History of Medicine Studies

ISSN:1300-669X
Volume 18 Issue 2 Sep 2022

Staphylococcus aureus and Enterobacteriaceae Biofilm Formation and Antibiotic Resistance in Clinical Samples Obtained from Patients With Urinary Tract Infections

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Abstract

Community and hospital-acquired infections are caused by Gram-negative and Gram-positive bacteria, respectively. Antimicrobial resistance is one of the world's leading health concerns because of its rapid development, appearance, and dissemination among microorganisms. Bacteria employ biofilm development as a method of resistance. This research set out to determine whether or not Staphylococcus aureus and Enterobacteriaceae isolates exhibited antibiotic resistance patterns and whether or not they were capable of forming biofilms.

Methods: Patients with urinary tract and surgical site infections at Hôpital Biamba Marie Mutombo and Saint Joseph Hospital provided a total of 18 Staphylococcus aureus and 60 Enterobacteriaceae clinical isolates. Disk-diffusion testing was used to identify the antibiotic resistance pattern of the isolates. The capacity of bacterial strains for producing and forming un biofilm was evaluated using the microtiter plate technique.

Antibiotic and biofilm producer resistance was found to be very common among clinical isolates of S. aureus and Enterobacteriacea. The ampicillin-sulbactam, piperacillin-tazobactam, vancomycin, amoxicillin-clavulanic acid, levofloxacin, and aztreonam susceptibilities of S. aureus strains were all at 100%. Antibiotics including amoxicillin-clavulanic acid, erythromycin, and tetracycline were completely ineffective against strains of Escherichia coli, Enterobacter sp., Citrobacter sp., and Serratia sp. The capacity to create a biofilm was not linked to resistance to antibiotics.

The current study's findings support the establishment of MDR-Os and recommend establishing a program to track the development of antibiotic resistance.

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

Introduction

Since fewer or, in some cases, no effective antimicrobial drugs are available to treat illnesses caused by pathogenic bacteria, the emergence of resistance to numerous antimicrobial agents in these bacteria has become a huge public health problem. 1). The establishment and growth of antibiotic resistance is a problem for both Gram-positive and Gram-negative bacteria [1]. Multidrug-resistant microorganisms have emerged as a global threat to effective illness treatment [2]. Increased mortality has been linked to infections caused by multidrug-resistant organisms (MDROs). antibiotics with varying degrees of resistance in terms of morbidity, duration of hospital stay, healthcare costs, and cost-effectiveness [3, 4]. Methicillin-resistant Staphylococcus aureus (MRSA), resistant gram-negative bacilli (RGNB), and vancomycin-resistant enterococci (VRE) are all examples of multidrug-resistant organisms [1]. Resistance to antibiotics in bacteria results from a number of different phenomena, including changes in the drug's target, the bacteria's inability to absorb the antibiotic, the molecule's destruction, the presence of an efflux system that can remove the antibiotic from the bacteria's cytoplasm, and genetically associated changes (mutational events, genetic transfer of resistance genes via plasmids, and mutations of target genes) [5].

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ISSN:1300-669X
Volume 18 Issue 2 Sep 2022

Since the development of extended-spectrum beta-lactamases (ESBL) and carbapenemase enzymes such oxacillinase (OXA)-48-like -lactamases [6, 7], Enterobacteriaceae have developed resistance to -lactam antibiotics and carbapenems. Nonetheless, this is not always the case when antibiotics fail to work. Biofilms may be formed by bacteria that have colonized host tissues or medical equipment. Biofilms are defined as sessile communities of microbes that are permanently attached to a surface or interface, are encased in a matrix of extracellular polymeric substances that they have synthesized, and display a modified phenotype in terms of growth rate and gene transcription [8]. Biofilms increase the likelihood of nosocomial infections by helping bacterial populations survive in hospitals and inside patients. Pathogenic bacteria that have formed a biofilm are more protected against the host's immune system and convectively delivered antibiotics [9]. Multiple drug resistance in clinical isolates has been linked to biofilm formation [10, 11].

Because drug-resistance monitoring is being performed in a small number of countries, we know very little about the real scope of the AMR issue in the African Region. Our team gathers bacterial strains from hospitals to track the antibiotic resistance of key diseases and offer statistics on antibiotic use. The current study's goals were to assess antibiotic resistance in S. aureus and Enterobacteriaceae strains isolated from patients with urinary tract and surgical site infections at Biamba Marie Mutombo Hospital and Saint Joseph Hospital in Eastern Kinshasa City, to identify the prevalence of OXA-48-producing Enterobacteriaceae, and to investigate the formation of biofilm by clinical strains isolated.

Material and Methods

Bacteria isolates

Thirteen S. aureus isolates (from urines, vaginal swabs, and other clinical sources) were collected from patients at Biamba Marie Mutombo Hospital. smears, prostatic fluid, infected devices, and surgical site infections [SSI]), and 19 clinical isolates of Enterobacteriaceae from urinary tract samples (UTI) (10 Escherichia coli and 9 Enterobacter sp.). Forty-one SSI isolates from Saint Joseph Hospital were examined, including five from S. aureus and nineteen from E. coli, eight from Enterobacter sp., nine from Citrobacter sp., and five from Serratia sp. These hospitals' bacteriology labs gathered clinical samples from both inpatients and outpatients for diagnostic testing. Gram stain, catalase, and coagulase tests were used originally to identify all Staphylococcus spp. The latex agglutination test (Pastorex Staph- Plus, BioRad, Marnes-la-Coquette, France) and the deoxyribonuclease (DNase) test were used at the microbiology lab of the Faculty of Pharmaceutical Sciences at the University of Kinshasa to identify Staphylococcus aureus strains. Coagulase-negative staphylococci were defined as all those strains that tested negative for both latex agglutination and DNase. Conventional microbiological techniques were used to identify the isolated strains of Gram-negative bacilli. These techniques included Gram staining, oxydase tests, indole and urease production, citrate utilization, hydrogen sulfide, gas production, and sugar fermentation; phenylalanine deaminase; lysine decarboxylase (L.D.C.); ornithine decarboxylase (O.D.C. We used the same methods to determine that Gram-negative bacilli in our lab belonged to the family Enterobacteriaceae. Trypticase soy agar (Liofilchen; Roseto degli Abruzzi; Italy) was used for all of the cultures.

Testing for antibiotic resistance

The following antibiotic disks (Liofilchen, Roseto degli Abruzzi, Italy) were used to create antibiograms for all of the Staphylococcus spp. strains isolated using the diffusion technique on Mueller Hinton Agar: antibiotics such as amoxicillin (500 mg) and amikacin (30 g) + 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). The methicillin resistance test used the diffusion technique with 1 g of oxacillin on 4% NaCl-containing Mueller Hinton agar.

Antibiotic disks (Liofilchen, Roseto degli Abruzzi, Italy) containing ampicillin (30 g), amikacin (10 g), amoxicillin (10 g), ampicillin (30 g), ampicillin-sulbactam (20 g), and aztreonam were used to investigate their efficacy against Enterobacteriaceae.

cefotaxime (5 g), cefixime (5 g), cefuroxime (30 g),

fosfomycin (200 mg), ceftazidime (30 mg), imipenem (10

piperacillin-tazobactam (100/10 g), norfloxacin (5 g), levofloxacin (5 g), tobramycin (10 g), and temocillin (30 g). Zone of inhibition diameters were calculated after 24-hour incubation of plates at 37°C. Clinical Laboratory Standards Institute (CLSI) [12] criteria were used in the analysis of the data. Bacteria strains ATCC 25922 (E. coli) and 25923 (S. aureus) were used to ensure consistent quality.

Identifying Sources of OXA-48

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On ChromaticTM OXA-48 chromogenic media (Liofilchem, Roseto degli Abbruzzi, Italy), OXA-48-producing Enterobacteriaceae were identified. Red colonies indicated E. coli producing OXA-48, blue-violet colonies indicated Klebsiella sp. producing OXA-48, blue-green colonies indicated Enterobacter sp. producing OXA-48, and blue colonies with a red halo indicated Citrobacter sp. producing OXA-48. For testing purposes, we employed E. coli ATCC 25922.

An Evaluation of Biofilms

In the current investigation, we screened all isolates for biofilm formation potential using a modified version of the Crystal Violet Staining technique (described in [13]). For each strain, a suspension was made in Trypticase Soya broth (Becton Dickinson, Franklin Lake) to meet the McFarland 0.5 turbidity criterion. By diluting the solution serially in logarithmic stages, we were able to verify that the bacterial count was really accurate. Strips of sterile polystyrene were injected with 200 L of each calibrated bacterial culture, and the strips were then incubated for 24 hours at 35°C in a humid environment. The medium in the control well was kept sterile. Each sample was tested three times. The wells were emptied of their medium and cleansed three times with 200 L of distilled water to ensure sterility. After 45 minutes of air drying, 200 L of 0.1% Crystal violet solution was used to stain the adhering cells on the strips. After 45 minutes, the dye was removed, and 300 L of sterile distilled water was used to wash the wells five times. Biofilm absorbance at 540 nm was measured using an enzyme-linked immunosorbent assay (ELISA) reader after dissolving the dye absorbed by the biofilm's cells in 200 L of 33% (v/v) glacial acetic acid. The findings were reported as the percentage difference between the sample and control optical densities at 540 nm (OD540 nm). These optical density measurements were used as a proxy for the prevalence of biofilms formed by adherent bacteria. Using the mean of the three wells, we were able to classify biofilm formation as either non-adherent (OD 0.12), moderate producer (0.12 OD 0.24), or strong producer (OD > 0.24), as suggested by Stepanovic et al. [14].

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 fromUTI 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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ISSN:1300-669X
Volume 18 Issue 2 Sep 2022

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,

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%)

Enterobacteriaceae and *S. aureus isolates* from SSI

Among 5 *S. aureus* strains isolated from SSI in Saint 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. cloaceae* strains, 3 (33.3%) were SBP, 4 (44.5%) MBP, and 2 (22.2%) NBP (Table 5).

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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%)

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 atotal 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 producersand 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

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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%)**

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)

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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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ISSN:1300-669X
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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 theirassociations with biofilm phenotype

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

Discussion

In both community and nosocomial settings, Enterobacteriaceae and Staphylococcus are recognized as major pathogens. Multidrug-resistant microbes (MDR-Microbes) have emerged as a serious health threat around the globe, especially in Africa [15]. This research looked at the biofilm-forming and -producing abilities of bacteria that cause community and hospital-acquired illnesses to determine their resistance profiles. Antibiotic resistance was shown to have increased at an alarming rate among Urinary tract infection (UTI) and staph aureus (SSI) strains detected at Biamba Marie Mutombo and Saint Joseph Hospitals.

UTI and SSI isolates were 100% MRSA. This study's findings are consistent with those of previous research on antibiotic resistance in S. aureus from the Central Africa area. Clinical samples (wounds, urine, pus) included MRSA 82% of the time [16]. Our results showed that all of the MRSA samples tested were resistant to the antibiotics ceftazidime, cefotaxime, amoxicillin-clavulanic acid, and cefixime. In Uganda, 57.2% of the population has been found to be infected with MRSA, and all strains of MRSA found there are resistant to the antibiotics amoxicillin/clavulanic acid, ceftriaxone, and imipenem (15). The total frequency of MRSA was found to be 53.4% in another research conducted in East Africa [17]. Recent investigations found that MRSA isolates were still highly sensitive to teicoplanin and vancomycin [18, 19], which contradicts our findings.

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Our findings show that many Enterobacteriaceae strains responsible for urinary tract infections and surgical site infections are highly resistant to ampicillin, imipenem, cephalosporins, and other commonly used antibiotics. antibacterial drugs include ciprofloxacin, levofloxacin, norfloxacin, amoxicillin-clavulanic acid, amoxicillin, ampicillin-sulbactam, aztreonam, and tobramycin. These findings line up with what has been reported before. High levels of resistance were shown by E. coli isolates in Nigeria to -lactam antibiotics other than carbapenems and piperacillin-tazobactam. Cefepime 70%, ampicillin 90%, aztreonam 80%, and cefotaxime 80% resistance were also found in E. cloacae. 60 percent ceftazidime and 100 percent cefuroxime = 17. Researchers in Rwanda found that 75.9% of the 241 Gram-negative isolates tested against ceftriaxone were resistant [20].

Many distinct species of enterobacteria were identified in this investigation, and among them were those that produced OXA-48. We found that 87.2% of the Enterobacteriaceae were OXA-48 producers, which is significantly higher than the 3.4% and 4.9% found in a Nigerian hospital and in Tanzania among multidrug-resistant Enterobacteriaceae isolates in other studies [11,15]. Researchers in many African nations found that K. pneumoniae was the most common OXA-48 producer [10]. These countries include Tunisia, Libya, Tanzania, Senegal, and Morocco. However, all of the strains of Enterobacter sp. and Citrobacter sp. tested positive for OXA-48 production in this investigation. In contrast, 22 out of the 29 E. coli strains tested produced OXA-48.

In this research, the Microtiter plate technique was used to identify biofilm development. Of the 20 UTI isolates tested, 11 (84.6%) were found to be S. aureus, 6 (60%) were E. coli, and 7 (77.7%) were Enterobacter sp. Four of the four SSI S. aureus isolates (80.0%) and all Enterobacteriaceae (100.0%) were biofilm producers. The process of infection begins with the adhesion of microbial cells to surfaces and the subsequent formation of multicellular communities. In addition, bacterial biofilms may play an important role in both recurrent UTI and SSI [21, 22]. Clinical strains from SSI had a much higher potential to build a biofilm than those from UTI, according to the findings of this investigation. We also showed that the amount of biomass produced by biofilms varied widely across UTI and SSI isolates. Environment, sugar content and concentration (glucose vs. lactose), geographical origin, specimen type, surface adhesion properties, proteolytic enzymes, and biofilm linked genes [23–27] are only a few of the numerous elements that influence biofilm development. This increased incidence of biofilm development in SSI bacterial strains may be attributable to the aforementioned causes. Because bacteria in biofilms are resistant to antibiotic agents, illnesses caused by them have significant therapeutic implications. Some biofilm-forming microbes have been shown to

be up to a thousand times more resistant to antibiotics than their planktonic analogs [28]. Antibiotic resistance was particularly high among the biofilm-forming Enterobacteriaceae and Staphylococcus aureus found in UTIs, as well as among the non-biofilm-forming bacteria that were also present. In contrast to the findings of Neaopane et al. [29], whereby 86.7% of S. aureus biofilm producers were MDR, we found that all MRSA strains that did not create biofilms were not MDR. Also, when compared to the dosage found by Neupane et al., [30], our findings stand in stark contrast. Antibiotic resistance was shown to be substantially greater in biofilm-producing E. coli than in non-biofilm-producing E. coli in this most recent investigation.

E. coli. Three E. coli strains in our investigation were resistant to a whopping twelve drugs (Table 7). Antibiotic resistance was shown to be increased in both strong and moderate biofilm producers of Enterobacteriaceae and S. aureus from SSI. Our findings are consistent with those of other studies [26, 30]. The present study's findings are consistent with a previous investigation [31] that found no association between MDR or global resistance and biofilm development.

The rising opposition in Kinshasa may have several causes. Some common social practices, such as self-medication, an 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 strains' biofilm abilities and the acquisition of resistance genes [33], all contribute.

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.

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