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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

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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 *Enterobacteriacea* 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, levofloxacin, and aztreonam. *E. coli, Enterobacter* sp., *Citrobacter* sp., and *Serratia* sp. 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,

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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 carbapenemase 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, oxydase 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), cefixime (5 µg), ciprofloxacin (5µg),clarithromycin(15µg),erythromycin(15µg),fosfomycin (200 µg), kanamycin (30 µg), levofloxacin (5 µg), netilmicin (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

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(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 *Enterobacteriaceae* were detected on Chromatic™ OXA-48 chromogenic medium (Liofilchem, Roseto degli Abbruzzi, 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 Soya 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, netilmycin 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%)
Netilmicin	4 (30.8%)	9 (69.2%)
Amoxicillin-clavulanic acid	13 (100.0%)	0 (0.0%)
***S. aureus* isolates from SSI (Saint Joseph Hospital)**		
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 (0.0%)
Aztreonam	4 (80.0%)	1 (20.0%)
Temocillin	4 (80%)	1 (20.0%)
Amoxicillin-clavulanic acid	5 (100.0%)	0 (0.0%)

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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 %), 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).

Table 2: Antibiotic susceptibility pattern of *Enterobacteriaceae* isolates from UTI (Biamba Marie Mutombo Hospital)

Antibiotics	*E. coli*		*Enterobacter* sp.	
	Resistant	Sensitive	Resistant	Sensitive
Imipenem	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)
Cefixime	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)
Cefotaxime	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)
Cefuroxime	10 (100.0%)	0 (0.0%)	7 (77,8)	2 (22.2%)
Ceftazidime	10 (100.0%)	0 (0.0%)	8 (88.9%)	1 (11.1%)
Fosfomycin	2 (20.0%)	8 (80.0%)	0 (0.0%)	10 (100.0%)
Amikacin	5 (50.0%)	5 (50.0%)	4 (44.4%)	5 (55.6%)
Tobramycin	7(70.0%)	3 (30.0%)	8 (88.9%)	1 (11.1%)
Aztreonam	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)
Levofloxacin	10 (100.0%)	0 (0.0%)	7 (77.8%)	2 (22.2%
Norfloxacin	10 (100.0%)	0 (0.0%)	8 (88.9%)	1 (11.1%)
Amoxicillin	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)
Ampicillin-sulbactam	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)
Piperacillin-tazobactam	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)
Temocillin	10 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)

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. cloaceae* 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

Table 3: Antibiotic susceptibility pattern of *Enterobacteriaceae* isolates from SSI Saint Joseph Hospital, Kinshasa

Antibiotics	*E. coli*		*Enterobacter* sp.		*Citrobacter* sp.		*Serratia* sp.	
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Ampicillin	19 (100.0%)	0 (0.0%)	8 (100.0%)	0 (0.0%)	9 (100.0%)	0(0.0%)	5 (100.0%)	0 (0.0%)
Amoxicillin – clavulanic acid	19 (100.0%)	0 (0.0%)	8 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)	5 (100.0%)	0 (0.0%)
Cefotaxime	19 (100.0%)	0 (0.0%)	8 (100.0%)	0 (0.0%)	8 (88,9%)	1(11.1%)	5 (100.0%)	0 (0.0%)
Norfloxacin	16 (84.2%)	3(15.8%)	4 (50.0%)	4 (50.0%)	5 (55.6%)	4 (44.4%)	0 (0.0%)	5 (100.0%)
Ciprofloxacin	16 (84.2%)	3 (15.8%)	5 (62.5%)	3 (37.5%)	6 (66.7%)	3 (33.3%)	2 (40.0%)	3 (60.0%)
Temocillin	19 (100.0%)	0 (0.0%)	8 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)	5 (100.0%)	0 (0.0%)
Imipenem	19 (100.0%)	0 (0.0%)	8 (100.0%)	0 (0.0%)	9 (100.0%)	0 (0.0%)	5 (100.0%)	0 (0.0%)
Amikacin	12 (63.3%)	7 (36.8%)	2 (22.2%)	6 (77.8%)	2 (22.2%)	7 (77.8%)	1 (20.0%)	4 (80.0%)
Kanamycin	19 (100.0%)	0 (0.0%)	8 (100.0%)	0 (0.0%)	6 (66.7%)	3 (33.3%)	5 (100.0%)	0 (0.0%)

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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).

Resistance 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 the 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 cloaceae*, resistance to ampicillin-sulbactam; cefotaxime, cefuroxime, amoxicillin, piperacillin-tazobactam, ceftazidime, cefixime, imipenem, aztreonam, levofloxacin, norfloxacin, amikacin, 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

Table 4: OXA-48-producing *Enterobacteriaceae* strains

Organisms	N°(%)OXA-48 type carbapenemase [Enterobacteriaceae isolates from UTI (Biamba Marie Mutombo Hospital)]	N° (%) OXA-48 type carbapenemase [Enterobacteriaceae isolates from SSI (Saint Joseph Hospital, Kinshasa)]	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%)	

Table 5: Biofilm phenotype of *Enterobacteriaceae* and *S. aureus* isolates from UTI and SSI

***Enterobacteriaceae* and *S. aureus* isolates from SSI (Saint Joseph Hospital)**					
Classification according to bacterial biofilm production	***E. coli***	***Enterobacter* sp**	***Citrobacter* sp**	***Serratia* sp**	***S. aureus***
	N°(%)	N°(%)	N°(%)	N°(%)	N°(%)
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)**
Biofilm phenotype of *Enterobacteriaceae* and *S. aureus* isolates from UTI (HBMM, Kinshasa)					
Adherent (strong biofilm producer) (OD > 0.24)	2(20%)	3(33.3%)	-	-	4(30.8%)
Moderate biofilm producer (0.12 < OD < 0.24)	4(40%)	4(44.5%)	-	-	7(53.8%)
Non-adherent (non-biofilm producer) (OD < 0.12)	4(40%)	2(22.2%)	-	-	2(15.4%)
TOTAL	**10(100%)**	**9(100%)**	-	-	**13(100%)**

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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-sulbatam	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

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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*		
	SBP	MBP	NBP	SBP	MBP	NBP	SBP	MBP	NBP	SBP	MBP	NBP	SBP	MBP	NBP
Oxacillin	100% (4/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ampicillin	100% (4/4)	0%	100% (1/1)	100% (10/10)	10% (9/9)	0%	100% (5/5)	100% (3/3)	0%	100% (6/6)	100% (3/3)	0%	100% (3/3)	100% (2/2)	
Amoxicillin-clavulanic acid	100% (4/4)	0%	100% (1/1)	100% (10/10)	100% (9/9)	0%	100% (5/5)	100% (3/3)	0%	100% (6/6)	100% (3/3)	0%	100% (3/3)	100% (2/2)	
Ceftazidime	75% (3/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cefixime	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cefotaxime	ND	ND	ND	100% (10/10)	10% (9/9)	0%	100% (5/5)	100% (3/3)	0%	6-May	100% (3/3)	0%	100% (3/3)	100% (2/2)	
Cefuroxime	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Amoxicillin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aztreonam	75% (3/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Vancomycin	100% (4/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Teicoplanin	100% (4/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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*		
	SBP	MBP	NBP	SBP	MBP	NBP	SBP	MBP	NBP	SBP	MBP	NBP	SBP	MBP	NBP
Amikacin	50% (2/4)	0%	0% (0/1)	90% (9/10)	33.3% (3/9)	0%	0% (2/5)	0% (0/3²)	0%	% (2/6)	0% (0/3)	0%	50% (1/3)	0% (0/2)	0%
Kanamycin	ND	ND	ND	100% (10/10)	100% (9/9)		100% (5/5)	100% (3/3)		100% (6/6)	%2/3	0%	100% (3/3)	100% (2/2)	
Levofloxacin	75% (3/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Norfloxacin	ND	ND	ND	100% (10/10)	66.6% (6/9)	0%	60% (3/5)	33.3% (1/3)	0%	50% (3/6)	33.3% (1/3)	0%	0%	0% (0/0)	0%
Ciprofloxacin	75% (3/4)	0%	100% (1/1)	100% (10/10)	66.6% (6/9)	0%	80% (4/5)	33.3% (1/3)	0%	66.6% (4/6)	33.3% (1/3)	0%	100% (3/3)	0% (0/2)	0%
Trimethoprim	50% (2/4)	0%	0% (0/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fosfomycin	100% (4/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Erythromycin	100% (4/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Imipenem	ND	ND	ND	100% (10/10)	100% (9/9)	0%	100% (5/5)	100% (3/3)	0%	100% (6/6)	100% (3/3)	0%	100% (3/)	100% (2/2)	0%
Temocillin	75% (3/4)	0%	100% (1/1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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

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Table 8: Occurrence of multidrug resistant pattern and their associations with biofilm phenotype in *Enterobacteriaceae* and *S. aureus* isolates from UTI (Biamba Marie Mutombo Hospital)

N° of antibiotic category	N°(%) of *E. coli* biofilm phenotype			Total number of isolates
	SBP	MBP	NBP	
14	1(50.0%)	1(25.0%)	0(0.0%)	2(20.0%)
13	1(50.0%)	1(25.5%)	0(0.0%)	2(20.0%)
12	0(0.0%)	2(50.0%)	3(75.0%)	5(50.0%)
11	0(0.0%)	0(0.0%)	1(25.0%)	1(10.0%)
TOTAL	**2 (20.0%)**	**4 (40%)**	**4 (40%)**	**10 (100%)**
	N°(%) of *E. cloaceae* biofilm phenotype			
13	2(66.7)	2(50.0%)	0(0.0%)	4(44.5)
12	1(33.3%)	1(25.0%)	0(0.0%)	2(22.2)
11	0(0.0%)	0(0.0%)	1(50.0%)	1(11.1%)
10	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
9	0(0.0%)	1(25%)	1(50.0%)	2(22.2%)
TOTAL	**3(33.3%)**	**4 (44.5%)**	**2 (22.2%)**	**9 (100.0%)**
	N°(%) of *S. aureus* biofilm phenotype			
16	1(25%)	0 (0%)	0(0%)	1(7.7)
15	1(25%)	0 (0%)	1(50%)	2(15.4)
14	1 (25%)	6(85.7%)	0(0%)	7(53.8%)
13	0 (%)	1(14.3%)	0(0%)	1(7.7)
12	0 (0%)	0 (0%)	1(50%)	1(7.7)
11	0(%)	0(%)	0(0%)	0(0%)
10	0(%)	0(%)	0(0%)	0(0%)
9	1(25%)	0(0%)	0(0%)	1(7.7)
TOTAL	**4(30.8%)**	**7(53.8%)**	**2(14.4%)**	**13(100%)**

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,

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ciprofloxacin, levofloxacin, norfloxacin, amoxicillin-clavulanic acid, amoxicillin, ampicillin-sulbactam, aztreonam, and tobramycin. These results are consistence with previous reports. In Nigeria, *E. coli* 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%), ceftazidime (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 *Enterobacteriaceae* 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 rate of OXA-48 producers among *Enterobacter* sp and *Citrobacter* sp 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 Neaopane 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

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Abbreviations

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

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