ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology

www.iajlb.com
Email : editor@iajlb.com or iajlb.editor@gamil.com

Indo-American Journal of Life Sciences and Biotechnology

Vol.6, Issue.1, March 2018

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

A.V.S. Ksheera Bhavani[1] , M.Madhuri[2] , L. Sangeetha kumara[3], B. Rajesh kumar[4]

Abstract

Community and hospital-acquired illnesses may be caused by either Gram-negative or Gram-positive bacteria. The rise, development, and dissemination of bacterial resistance to antimicrobials are among the world's leading health concerns. Bacteria employ biofilm development as a method of resistance. The purpose of this research was to examine Staphylococcus aureus and Enterobacteriaceae isolates for their antibiotic resistance profile and their capacity to produce biofilms.

Methods: Patients at Hôpital Biamba Marie Mutombo and Saint Joseph Hospital were sampled for urinary tract and surgical site infections, yielding 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 to create and form 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. Complete resistance to ampicillin-sulbactam, piperacillin-tazobactam, vancomycin, amoxicillin-clavulanic acid, levofloxacin, and aztreonam was also seen in S. aureus strains. Third-generation cephalosporins, imipenem, and amoxicillin-clavulanic acid were all completely ineffective against strains of E. coli, Enterobacter sp., Citrobacter sp., and Serratia sp. The capacity to create a biofilm was not linked to resistance to antibiotics.

The findings of the current research show that MDR-TB is on the rise, and they recommend setting up a program to track the development of resistance to antibiotics.

Keywords: Staphylococcus aureus, Enterobacteriaceae, Biofilm, and Antibiotic Resistance

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). Emerging and increasing antibiotic resistance affects both Gram-positive and Gram-negative bacteria [1]. Multidrug-resistant microorganisms

have emerged as a global threat to effective illness treatment [2]. The cost-effectiveness of antibiotics with varying degrees of resistance [3, 4] is negatively impacted by the prevalence of infections caused by multidrug-resistant organisms (MDROs), including higher mortality, morbidity, duration of hospital stay, and overall healthcare costs. Methicillin-resistant Staphylococcus aureus (MRSA), resistant gram-negative bacilli (RGNB), and vancomycin-resistant enterococci (VRE) are all examples of multidrug-resistant organisms [1]. Several phenomena, including bacterial impermeability to the drug, bacterial destruction of the antibiotic molecule, an efflux system that can pump antibiotic out of the cytoplasm of bacteria, and genetically associated changes (mutational events, genetic transfer of resistance genes via plasmids, and mutations of target genes), all contribute to the development of antibiotic resistance in bacteria [5]. Extended-spectrum beta-lactamases (ESBL) and carbapenemase enzymes, such as oxacillinase (OXA)-48-like -lactamases, were produced by Enterobacteriaceae

1.A.V.S. Ksheera Bhavani, Assistant professor, Department of Pharmaceutical Biotechnology,
Email:andavarapu.bhavani@gmail.com
2. M.Madhuri, Assistant professor, Department of Pharmaceutical Analysis,
3. L. Sangeetha kumari, Assistant professor, Department of Pharmaceutical Analysis,
4. B. Rajesh kumar, Assistant professor, Department of Pharmaceutical Analysis,
Sri Venkateswara College of Pharmacy, Etcherla, Srikakulam

, making them resistant to -lactam antibiotics and carbapenems [6, 7]. However, this isn't the sole explanation for unsuccessful antimicrobial therapy. Biofilms may be formed by bacteria that have colonized host tissues or medical equipment. An altered phenotype in terms of growth rate and gene transcription characterizes the cells that make up biofilms, which are defined as sessile communities derived from microorganisms and characterized by cells that are irreversibly attached to a substratum or interface or each other and are embedded in a matrix of extracellular polymeric substances that they have produced [8]. Nosocomial infections are more likely to occur when bacterial populations in hospitals or on patients are allowed to thrive in biofilms. 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. In order to track the antibiotic resistance of key infections, our lab gathers bacterial samples from hospitals throughout the world. In this study, we aimed to determine the prevalence of OXA-48-producing Enterobacteriaceae, evaluate antibiotic resistance in S. aureus and Enterobacteriaceae strains isolated from patients with urinary tract and surgical site infection at Biamba Marie Mutombo Hospital and Saint Joseph Hospital in Eastern Kinshasa city, and examine the formation of biofilm by clinical strains isolated.

Material and Methods

Bacteria isolates

From Biamba Marie Mutombo Hospital, a total of 13clinical 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 % *Enterobacteriaceae* were tested against 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

***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%)

(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

form biofilm by Crystal Violet Staining method as previouslydescribed [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-

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, andthe 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 forfosfomycin, 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 wereMRSA and resistant to glycopeptide antibiotics, vancomycin and teicoplanin (Table 1).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 wereMRSA and resistant to glycopeptide antibiotics, vancomycinand teicoplanin (Table 1).

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

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%)

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).

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%)

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 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

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 MutomboHospital

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 MarieMutombo 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

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

Table 8: Occurrence of multidrug resistant pattern and their associations with biofilm phenotype in *Enterobacteriaceae* and *S. aureus* isolatesfrom 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 (Table8 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 andtheir capability to form and to produce a biofilm. The results showed an alarmingly increase of antibiotic resistance among E*nterobacteriaceae* 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 resistancein 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 MRSAstrains 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 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

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 resistance*;* 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 expert 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, Bjarnsholt T, Givskov M, Molin S, Ciofu Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35 (2010): 322-332.
9. 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.
10. Nirwati H, Sinanjung K, Fahrunissa 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.
11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement. CLSI document (2012): M100-S22.Chavant P1, Gaillard-Martinie 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.
12. 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.
13. Tadesse et al. Antimicrobial resistance in Africa: a systematic

review. BMC Infectious Diseases 17 (2017): 616.

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

15. Baguma A, Tibyange 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.

16. 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

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

18. 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.

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

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

21. Kokare CR, Chakraborty S, Khopade AN, Mahadik KR. Biofilm: importance and applications. Indian J Biotechnol8 (2009): 159–168.

22. 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.

23. 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.

24. hang 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.

25. Kawamura H, Nishi J, Imuta 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.

26. 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.

27. Neopane P, Nepal HP, Shrestha R, Uehara 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.

28. 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.

29. 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.

30. 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.

31. 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.