Biofilm Formation and Antibiotic resistance of Enterobacteriaceae isolates from Surgical Site infections in Hôpital de Référence Saint Joseph, Kinshasa, Democratic Republic of Congo

Jean-Marie Liesse Iyamba (✉ liesseiyamba@gmail.com)

Universite de Kinshasa <https://orcid.org/0000-0002-5942-8365>

Rodriguez Musomoni Mabankama

Faculty of Pharmaceutical Sciences, University of Kinshasa

Cyprien Mbundu Lukukula

University of Kinshasa

Joseph Welo Unya

Faculty of Pharmaceutical Sciences, University of Kinshasa

Daniel Tassa Okombe

Faculty of Pharmaceutical Sciences, University of Kinshasa

Benjamin Kodondi Ngbandani

Faculty of Pharmaceutical Sciences, University of Kinshasa

Grégoire Mbusa Vihembo

Faculty of Pharmaceutical Sciences: Siksha O Anusandhan University School of Pharmaceutical Sciences

Nelson Nsiata Ngoma

Hopital de Référence Saint Joseph

Thierry Mukendi Kajinga

Hôpital Saint Joseph

Odette Mboma Mapipi

Centre National de Pharmacovigilance

Jacques Onokodi Kasongo

Faculty of Pharmaceutical Sciences: Siksha O Anusandhan University School of Pharmaceutical Sciences

NB Takaisi-Kikuni

Faculty of Pharmaceutical Sciences: Siksha O Anusandhan University School of Pharmaceutical Sciences

Keywords: Antibiotic susceptibility, multidrug resistant bacteria, Surgical site infections, Biofilm formation, OXA-48 producing Enterobacteriaceae, Democratic Republic of Congo.

Posted Date: October 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-96885/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

Background: *Enterobacteriaceae* are one of the most predominant pathogen in surgical site infections. In recent years we observed increase in resistance among bacteria from surgical site infections. The aim of this study was to evaluate antimicrobial susceptibility pattern of *Enterobacteriaceae* isolates from surgical site infections, the biofilm formation and the production of OXA-48 carbapenemase.

Methods: A total of 41 *Enterobacteriaceae* (19 *Escherchia coli*, 8 *Enterobacter* sp., 9 *Citrobacter* sp., and 5 *Serratia* sp.) clinical isolates were collected from patients with SSI in Hôpital Saint Joseph (Kinshasa) for diagnostic purposes. The pus samples were cultured and the antibiotic susceptibility profile of the isolates were determined by disk-diffusion method following Clinical and Laboratory Standards Institute 2012 recommendations. OXA-48-producing *Enterobacteriaceae* were detected using Chromatic™ OXA-48 chromogenic medium. Crystal Violet Staining Method was used to assess the ability of bacteria strains to form a biofilm.

Results: All *Enterobacteriaceae* isolates studied were biofilm producers and highly resistant to the majority of antibiotics tested. *E. coli*, *Enterobacter* sp., *Citrobacter* sp., and *Serratia* sp. were 100 % resistant cefotaxime, imipenem, and amoxicillin-clavulanic acid, and ampicillin. *Serratia* sp. isolates were 100% and 80% sensitive to norfloxacin and amikacin respectively. There was relationship between antibiotic resistance and biofilm production. *E. coli*, *Enterobacter* sp., and *Citrobacter* sp. strains were all OXA-48 producers.

Conclusion: The results of the present study demonstrate the emergence of multidrug resistant organisms, the correlation between antibiotic resistance- biofilm formation and OXA-48 production. These results suggest the implementation of antimicrobial resistance survey program in order to prevent and combat the spread of multidrug resistant organisms in hospital and community in Democratic Republic of Congo.

Introduction

Surgical site infections (SSIs) are the common postoperative complications and has been estimated to occur in up to 5% of all procedures [1]. More than 30% of the Healthcare-associated infections are surgical site infections defined as infections related to operative procedures that occur at or near surgical incisions within 30 days of the procedure or within 90 days if prosthetic materials are implanted at surgery [2, 3]. SSIs are associated with increased morbidity and mortality. Furthermore, the development of an SSI causes a substantial increase in the clinical and economic burden of surgery. The financial burden of surgery is increased due to the direct costs incurred by prolonged hospitalization of the patient, diagnostic tests, and treatment. Certain patients may also require reoperation after the contraction of an SSI, which is associated with considerable additional costs [4,5]. The outcome of SSIs is partly attributed to increase in antimicrobial resistant bacterial pathogens, which make the choice of empirical therapy more difficult [6]. Studies conducted in many developing countries and in Africa, have indicated high

antibiotic resistance among Gram-negative bacteria to commonly used antibiotics, leading to a loss of efficacy for treatment of common infections [7–9]. Infections due to antibiotic-resistant gram-negative organisms, including those caused by extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, are increasing worldwide and represent a significant public health threat [10]. The standard treatment of infections caused by ESBL-secreting *Enterobacteriaceae* is based on the use of carbapenems [11]. However, their usefulness is threatened by the emergence and spread of bacteria that produce carbapenemase enzymes hospital and community [12, 13]. Carbapenemases come from ambler class A or D serine β -lactamases and ambler class B metallo- β -lactamases (MBLs). *K. pneumoniae* carbapenemase (KPC-Class A), New Delhi metallo- β -lactamase (NDM-Class B), Verona integron-encoded metallo- β -lactamase (VIM-Class B), Imipenemase metallo- β -lactamase (IMP-Class B), and Oxacillinase-48 (OXA-48-Class D) variants are the most common carbapenemases in carbapenemase producing *Enterobacteriaceae* (CPE) [12,13]. OXA-48 carbapenemase is one of the several carbapenemases that have been described so far; the high prevalence of infections caused by OXA-48 producers is well established in Africa, Europe and elsewhere [14].

Pathogenesis of *Enterobacteriaceae* is attributable not only to antibiotic resistance, which complicates the treatment of SSIs, but also to other factors. One of these factors is the ability of bacteria to adhere to a biotic or an abiotic surface and to form a biofilm [15]. There is a high probability that microorganisms will contaminate the site of incision or excision in a surgical

wound. If a bacterium is able to attach onto a biological surface it rapidly changes the proteins it expresses and as such becomes sessile and significantly different phenotypically, when compared with its planktonic counterpart [16].

A biofilm can be defined as a community of microorganisms that are attached to each other, and/or a surface, encased within a matrix of extracellular polymeric substance, appearing as microcolonies or aggregates of cells [17]. The presence of biofilms as a potential cause of SSIs may explain the sometimes-disappointing response obtained from traditional approaches such as promoting drainage, systemic antibiotics or delayed closure [18].

The goal of the present study was to analyze the capacity to form biofilm of 41 *Enterobacteriaceae* strains isolated from SSIs and to evaluate the production of OXA-48 carbapenemase. We investigated also the correlation between biofilm formation, antimicrobial resistance and OXA-48 production in *Enterobacteriaceae* isolated from patients with SSIs.

Material And Methods

A total of 41 *Enterobacteriaceae* strains from patients with SSI (19 *E. coli*, 8 *Enterobacter* sp., 9 *Citrobacter* sp., and 5 *Serratia* sp.) were studied. Isolates were received from Hôpital de Référence Saint Joseph (Kinshasa). The clinical samples were collected for diagnostic purposes by the bacteriology laboratories of this hospital, and were from hospitalized patients.

Isolated strains were identified using microbiological conventional methods including Gram staining, oxidase tests, indole and urease production, citrate utilization, hydrogen sulphide, gas production and fermentation of sugars, phenylalanine deaminase, lysine decarboxylase (L.D.C.), ornithine decarboxylase (O.D.C.), arginine dihydrolase (A.D.H.) tests, and methyl red reaction. In our laboratory, strains were picked on MacConkey agar (Liofilchen, Roseto degli Abruzzi, Italy), and 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 *Enterobacteriaceae* strains using the diffusion method on Mueller Hinton Agar were realized with the following antibiotic disks (Liofilchen, Roseto degli Abruzzi, Italy): ampicillin (30 µg), amoxicillin-clavulanic acid (30 µg), amikacin (30 µg), cefotaxime (30 µg), imipenem (10 µg), norfloxacin (5 µg), ciprofloxacin (5 µg), kanamycin (30 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) [19]. *E. coli* ATCC 25922 were used for quality control. The results of antibiotic susceptibility tests from hospital were confirmed in our laboratory using the same antibiotic disks.

Biofilm formation assay

In present study, we screened all isolates for their ability to form biofilm by Crystal Violet Staining Method (CVSM) as previously described [20], with modifications. A suspension equivalent to the McFarland 0.5 turbidity standard was prepared in trypticase soya broth (Liofilchen, Roseto degli Abruzzi, Italy) 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 Ramos-Vivas et al. [21] was adopted: OD ≤ 0.05, non-biofilm producer; OD > 0.05–0.1 weak biofilm producer; OD > 0.1–0.3 moderate biofilm producer; and OD > 0.3 strong biofilm producer.

Detection of OXA-48 producers

OXA- 48-producing *Enterobacteriaceae* were detected on Chromatic™ OXA-48 chromogenic medium (Liofilchem, Roseto degli Abruzzi, Italy). After incubation at 37°C/24 - 48 hours, the color and the morphology of the colonies were observed and the results interpreted according to the supplier's instructions 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.

Statistical analysis

GraphPad software package was used for statistical analysis. Chi-square test was applied. *P*-value < 0.05 was considered statistically significant.

Results

Antibiotic susceptibility

The *E. coli*, *Citrobacter* sp., *Enterobacter* sp., *Serratia* sp. strains from SSIs were highly resistant to the majority of antibiotics tested. *E. coli* isolates were particularly 100 % resistant to ampicillin, kanamycin, amoxicillin – clavulanic acid, cefotaxime, and imipenem. *Serratia* sp. isolates were fully sensitive to norfloxacin and 80 % sensitive to amikacin. Multidrug resistance (MDR) was observed in *Enterobacteriaceae* isolated from SSIs (Table 1).

Table1 Antibiotic susceptibility pattern of *Enterobacteriaceae* isolated from SSIs

Antibiotics	<i>E. coli</i>		<i>Enterobacter</i> sp.		<i>Citrobacter</i> sp.		<i>Serratia</i> sp.	
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
	N°(%)	N°(%)	N°(%)	N°(%)	N°(%)	N°(%)	N°(%)	N°(%)
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)
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)

Biofilm formation

The results of biofilm formation of different clinical isolates studied are presented in Table 2 and Figure 1. Ten (52.6%), 9 (47.4%) of *E. coli* strains were strong biofilm producers (SBP) and moderate biofilm producers (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%) *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 2 Summary of biofilm producers isolates

Classification according to bacterial biofilm production	<i>Enterobacteriaceae</i> strains isolated from SSIs			
	N (%)	N (%)	N(%)	N(%)
	<i>E. coli</i>	<i>Enterobacter</i> sp.	<i>Citrobacter</i> sp.	<i>Serratia</i> sp.
Strong biofilm producer (OD > 0.3)	10 (52.6)	5(62.5)	6 (66.7)	3 (60.0)
Moderate biofilm producer (OD > 0.1–0.3)	9 (47.4)	3(37.5)	3(33.3)	2(40.0)
Weak biofilm producer (OD > 0.05–0.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Non-biofilm producer (OD ≤ 0.05)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
TOTAL	19(100.0)	8(100.0)	9(100.0)	5(100.0)

Resistance pattern of *Enterobacteriaceae* strains among biofilm producers

Resistance to ampicillin, amoxicillin-clavulanic acid, cefotaxime, amikacin, kanamycin, norfloxacin, and imipenem were higher in SBP than in MBP in *E. coli* isolates. Similar results were obtained for *Enterobacter* sp., *Citrobacter* sp., and *Serratia* sp. isolates (Table 3). Regarding MDR, relationship was found between multidrug resistance and biofilm formation (Table 4).

Table 3 Antibiotic susceptibility pattern of <i>Enterobacteriaceae</i> isolates from SSIs among biofilm producers												
Antibiotics	<i>E. coli</i>			<i>Enterobacter sp.</i>			<i>Citrobacter sp.</i>			<i>Serratia sp.</i>		
	SBP, N=10	MPB, N=9	Total resistant	SBP, N=5	MBP, N=3	Total resistant	SBP, N=6	MBP, N=3	Total resistant	SBP, N=3	MBP, N=2	Total resistant
	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)	N° (%)
Ampicillin	10(100)	9(100)	19(100)	5(100)	3(100)	8(100)	6(100)	3(100)	9(100)	3(100)	2(100)	5(100)
Amoxicillin – clavulanic acid	10(100)	9(100)	19(100)	5(100)	3(100)	8(100)	6(100)	3(100)	9(100)	3(100)	2(100)	5(100)
Cefotaxime	10(100)	9(100)	19(100)	5(100)	3(100)	8(100)	5(83.3)	3(100)	8(88.8)	3(100)	2(100)	5(100)
Norfloxacin	10(100)	6(66.6)	16(84.2)	3(60)	1(33.3)	4(50)	3(50)	1(33.3)	4(44.4)	0(0)	0(0)	0(0)
Ciprofloxacin	10(100)	6(66.6)	16(84.2)	4(80)	1(33.3)	5(62.5)	4(66.6)	1(33.3)	5(55.5)	3(100)	0(0)	3(60)
Imipenem	10(100)	9(100)	19(100)	5(100)	3(100)	8(100)	6(100)	3(100)	9(100)	3(100)	2(100)	5(100)
Amikacin	9(90)	3(33.3)	12(63.1)	2(40)	0(0)	2(25)	2(33.3)	0(0)	2(22.2)	1(33.3)	0(0)	1(20)
Kanamycin	10(100)	9(100)	19(100)	5(100)	3(100)	8(100)	6(100)	2(66.6)	8(88.8)	3(100)	2(100)	5(100)

SBP: strong biofilm producers; MBP: moderate producers; ND: not determine

Table 4 Occurrence of multidrug resistant pattern and their associations with biofilm phenotype in *Enterobacteriaceae* isolates from SSIs

Number of antibiotic category	Number of <i>E. coli</i> biofilm phenotype			Total number of isolates
	SBP	MBP	NBP	
	N°(%)	N° (%)	N° (%)	N° (%)
8	9(90.0)	1(11.1)	0(0.0)	10(52.6)
7	1(10.0)	6(66.7)	0(0.0)	7(36.9)
6	0(0.0)	2(22.2)	0(0.0)	2(10.5)
TOTAL	10(52.6)	9(47.4)	0 (0.0)	19(100.0%)
	Number of <i>Enterobacter</i> sp. biofilm phenotype			
8	2(40.0)	0(0.0)	0(0.0)	2(25.0)
7	1(20.0)	1(33.3)	0(0.0)	2(25.0)
6	1(20.0)	0(0.0)	0(0.0)	1(12.5)
5	1(20.0)	2(66.7)	0(0.0)	3(37.5)
TOTAL	5(62.5)	3(37.5)	0(0.0)	8(100.0)
	Number of <i>Citrobacter</i> sp. biofilm phenotype			
8	2(33.2)	0(0.0)	0(0.0)	2(22.2)
7	1(16.7)	1(33.3)	0(0.0)	2(22.2)
6	1(16.7)	0(0.0)	0(0.0)	1(11.1)
5	1(16.7)	2(66.7)	0(0.0)	3(33.4)
4	1(16.7)	0(0.0)	0(0.0)	1(11.1)
TOTAL	6(66.7)	3(33.3)	0(0.0)	9(100.0)
	Number of <i>Serratia</i> sp. biofilm phenotype			
7	1(33.3)	0(0.0)	0(0.0)	1(20.0)
6	2(66.7)	0(0.0)	0(0.0)	2(40.0)
5	0(0.0)	2(100)	0(0.0)	2(40.0)
TOTAL	3(60.0)	2(40.0)	0 (0.0)	5(100.0)

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

OXA- 48 Producers

All *E. coli*, *Enterobacter* sp., and *Citrobacter* sp. strains isolated from SSI were found to be OXA-48 producers.

Association between OXA-48 production and biofilm among isolates from SSIs

Out of 19 OXA-48 producing *E. coli* strains isolated from SSI, 10 were SBP and 9 were MBP. Five OXA-48 producing *Enterobacter* sp. isolates from SSI were SBP and 3 were MBP. Out of 9 OXA-48 producing *Citrobacter* sp. strains isolated from SSI, 6 were SBP and 3 were MBP.

Table 5 OXA-48-producing *Enterobacteriaceae* strains and biofilm phenotype

Organisms	OXA-48 producers according to biofilm phenotype				Typical color colony
	SBP, n (%)	MBP, n (%)	NBP, n (%)	Total OXA-48 producers, n (%)	
<i>Escherichia coli</i>	10 (52.6)	9 (47.4)	0 (0.0)	19 (100.0)	Red
<i>Enterobacter</i> sp.	5 (62.5)	3 (37.5)	0 (0.0)	8 (100.0)	Blue-green
<i>Citrobacter</i> sp.	6 (66.7)	3 (33.3)	0 (0.0)	9 (100.0)	Blue with red halo
<i>Serratia</i> sp.	ND	ND	ND	ND	ND

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

Discussion

Enterobacteriaceae are known as a significant cause of SSIs [22, 23]. 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 [24]. Antibiotic sensitivity testing of the isolates showed higher rates of multidrug resistant (MDR) strains of these organisms in SSIs. *E. coli*, *Enterobacter* sp., *Citrobacter* sp., and *Serratia* showed higher resistance to β -lactam antibiotics (ampicillin, amoxicillin-clavulanic acid, cefotaxime), quinolones (ciprofloxacin, norfloxacin), aminoglycosides (amikacin and kanamycin), carbapenem (imipenem). This high resistance of organisms to the most commonly used antibiotics was reported from many studies [25-29]. 100% of *Enterobacteriaceae* strains were resistant to imipenem. Our results are not consistent with a report from Iran in which 100% of *Enterobacteriaceae* isolated from SSIs were sensitive to this antibiotic [30]. In this study, we detected OXA-48-producing strains among different enterobacterial species isolated in samples from patients with SSIs. *E. coli*, *Citrobacter* sp., and *Enterobacter* sp. strains were 100% OXA-48-producers. Reports from many African studies showed the spread of carbapenemase-producing bacteria [31]. Investigations done in many African countries such as Tunisia, Libya, Tanzania, Senegal, and Morocco, have shown that *K. pneumoniae* was the most frequently OXA-48 producer [32]. But in this study, we observed a high rate of OXA-48 producers among *E. coli*, *Enterobacter* sp and *Citrobacter* sp strains (100%). Due to limited laboratory facilities (molecular tools), we were unable to investigate the production of OXA-48 by *Serratia* sp. strains. This organism is inhibited in ChromaticTM OXA-48 medium. Only *Klebsiella* sp, *E. coli*, *Citrobacter* and

Enterobacter sp. produce colonies on this medium. Using Polymerase Chain Reaction, other reports have demonstrated that *K. pneumoniae*, *E. coli*, *C. freundii*, *P. mirabilis*, *Enterobacter* sp. and *Serratia marcescens* are OXA-48 producers [33, 34]. The results of the current study demonstrated that SSIs due OXA-48-carbapenemase-producing *Enterobacteriaceae* is a major clinical challenge.

In this study the detection of biofilm formation was performed using CVSM. All *Enterobacteriaceae* (100%) isolates from SSIs 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 SSIs [35] and can delay wound healing (18, 36). We demonstrated also a high variability in biofilm biomass production among isolates from SSIs (Figure 1). 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 [37-39]. These factors could be involved in the high number of biofilm positive strains as observed in the present study. The biofilm producing-*Enterobacteriaceae* from SSIs were very resistant to antibiotics. A biofilm creates a protected mode of growth that allows bacteria to survive in hostile environments protected from host defenses and the action of antibiotics. Bacteria in biofilms show high antibiotic resistance because of the difficulty the drugs have in penetrating the biofilm (antibiotics do not reach sufficient concentrations in any part of the biofilm) and the metabolism of bacteria at the base of the biofilm (they are metabolically inactive and therefore resistant to some antibiotics [16]. The association between biofilm and antibiotic resistance was noted to be statistically significant ($P < 0.05$) in most of the antibiotics (ampicillin, amoxicillin-clavulanic acid, cefotaxime, imipenem, and kanamycin). The correlation between antibiotic resistance and biofilm production has been documented in other studies [40-42].

The association between OXA-48 production and biofilm formation was observed for *E. coli*, *Citrobacter* sp., and *Enterobacter* sp. ($P < 0.05$). Imipenem, ampicillin, amoxicillin-clavulanic acid, and cefotaxime was 100% ineffective against OXA-48 positive strains. OXA-48 are enzymes that are responsible for resistance of bacteria toward carbapenems. It is well known that carbapenems are the most effective antibiotics for the treatment of infections resulted from MDR strains; recently, the increasing occurrence of carbapenem-resistant isolates of *Enterobacteriaceae* has become a global concern (12-14). Many factors could be responsible for the increasing of resistance in DRC as demonstrated in the present study. 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 [43], as well as biofilm ability of strains and the acquisition of resistance genes [40-42].

Conclusion

There was higher resistance rate among biofilm producing *E. coli*, *Citrobacter* sp, *Enterobacter* sp , and *Serratia* sp. isolates to the majority of antibiotic tested except norfloxacin and amikacin for *Serratia* sp. The higher resistance rate of strains to imipenem and to other antibiotics suggests the implementation

and development of national policies and plans to prevent and combat the spread of resistant bacteria in community and hospital setting.

Abbreviations

ADH: Arginine dihydrolase; ATCC: American type culture collection; CLSI: Clinical and laboratory standards institute; CVSM: *Crystal Violet Staining Method*; DRC: Democratic Republic of Congo; ELISA: Enzyme-linked immunosorbent assay; L.D.C: Lysine decarboxylase; MBP: Moderate biofilm producer; MDR: Multidrug resistance; NBP: Non-biofilm producer; OD: *Optical density*; ODC: Ornithine decarboxylase; *OXA-48*: Oxacillinase-48; SSIs: Surgical site infections; SBP: Strong biofilm producer.

Declarations

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are not publicly available. Laboratory has not provided us information on patients. Results of antibiograms and biofilm tests can be provided by the corresponding author on reasonable request.

Funding

Not applicable

Acknowledgements

Not applicable

Authors' contributions

Jean-Marie LIESSE IYAMBA contributed to the conception of the study, the preparation of the manuscript and the statistical analyses. Ntongo za Balega Takaisi-Kikuni and Jacques Onokodi Kasongo contributed to analysis and the preparation of manuscript. Thierry Mukendi Kajinga and Nelson Nsiata contributed to the collection of samples and to antimicrobial susceptibility tests at Hôpital Saint Joseph. Cyprien Mbundu Lukukula and Joseph Welo Unya contributed to the processing of samples and to antibiotic susceptibility tests in our laboratory. Daniel Tassa Okombe, Benjamin Kodondi Ngbandani, and Grégoire Mbusa Vihembo contributed to OXA-48 carbapenemase screening and biofilm formation tests. Odette Mboma Mapipi analysed literature data. All the authors have read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical approval

Not required.

Author details

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

²Service de Microbiologie, Hôpital de Référence Saint Joseph, Limete-Kinshasa, Democratic Republic of Congo

³Service de Médecine Interne, Hôpital de Référence Saint Joseph, Limete-Kinshasa, Democratic Republic of Congo

⁴Centre National de Pharmacovigilance, University of Kinshasa, Kinshasa, Democratic Republic of Congo.

References

1. Cheadle WG. Risk factors for surgical site infection. *Surg Infect (Larchmt)*. 2006;7 Suppl 1:S7-11. <https://doi.org/10.1089/sur.2006.7.s1-7>.
2. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. *Infect Control Hosp Epidemiol*. 1992;13(10):606-8.
3. Allegranzi B, Bischoff P, de Jonge S, et al. New WHO recommendations on preoperative measures for surgical site infection prevention: an evidence-based global perspective. *Lancet Infect Dis*. 2016;16(12):e276–e87. [https://doi.org/10.1016/s1473-3099\(16\)30398-x](https://doi.org/10.1016/s1473-3099(16)30398-x).
4. Badia JM, Casey AL, Petrosillo N, Hudson PM, Mitchell SA, Crosby C. Impact of surgical site infection on healthcare costs and patient outcomes: a systematic review in six European countries. *J Hosp Infect*. 2017;96(1):1-15. <https://doi.org/10.1016/j.jhin.2017.03.004>.
5. O'Keefe AB, Lawrence T, Bojanic S. Oxford craniotomy infections database: a cost analysis of craniotomy infection. *Br J Neurosurg*. 2012;26:265-9. <https://doi.org/10.3109/02688697.2011.626878>.
6. Weigelt JA, Lipsky BA, Tabak YP, Derby KG, Kim M, Gupta V: Surgical site infections: causative pathogens and associated outcomes. *Am J Infect Control*. 2010; 38:112–120. <https://doi.org/10.1016/j.ajic.2009.06.010>.
7. Le Doare K, Bielicki J, Heath PT, Sharland M. Systematic review of antibiotic resistance rates among gram-negative bacteria in children with sepsis in resource-limited countries. *J Pediatric Infect Dis Soc*. 2015;4:11–20. <https://doi.org/10.1093/jpids/piu014>.

8. Carroll M, Rangaiahagari A, Musabeyezu E, Singer D, Ogbuagu O. Five-year antimicrobial susceptibility trends among bacterial isolates from a tertiary health-care facility in Kigali, Rwanda. *Am J Trop Med Hyg.* 2016;95:1277–83. <https://doi.org/10.4269/ajtmh.16-0392>.
9. Kumburu HH, Sonda T, Mmbaga BT, Alifrangis M, Lund O, Kibiki G, Aarestrup FM. Patterns of infections, aetiological agents, and antimicrobial resistance at a tertiary care hospital in northern Tanzania. *Tropical Med Int Health.* 2017; 22:454–64. <https://doi.org/10.1111/tmi.12836>.
10. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clin Infect Dis.* 2006 Jan 15;42 Suppl 2:S82-9. <https://doi.org/10.1086/499406>.
11. Pana ZD and Zaoutis T. Treatment of extended-spectrum β -lactamase-producing *Enterobacteriaceae* (ESBLs) infections: what have we learned until now? *F1000Res.* 2018;7:F1000 Faculty Rev-1347. <https://doi.org/10.12688/f1000research.14822.1>.
12. Potter RF, D'Souza AW, Dantas G. The rapid spread of carbapenem-resistant *Enterobacteriaceae*. <https://doi.org/10.1016/j.drug.2016.09.002>.
13. van Duin D and Doi. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. *Virulence.* 2017; 8(4): 460-69. <https://doi.org/10.1080/21505594.2016.1222343>.
14. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother.* 2012; 67 (7): 1597-606. <https://doi.org/10.1093/jac/dks121>.
15. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis.* 2002; 8 (9): 881-90. <https://doi.org/10.3201/eid0809.020063>.
16. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science.* 1999; 284(5418): 1318–22. <https://doi.org/10.1126/science.284.5418.1318>.
17. Percival et al. Biofilms and Wounds: An Identification algorithm and potential treatment options. *Adv Wound Care (New Rochelle).* 2015;4(7):389-397. <https://doi.org/10.1089/wound.2014.0574>.
18. Wolcott R, Cutting KF, Dowd SE. Surgical site infections: biofilms, dehiscence and delayed healing. *Wounds UK.* 2008; 4 (4):108-13.
19. Clinical Laboratory Standards Institute (CLSI). CLSI document M100S-S22. Performance standards for antimicrobial susceptibility testing: Twenty second informational supplement ed. Wayne: CLSI; 2012.
20. Chavant P, Gaillard-Martinie B, Talon R, Hébraud M, Bernardi T. A new device for rapid evaluation of biofilm formation potential by bacteria. *J Microbiol Methods.* 2007;68(3):605-612. <https://doi.org/10.1016/j.mimet.2006.11.010>.
21. Ramos-Vivas J, Chapartegui-González I, Fernández-Martínez M, et al. Biofilm formation by multidrug resistant *Enterobacteriaceae* strains isolated from solid organ transplant recipients. *Sci Rep.* 2019;9(1):8928. <https://doi.org/10.1038/s41598-019-45060-y>.
22. Lehner S, Grabein B, Pfaller P, Kopp R. Relevance of ESBL-producing pathogens for clinical surgery: diagnostics, therapy, and prevention. *Chirurg.* 2009;80(6):527-36. <https://doi.org/10.1007/s00104-008-1664-x>.

23. Jolivet S, Lescure FX, Armand-Lefevre L, Raffoul R, Dilly MP, Ghodbane W, Nataf P, Lucet JC. Surgical site infection with extended-spectrum β -lactamase-producing *Enterobacteriaceae* after cardiac surgery: incidence and risk factors. Clin Microbiol Infect. 2018;24(3):283-28. <https://doi.org/10.1016/j.cmi.2017.07.004>.
24. Tadesse BT, Ashley EA, Ongarello S, et al. Antimicrobial resistance in Africa: a systematic review. BMC Infect Dis. 2017;17(1):616. <https://doi.org/10.1186/s12879-017-2713-1>.
25. Dessie W, Mulugeta G, Fentaw S, Mihret A, Hassen M, Abebe E. Pattern of bacterial pathogens and their susceptibility isolated from surgical site infections at selected referral hospitals, Addis Ababa, Ethiopia. Int J Microbiol. 2016; 2016:2418902. <https://doi.org/10.1155/2016/2418902>.
26. Mengesha RE, Kasa BG, Saravanan M, Berhe DF, Wasihun AG. Aerobic bacteria in post-surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. BMC Res Notes. 2014;7:575. <https://doi.org/10.1186/1756-0500-7-575>.
27. Anguzu JR, Olila D: Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. Afr Health Sci. 2007;7(3):148-154. <https://doi.org/10.5555/afhs.2007.7.3.148>.
28. Mulu W, Kibru G, Damtie M: Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. Ethiop J Health Sci. 2012, 22(10):7–18.
29. Manyahi J, Matee MI, Majigo M, Moyo S, Mshana SE, Lyamuya EF. Predominance of multi-drug resistant bacterial pathogens causing surgical site infections in Muhimbili national hospital, Tanzania. BMC Res Notes. 2014;7:500. <https://doi.org/10.1186/1756-0500-7-500>.
30. Akhi MT, Ghotaslou R, Beheshtirouy S, et al.. Antibiotic Susceptibility Pattern of Aerobic and Anaerobic Bacteria Isolated From Surgical Site Infection of Hospitalized Patients. Jundishapur J Microbiol. 2015;8(7):e20309.<https://doi.org/10.5812/jjm.20309v2>.
31. Manenzhe RI, Zar HJ, Nicol MP, Kaba M. The spread of carbapenemase-producing bacteria in Africa: a systematic review. J Antimicrob Chemother. 2015;70(1):23-40. <https://doi.org/10.1093/jac/dku356>.
32. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect. 2002; 8(6):321-331.<https://doi.org/10.1046/j.1469-0691.2002.00401.x>.
33. Tzouveleakis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25(4):682-707. <https://doi.org/10.1128/cmr.05035-11>.
34. Regev-Yochay G, Smollan G, Tal I, et al.. Sink traps as the source of transmission of OXA-48–producing *Serratia marcescens* in an intensive care unit. Infect Control Hosp Epidemiol. 2018;39(11):1307-1315. <https://doi.org/10.1017/ice.2018.235>.
35. Percival S.L. 2017. Importance of biofilm formation in surgical infection. Br J Surg. 2017;104(2):e85-e94. <https://doi.org/10.1002/bjs.10433>.

36. Metcalf DG, Bowler PG. Biofilm delays wound healing: A review of the evidence. *Burn Trauma*. 2013;1:5-12.
37. Kokare CR, Chakraborty S, Khopade AN, Mahadik KR. Biofilm: importance and applications. *Indian J Biotechnol*. 2009; 8:159–168.
38. Characklis W G, McFeters G A, Marshall K C. Physiological ecology in biofilm systems. In: Characklis W G, Marshall K C, editors. *Biofilms*. New York, N.Y: John Wiley & Sons, Inc.; 1990. pp. 341–394.
39. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. *Annu Rev Microbiol*. 2000; 54:49-79. <https://doi.org/10.1146/annurev.micro.54.1.49>.
40. 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*. 2016; 5:5. <https://doi.org/10.1186/s13756-016-0104-9>.
41. Karami, et al. The correlation between biofilm formation capability and antibiotic resistance pattern in *Pseudomonas aeruginosa*. *Gene Reports* 18 (2020) 100561. <https://doi.org/10.1016/j.genrep.2019.100561>.
42. Dumar 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*. 2019;12(1):38. <https://doi.org/10.1186/s13104-019-4084-8>.
43. 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*. 2017;27(2):147-154. <https://doi.org/10.1684/mst.2017.0678>.

Figures

Figure 1

Biofilm formation by Enterobacteriaceae isolates was performed using CVSM. Data are the mean $\pm$ standard deviation of 3 independent experiments.