprim                | 100%(4/4) | 85.7%(6/7) | 100%(2/2) | ND | ND | ND | ND | ND | ND |
| Fosfomycin                  | 0%(0/4) | 28.6%(2/7) | 100%(2/2) | 50%(1/2) | 25%(1/4) | 0%(0/4) | 0%(0/3) | 0%(0/4) | 0%(0/2) |
| Clarithromycin              | 75%(3/4) | 71.4%(5/7) | 50%(1/2) | ND | ND | ND | ND | ND | ND |
| Azithromycin                | 75%(3/4) | 85.7%(6/7) | 50%(1/2) | ND | ND | ND | ND | ND | ND |

SBP: strong biofilm producers; MBP: moderate producers; NBP: non-biofilm producers; ND: not determined

Citation: Jean-Marie Liesse Iyamba, Cyprien Mbundu Lukukula, Joseph Welo Unya, Benjamin Kodondi Ngbandani, Edouard Bissingou, Musomoni Mabanakama, Nelson Nsiata Ngoma, Thierry Mukendi Kajinga, Blaise Mamou Maya, Aline Diza Lubonga, NB Takaisi-Kikuni. Antibiotic Resistance Pattern and Biofilm Formation of *Staphylococcus* and *Enterobacteriaceae* Isolates from Clinical Samples of Patients with Urinary Tract and Surgical Site Infections in Kinshasa, Democratic Republic of Congo. Journal of Pharmacy and Pharmacology Research 6 (2022): 159-168.
### Table 7: Biofilm formation and antibiotic resistance pattern of Enterobacteriaceae and S. aureus isolates from SSI (Saint Joseph Hospital)

| Antibiotic agent | Percentage of antibiotic-resistant strains in different biofilm phenotype |
|------------------|-------------------------------------------------------------------------|
|                  | S. aureus | E. coli | E. cloaceae | Citrobacter | Serratia |
| Oxacillin        | 100%      | 0%      | ND         | ND          | ND       |
| Ampicillin       | 100%      | 0%      | 100%       | ND          | ND       |
| Amoxicillin-clavulanic acid | 100% | 0%      | 100%       | ND          | ND       |
| Ceftazidine      | 75%       | 0%      | ND         | ND          | ND       |
| Cefotaxime       | ND        | ND      | ND         | ND          | ND       |
| Cefuroxime       | ND        | ND      | ND         | ND          | ND       |
| Amoxicillin      | ND        | ND      | ND         | ND          | ND       |
| Aztreonam        | 75%       | 0%      | ND         | ND          | ND       |
| Vancomycin       | 100%      | 0%      | ND         | ND          | ND       |
| Teicoplanin      | 100%      | 0%      | ND         | ND          | ND       |

SBP: strong biofilm producer; MBP: moderate biofilm producer; NBP: non-biofilm producer

### Table 7 Continued: Biofilm formation and antibiotic resistance pattern of Enterobacteriaceae and S. aureus isolates from SSI (Saint Joseph Hospital)

| Antibiotic agent | Percentage of antibiotic-resistant strains in different biofilm phenotype |
|------------------|-------------------------------------------------------------------------|
|                  | S. aureus | E. coli | E. cloaceae | Citrobacter | Serratia |
| Amikacin         | 50%       | 0%      | 90%        | 33.3%       | 0%       |
| Kanamycin        | ND        | ND      | 100%       | 100%        | 0%       |
| Levofoxacin      | 75%       | 0%      | ND         | ND          | ND       |
| Norfloxacin      | ND        | ND      | 100%       | ND          | ND       |
| Ciprofloxacin    | 75%       | 0%      | 100%       | ND          | ND       |
| Trimethoprim     | 50%       | 0%      | ND         | ND          | ND       |
| Fosfomycin       | 100%      | 0%      | ND         | ND          | ND       |
| Erythromycin     | 100%      | 0%      | ND         | ND          | ND       |
| Imipenem         | ND        | ND      | 100%       | 100%        | 0%       |
| Temocillin       | 75%       | 0%      | ND         | ND          | ND       |

SBP: strong biofilm producer; MBP: moderate biofilm producer; NBP: non-biofilm producer

Citation: Jean-Marie Liesse Iyamba, Cyprien Mbundu Lukukula, Joseph Welo Unya, Benjamin Kodondi Ngbandani, Edouard Bissingou, Musomoni Mabankama, Nelson Nsiata Ngoma, Thierry Mukendi Kajinga, Blaise Mamamu Maya, Aline Diza Lubonga, NB Takaisi-Kikuni. Antibiotic Resistance Pattern and Biofilm Formation of Staphylococcus and Enterobacteriaceae Isolates from Clinical Samples of Patients with Urinary Tract and Surgical Site Infections in Kinshasa, Democratic Republic of Congo. Journal of Pharmacy and Pharmacology Research 6 (2022): 159-168.
in SBP than in MBP in *E. coli* isolates. Similar results were obtained for *Enterobacter* sp., *Citrobacter* sp., and *Serratia* sp. isolates (Table 7).

**Occurrence of multidrug resistant pattern and their associations with biofilm phenotype**

Regarding MDR, no relationships were found between the ability to form biofilm and antimicrobial resistance (Table 8 and Table 9).

**Discussion**

*Enterobacteriaceae* and *Staphylococcus* are known as a significant cause of infections in both community and nosocomial settings. The emergence of microorganisms resistant to multiple antibiotics used in the treatment of infections has become an important health problem worldwide, particularly in African countries [15]. The present study analyzed the resistance profile of pathogens involved in community and hospital acquiring infections and their capability to form and to produce a biofilm. The results showed an alarmingly increase of antibiotic resistance among *Enterobacteriaceae* and *Staphylococcus aureus* strains from UTI and SSI isolated in Biamba Marie Mutombo and Saint Joseph Hospitals.

All *S. aureus* isolates from UTI and SSI were MRSA. The results of studies conducted on *S. aureus* antibiotic resistance in Central Africa region are in concordance with the results of the present study. 82 % of *S. aureus* strains isolated from different clinical samples (wounds, urines, pus) were MRSA [16]. 100 % of these MRSA strains were also resistant to ceftazidime, cefotaxime, amoxicillin-clavulanic acid and cefixime as demonstrated in our study. Reports from Uganda showed MRSA prevalence of 57.2%, where 100% of MRSA strains resistant to amoxicillin-clavulanic acid, ceftriaxone, and imipenem (15). Another study from East Africa revealed an overall MRSA prevalence of 53.4% [17]). In contrast to our data, MRSA isolates from these last studies remained highly susceptible to teicoplanin and vancomycin [18, 19].

Our data demonstrates very high prevalence rates of antibiotic resistance of *Enterobacteriaceae* strains from UTI and SSI to ampicillin, imipenem, cephalosporins,
ciprofloxacin, levofloxacin, norfloxacin, amoxicillin-
clavulanic acid, amoxicillin, ampicillin-sulbactam, aztreonam, and tobramycin. These results are consistent with previous 
reports. In Nigeria, Enterobacteriaceae isolates demonstrated remarkable high rates of resistance to the β-lactam antibiotics, except the 
carbapenems and piperacillin-tazobactam. High resistance rates were also observed for E. cloacae against ampicillin 
(90%), aztreonam (80%), cefepime (70%), cefotaxime (80%), 
cefazidime (60%), and cefuroxime (100%) [17]. A study 
conducted in Rwandan referral hospital have demonstrated 
that out of 241 Gram-negative isolates tested for ceftriaxone, 
183 (75.9%) were resistant [20].

In this study, we detected OXA-48-producing strains 
among different enterobacterial species isolated in samples 
from patients with UTI and SSI. The prevalence of 87.2% 
of OXA-48-producing E. coli was observed in our 
study was higher than those obtained from studies conducted 
in some African countries, such as in a Nigerian hospital and 
in Tanzania with respectively 3.4 % and 4.9 % of OXA-48 
producers among multidrug-resistant Enterobacteriaceae 
isolates [11,15]. Investigations done in many African countries 
such as Tunisia, Libya, Tanzania, Senegal, and Morocco, had 
shown that K. pneumoniae was the most frequently OXA-48 
producer [10]. But in this study, we observed an emerging 
rates of OXA-48 producers among Enterobacter and 
Citrobacter strains (100%). In contrast, 22 of the 29 strains 
of E. coli were OXA-48 producers.

In this study the detection of biofilm formation was 
performed using Microtiter plate method. The results showed 
that 11 (84.6%) S. aureus, 6 (60%) E. coli, and 7 (77.7%) 
Enterobacter sp. isolates from UTI were biofilms producers. All Enterobacteriaceae and 4 (80.0%) S. aureus isolates 
from SSI were biofilm producers. Microbial cell adherence to 
surfaces and the development of multi-cellular communities 
is a key step in infection. Furthermore, bacteria biofilms can 
play a critical role in SSI and in in recurrent UTI [21, 22]. In 
this study the results showed that the capability of bacteria 
isolates to form a biofilm was very high in clinical strains 
from SSI than those from UTI. We demonstrated also a high 
variability in biofilm biomass production among isolates from 
UTI and SSI. Biofilm formation depends on many factors such 
as environment, sugar content and concentration (glucose 
versus lactose), geographical origin, types of specimen, 
surface adhesion characteristics, proteolytic enzymes, and 
biofilm associated genes [23 - 27]. These factors could be 
involved in the high prevalence of biofilm formation in 
bacteria strains from SSI as observed in the present study. 
Biofilm infections are clinically important because bacteria 
in biofilms exhibit recalcitrance to antimicrobial compounds. 
Microbes growing within a biofilm have been reported to 
be up to 1000 times more tolerant to antimicrobials than 
their planktonic counterparts [28]. The biofilm producing - 
Enterobacteriaceae and Staphylococcus aureus as well as non-
biofilm producers from UTI were very resistant to antibiotics. 
Our results are in contrast with those obtained by Neupane 
et al. in which 86.7% of biofilm-producing S. aureus were 
MDR; whereas all MRSA non-biofilm producers were non-
MDR [29]. Our results are also in contrast with dose obtained 
by Neupane et al., [30]. In this last study authors showed that 
the antibiotic resistance of biofilm producing - E. coli was 
found significantly higher than that of biofilm non-producing 
E. coli. In our study 3 E. coli negative for biofilm formation 
were resistant to 12 different antibiotics (Table 7). Among 
biofilm producing-Enterobacteriaceae and S. aureus from 
SSI, higher antibiotic resistance was observed in strong and 
moderate biofilm producers. In this case, our results are in 
agreement with previous reports [26, 30]. Globally, the results 
of the current study are in agreement with report in which no 
relationship was observed between global resistance or MDR 
and biofilm formation [31].

Many factors could be responsible for the increasing 
of resistance in Kinshasa. Among them are some frequent 
societal behaviors (such as self-medication), inadequate 
healthcare infrastructure (insufficiently trained prescribers 
and inadequate diagnostic tools), and an uncontrolled drug 
sector (antibiotics sold over-the-counter, improperly stored, 
counterfeit, and/or expired [32] as well as biofilm ability of 
strains and the acquisition of resistance genes [33].

Conclusion

The alarming increase of S. aureus and Enterobacteriaceae 
isolates from Biamba Marie Mutombo and Saint Joseph 
Hospital to antibiotics limits the treatment of patients with 
UTI and SSI. The study showed that non-biofilm and biofilm 
producers were MDROs. The results of the present study 
showed that antibiotic resistance is a major public health 
problem that requires a range of urgent interventions. So, 
public health authorities should implement and develop 
comprehensive national policies and plans to prevent and 
combat the spread of MDROs in community and hospital 
setting.

Conflict of Interest

None

Acknowledgments

We thank Microbiology Laboratory staff members of 
Biamba Marie Mutombo and Saint Joseph Hospitals, 
Kinshasa, for their cooperation and technical assistance 
during the study.
Abbreviations

MDROs-Multidrug-Resistant Organisms; MRSA- methicillin-resistant Staphylococcus aureus; MDR- Multidrug-resistant; OXA-oxacillinase; UTI-Urinary tract infection; SSI-Surgical site Infections, SBP-Strong biofilm producers; MBP-Moderate producers; NBP-Non-biofilm producers.

References

1. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18 (2012): 268-81.

2. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell 128 (2007): 1037-50.

3. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin Infect Dis 42 (2006): S82-9.

4. Eandi M, Zara GP. Economic impact of resistance in the community. Int J Clin Pract Suppl 95 (1998): 27-38.

5. Munita J. M and Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr 4 (2016): 10.1128.

6. Van Duin D, Paterson DL. Multidrug-resistant bacteria in the community: Trends and lessons learned. Infect Dis Clin North Am 30 (2016): 377-390.

7. van Duin D and Doi. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 8 (2017): 460-469.

8. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis 8 (2002): 881-890.

9. Høiby N, Bjarsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35 (2010): 322-332.

10. Agnes Bedie Eyoh et al. Relationship between multiple drug resistance and biofilm formation in Staphylococcus aureus isolated from medical and non-medical personnel in Yaounde, Cameroon. Pan African Medical Journal (2014): 186.

11. Nirwati H, Sinanjung K, Fahrurinissa F, et al. Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC Proc 13 (2019): 20.

12. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement. CLSI document (2012): M100-S22.

13. Chavant PI, Gaillard-Martineau B, Talon R, Hébraud M, Bernardi T. A new device for rapid evaluation of biofilm formation potential by bacteria. J Microbiol Methods 68 (2007): 605-12.

14. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 40 (2000): 175-9.

15. Tadesse et al. Antimicrobial resistance in Africa: a systematic review. BMC Infectious Diseases 17 (2017): 616.

16. Achianga et al. Antibiotic resistance in the central African Region. A review. J Environ Sci Public Health 3 (2019): 358-378.

17. Baguma A, Tbyange J, Owalla T, Kagirita A, et al. Highly resistant Staphylococcus aureus isolated from patients attending a tertiary hospital, south western Uganda. Microbiol Res J Internat 26 (2019): 1-10.

18. Wangai FK, Masika MM, Maritim MC, Seaton RA. Methicillin-resistant Staphylococcus aureus (MRSA) in East Africa: red alert or red herring? BMC Infectious Diseases 19 (2019): 596.

19. Raji MA, Jamal W, Ojemhen O, Rotimi VO. Point-surveilllance of antibiotic resistance in Enterobacteriaceae isolates from patients in a Lagos Teaching Hospital, Nigeria. J Infect Public Health 6 (2013): 431-7.

20. Sutherland T, Mpirimbanyi C, Nziyomaze E, Niyomugabo JP, et al. Widespread antimicrobial resistance among bacterial infections in a Rwandan referral hospital. PLoS One 14 (2019): e0221121.

21. Percival SL. Importance of biofilm formation in surgical infection. BJS 104 (2017): e85–e94.

22. Delecaru C, Alexandru I, Podgoreanu P, Grosu M. Microbial biofilms in urinary tract infections and prostatitis: Etiology, pathogenicity, and combating strategies. Pathogens 5 (2016): 65.

23. Kokare CR, Chakraborty S, Khopade AN, Mahadik KR. Biofilm: importance and applications. Indian J Biotechnol One 8 (2009): 159–168.

24. Coelho LR, Souza RR, Ferreira FA, Guimaraes MA, et al. (2008). Agr RNAIII divergently regulates glucose-induced biofilm formation in clinical isolates of Staphylococcus aureus. Microbiology 154 (2008): 3480–3490.

25. Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, et al. Correlation between virulence factors and in vitro biofilm formation by Escherichia coli strains. Microb Pathog 45 (2008): 86-91.
26. Zhang Y, Xu D, Shi L, Cai R, Li C and Yan H. Association between agr type, virulence factors, biofilm formation and antibiotic resistance of Staphylococcus aureus Isolates From Pork Production. Front. Microbiol 9 (2018): 1876.

27. Kawamura H, Nishi J, Inuma N, Tokuda K, Miyanohara H, et al. Quantitative analysis of biofilm formation of methicillin-resistant Staphylococcus aureus (MRSA) strains from patients with orthopaedic device-related infections. FEMS Immunol Med Microbiol 63 (2011): 10-5.

28. Luppens S, Rombouts F, Abee T. The effect of the growth phase of Staphylococcus aureus on resistance to disinfectants in a suspension test. J Food Prot 65 (2002): 124–129.

29. Neopane P, Nepal HP, Shrestha R, Uelhara O, Abiko Y. In vitro biofilm formation by Staphylococcus aureus isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. Int J Gen Med 11 (2018): 25-32.

30. Neupane S, Pant ND, Khatiwada S, Chaudhary R, Banjara MR. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic Escherichia coli isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. Antimicrob Resist Infect Control 5 (2016): 5.

31. Cepas V, López Y, Muñoz E, Rolo D, Ardanuy C, Martí S, Xercavins M, Horcajada JP, Bosch J, Soto SM. Relationship Between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria. Microb Drug Resist 25 (2019): 72-79.

32. Ouedraogo AS, Jean Pierre H, Bañuls AL, Ouédraogo R, Godreuil S. Emergence and spread of antibiotic resistance in West Africa: contributing factors and threat assessment. Med Sante Trop 27 (2017): 147-154.

33. Dumaru R, Baral R, Shrestha LB. Study of biofilm formation and antibiotic resistance pattern of gram-negative Bacilli among the clinical isolates at BPKIHS, Dharan. BMC Res Notes 12 (2019): 38.