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Profile of Multidrug Resistant Bacteria in Bukavu Hospitals and Antimicrobial Susceptibility to *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis and Staphylococcus aureus*

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Abstract

Objective: To evaluate the spread of Multidrug-Resistant (MDR) bacterial infections in Bukavu hospitals and test antimicrobial susceptibility patterns of some isolates to usual marketed antibiotics. **Methods:** The prevalence of MDR strains was determined by using general antimicrobial susceptibility data collected from 3 hospital laboratories. The susceptibility of some isolates to usual antibiotics was processed by agar diffusion method with standard *E. coli* ATCC8739 and standard antibiotics discs as controls. The tested antibiotics were ampicillin, ceftriaxone, gentamicin, chloramphenicol and ciprofloxacin. **Results:** At the 3 hospitals, 758 tests were realized in urine, pus, stool, FCV, blood, LCR, split and FU specimens; 46 strains were unidentified and 712 strains were identified. Of 712 identified strains, 223 (31.4%) were MDR or XDR strains including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter*, *Proteus mirabilis*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Morganella morganii*, *Enterococcus faecalis and E. faecium*, *Neisseria gonorrohoae*, *Staphylococcus aureus*, coagulase-negative, staphylococci, *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Of the infected patients, 36 (21.5%) children were under 16 years and 188 (78.5%) adults were predominately women (58.5%). The susceptibility test showed that all strains

but *S. aureus* were resistant to ampicillin and amoxicillin and ciprofloxacin. Gentamicin, ceftriaxone, and chloramphenicol remain partially active (27% - 80%) against *P. mirabilis*, *E. coli* and *P. aeruginosa*. The resistance is more likely related to strain mutation than to pharmaceutical quality of the antibiotics prescribed. **Conclusion:** Both data from hospital laboratories and *in vitro* post-testing findings confirmed the ongoing elevated prevalence of MDR strains in Bukavu. The causes of antibiotic misuse and socio-economic determinants of the phenomenon of resistance should be scrutinized in order to take adequate strategies in the prospective of establishing an effective control system against this threat to overall health. The results of this work on MDR profiles have various implications for the management of infectious diseases. It provides indicators for the surveillance of antimicrobial resistance, practical guidelines for antibiotic susceptibility testing in biomedical laboratories, and guidance for antibiotic therapy.

Keywords

Prevalence, Antimicrobials, Multi-Resistance, Bacterial Sensitivity, Bukavu, DRC

1. Introduction

Multidrug-Resistant (MDR) strain is defined in the medical literature as a non-susceptibility of such microbe strain to at least one agent in three or more antimicrobial categories [1]. The terms Extensively Drug Resistant (XDR) and Pan-drug-Resistant (PDR) have also been introduced to recognize different degrees of multidrug resistance [2].

The World Organization for Animal Health (WOAH), the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have reported the spread of multiple pathogenic bacteria that are resistant to multiple antimicrobial agents, underlining that multidrug resistance is now reaching dangerously high levels in all regions of the world. The pathogens that cause tuberculosis, malaria, sexually transmitted infections, typhoid fever, bacterial dysentery and pneumonia present now the characteristics of MDR [3] [4]. Up to 17% of TB cases are MDR and more and more, XDR of TB is being seen all over the world [5] [6]. The hospital is a major source of drug-resistant infections caused by *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Citrobacter* spp., *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* [7]. Up to 10% of inpatients contract nosocomial infections [8], but community-based transmission is also dangerously underway.

Bacterial resistance often leads to treatment failure and thus increases the mortality associated with infections [9]. Other studies have reported that the mortality rate associated with an infection caused by an antibiotic-resistant bacterium is 2 to 4 times higher than that associated with a sensitive bacterium [6] [10]. In

addition, the prolongation of illness and treatment increases health expenditures, as well as the financial burden on families and society. When an infection can no longer be treated with a first-line antibiotic, more expensive drugs shall be used.

Many factors triggering microbial resistance are known. Antimicrobials are among the most widely and most irrationally used drugs and this is the leading cause for resistance breakout. In 20% - 50% of cases, the use in humans is useless and in 40% - 80% of cases the use in animals is of doubtful interest [5] [11]. The use of ineffective or poor-quality antimicrobial drugs has multiple disadvantages leading to microbial resistance, treatment failure, exacerbation of the disease, and increased mortality rates. An increasing rate of low-quality generic medicines is of growing concern in Sub-Saharan Africa [12] [13]. The WHO, the United States Center for Disease Control and Prevention (CDCP), and the European Commission have recognized the importance of studying the emergence and risk factors of resistance as well as the need to establish strategies for its control. It is therefore essential to prioritize the rational use of antibiotics as well as the prevention of infections through rapid detection and epidemiological surveillance of bacterial resistance [14].

A review of literature was conducted to assess the prevalence and mechanisms of antibiotic resistance mainly to β-lactam antibiotics, cephalosporins, carbapenems, colistin, and tigecycline in the Democratic Republic of the Congo (DRC). The studies found that bacterial resistance to antibiotics concerned both Gram-negative and Gram-positive bacteria; multidrug resistance prevalence was the same in half of *Streptococcus pneumoniae* isolates; a worrying prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) was noted, which is associated with co-resistance to several other antibiotics; and resistance to third-generation cephalosporins was very high in Enterobacteriaceae, mainly because of blaCTX-M-1 group and blaSHV genes [15]. A study conducted on *Salmonella* spp. strains isolated in Bukavu (capital of South Kivu province, DRC) are sensitive to ciprofloxacin, ceftazidime, ceftriaxone, norfloxacin, amikacin and cefuroxime. They remain resistant to amoxicillin, augmentin, chloramphenicol, cotrimoxazole, doxycycline, gentamicin and negram [16]. The challenges encountered by the health system in DRC are a lack of a national action plan for combating antimicrobial resistance including the establishment of ABR surveillance and monitoring systems, and building laboratory capacity [17]. To complete the existing literature through this study, the following research questions were raised. What's the profile and prevalence of Multidrug-Resistant (MDR) bacterial infections isolated in hospital laboratories of Bukavu? What's the *vitro* susceptibility profile of some MDR isolates to usual antibiotics marketed in Bukavu, DRCongo?

2. Methods

2.1. Study Design

The general design of the study is shown in **Figure 1**. The aim was to determine

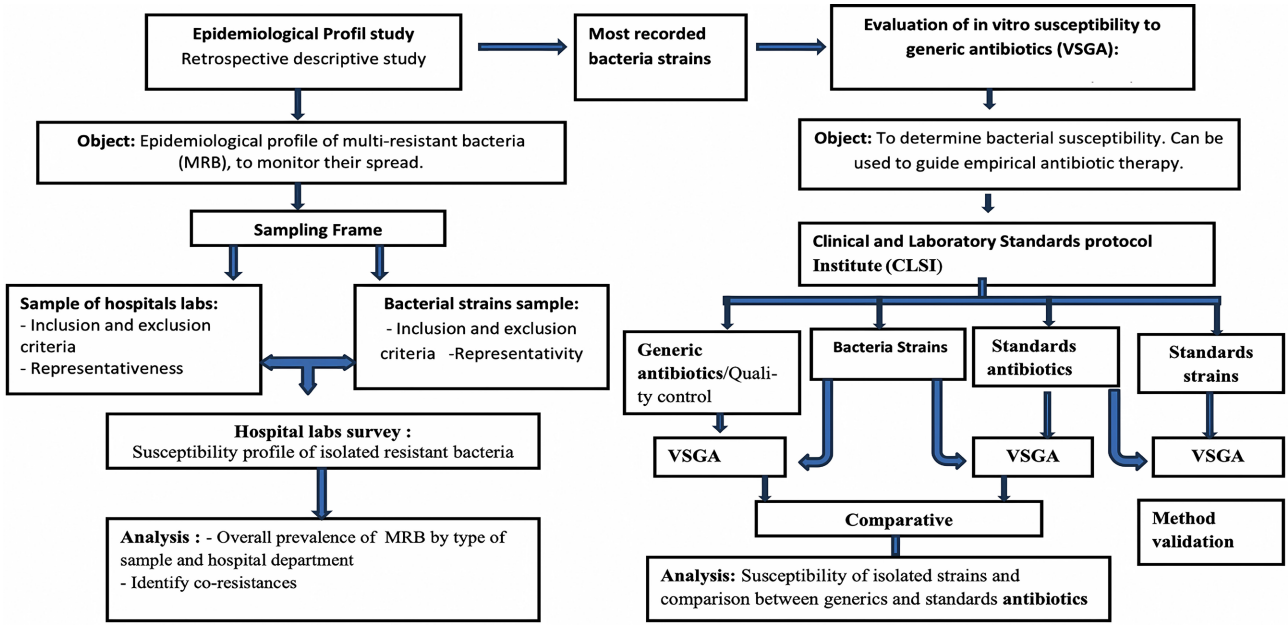

Figure 1. Diagram of the study design.

the epidemiologic prevalence of multi-resistant bacterial infections identified in hospitals and to test *in vitro* the susceptibility of some isolates to usual generic antibiotics in comparison with standard antibiotics disks. The epidemiologic prevalence study was retrospective, conducted by collecting data from hospitals' laboratory records, over a period of 2 years (January 2016 to December 2017). For susceptibility study, the most prevalent strains were isolated, cultivated and subjected to *in vitro* susceptibility testing by agar diffusion method using *E. coli* ATCC8739 standard strain and antibiotics standard disks, following the recommendations of EUCAST, CA-SFM, and CLSI [18] [19]. Some generic antibiotic brands marked in the city were purchased and also tested for susceptibility against MDR isolates.

2.2. Sampling of Bacteria Cases

As shown in **Figure 2**, the bacterial isolates were collected from microbial cultures carried out in the bacteriology departments of 3 hospitals-Panzi Hospital (HGP), BIOPHARM Hospital (HBP) and Cliniques Saint Luc (CSL)—selected by convenience in accordance with the WHO Guide to Training in Methods of Scientific Research (WHO, 2003). We have recorded the results of all antibiotic susceptibility tests carried out during the study period. During the period of the study, 758 cases were found of which 712 strains were identified and 46 strains unidentified. In the respective hospitals, antibiotic susceptibility testing was carried out using the modified Kirby Bauer method, recommended by the WHO and based on diffusion from antibiotic-impregnated discs on Muller-Hinton agar. We have recorded the result of the susceptibility testing performed on 712 specimens identified in the 3 hospitals. For the experimental part of the study, the sensitive test was carried out in the Microbiology Laboratory of the Faculty of

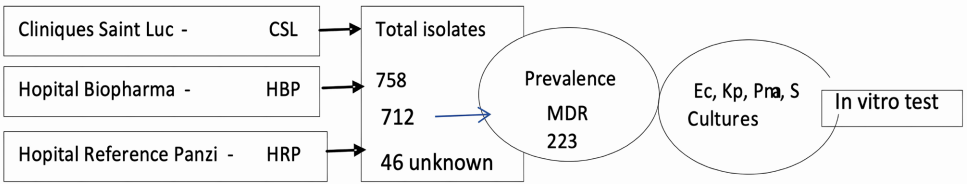

Figure 2. Sampling of bacteria cases. Ec = *Escherichia coli*; Kp = *Klebsiella pneumonia*; Pm = *Proteus mirabilis*; S = *Staphylococcus aureus*.

Pharmacy and Public Health of the Official University of Bukavu. We performed the sensitivity test *on Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis and Staphylococcus aureus* strains sampled from isolates of the 3 hospitals. The choice to perform susceptibility testing on these strains was made by convenience [20] as they recorded high isolation frequencies on all 758 strains.

2.3. Sampling of Generic Antibiotics

Generic products of ampicillin, gentamicin, chloramphenicol, ceftriaxone and ciprofloxacin were purchased randomly in triplicate from few community pharmacies in the city of Bukavu. The selection was made according to their sensitivity to the corresponding standard bacteria in accordance with the recommendations of the CA-SFM. Primary quality control of the brands purchased was done via visual inspection and assays test as described in the British Pharmacopoeia and GPHF-MiniLab 2012 manual. The antibiotic products were powdered and exactly weighted aliquots were dissolved in appropriate solvents according to pharmacopeial instructions. Appropriate dilutions were made to have desired concentrations, which should be the minimum inhibitory concentration of each antibiotic. The solutions were used to prepare the disks of 6 mm in diameter, which were in a hot air oven and kept in aseptic conditions [13].

2.4. Procedure of Antimicrobial Susceptibility Test

The authentication of bacteria isolates was done according to the method of the Health Care Infection Control Practices Advisory Committee (HICPAC) [21]. For quality control of the test system, *E. coli* ATCC 8739 standard strain (Certificate Number 61726097) was used (provided by Modern Diagnostic Uganda Ltd.) according to the protocol of the European Committee for Antimicrobial Susceptibility Testing. The standard reference discs of antibiotics were obtained from Modern Diagnostic Uganda Ltd. To test the sensitivity of different bacterial strains to antibiotics, we used Kirby-Bauer's *in vitro* antibiotic disk diffusion method based on the observation that there is a correlation between the Minimum Inhibitory Concentration (MIC) and the diameter of the bacterial growth inhibition zone around a disk. The procedure was described elsewhere [13]. All sources of eventual error were investigated and corrected according to EUCAST.

2.5. Data Analysis

The data were analyzed statistically using Excel and SPSS v20 statistical software

for descriptive statistics. The inhibition zones of generic antibiotics were compared to the inhibition zones of reference discs. The susceptibility of the bacteria to antibiotics was categorized as S, I or R based on the diameter of the inhibition zone (rounded to the nearest millimeter) and according to the data published in the CA-SFM, 2017 recommendation.

3. Results

3.1. Identification and Prevalence MDR Strains

Table 1 shows that out of 758 strains found, 46 were unidentified and 712 were identified, of which 223 (29.4%) were categorized as MDR strains (resistant to at least 3 antibiotics of different groups). These were *E. coli* (87/374; 23.3%), *Enterobacter* ssp *or Enterobacter agglomerans (*44/88; 50%), *Staphylococcus aureus or Staphylococcus coagulase negative* (12/88; 13.6%), *Salmonella enterica or Salmonella* ssp (9/21; 42.9%), *Proteus mirabilis (*14/28; 50%), *Klebsiella pneumoniae or Klebsiela* ssp (46/90; 51.1%), *Neisseria gonorrhea* (1/1; 100%), *Streptococcus B-hemolytic or Streptococcus* ssp (3/7; 42.9%), *Pseudomonas aeruginosa* (4/12; 33.3%), *Citobacter* ssp (1/1; 100%), *Enterococcus* (1/1; 100%) and *Morgella morcali* (1/1; 100%).

Table 2 shows that in total, 53.8% of 223 MDR strains were collected at HGP hospital, 30.9% at HGP hospital and 15.2% at CSL hospital. The predominance of each MDR strain varied from hospital to hospital and not all strains were found in all hospitals; the difference was found statistically significant ($p < 0.001$).

The majority of patients infected were adults with 73.1% against 21.5% of children for valid cases (where the information was given). Likewise, the majority were females with 63.7% against 30.9% of males. The difference in the predominance of each MDR strain was significant for age group ($p = 0.036$) and not significant for gender group ($p = 0.271$).

The majority of biological specimens tested were urine with 33.6% and CBEU with 27.8%, followed by pus (22%), VS (4.5%), stool (4.5%), PL (3.1%), CS (1.8%), HC (0.9%), CR (0.9%), LRC (0.4%) and FU (0.4%). Again, the preponderance of each strain in the various samples was statistically significant ($p < 0.001$).

Table 1. Prevalence of MDR strains out of the total isolates.

	EC	EB	SA	SE	PM	KB	NG	SP	PA	CB	ET	MN	Other	Total
Total N	374	88	88	21	28	90	1	7	12	1	1	1	46	758
Total %	49.3	11.6	11.6	2.8	3.7	11.9	0.1	0.9	1.6	0.1	0.1	0.1	6.1	100
MDR N	87	44	12	9	14	46	1	3	4	1	1	1	0	223
MDR %	23.3	50.0	13.6	42.9	50.0	51.1	100	42.9	33.3	100	100	100	0.0	29.4

EC = *Escherichia coli*; EB = *Enterobacter*; SA = *Staphylococcus aureus*, coagulase-negative staphylococci; SE = *Salmonella enteric*; PM = *Proteus mirabilis*; KB = *Klebsiella pneumoniae*; *NG* = *Neisseria gonorrohoae*; SP = *Streptococcus pneumoniae* and *Streptococcus pyogenes*; PA = *Pseudomonas aeruginosa*; CB = *Citrobacter freundii*; ET = *Enterococcus faecalis* and *E. faecium*; MM = *Morganella morganii*.

Table 2. Frequencies (%) of MDB strains disaggregated by hospital, age, sex and samples.

	EC	EB	SA	SE	PM	KB	NG	SP	PA	CB	ET	MN	Total	p-value
Hospital N	87	44	12	9	14	46	1	3	4	1	1	1	**223**	0.000
CSL %	14.9	20.5	41.7	33.3	7.1	2.2	100	33.3	0.0	0.0	0.0	0.0	**15.2**	
HBM %	56.3	29.5	0.0	0.0	0.0	15.2	0.0	0.0	0.0	0.0	0.0	0.0	**30.9**	
HGP %	28.7	50.0	58.3	66.7	92.9	82.6	0.0	66.7	100	100	100	100	**53.8**	
Age N	87	42	12	7	13	39	1	3	4	1	1	1	**211**	0.036
Adult %	83.9	54.5	75.0	33.3	78.6	69.6	100	100	100	100	100	100	**73.1**	
Child %	16.1	40.9	25.0	44.4	14.3	15.2	0.0	0.0	0.0	0.0	0.0	0.0	**21.5**	
Gender N	86	41	12	7	13	41	1	3	4	1	1	1	**211**	0.271
Female %	73.6	61.4	66.7	44.4	50.0	60.9	0.0	66.7	25.0	0.0	100	0.0	**63.7**	
Male %	25.3	31.8	33.3	33.3	42.9	28.3	100	33.3	75.0	100	0.0	100	**30.9**	
Specimen N	87	44	12	9	14	46	1	3	4	1	1	1	**223**	0.000
Urine %	31.0	31.8	8.3	22.2	28.6	54.3	0.0	0.0	25.0	0.0	100	0.0	**33.6**	
CBEU %	49.4	15.9	41.7	33.3	7.1	2.2	100	33.3	0.0	0.0	0.0	0.0	**27.8**	
Pus %	8.0	18.2	33.3	22.2	50.0	37.0	0.0	33.3	50.0	0.0	0.0	100	**22.0**	
VS %	10.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**4.5**	
Stool %	0.0	22.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**4.5**	
PL %	1.1	2.3	16.7	11.1	7.1	0.0	0.0	0.0	25.0	0.0	0.0	0.0	**3.1**	
CS %	0.0	2.3	0.0	11.1	0.0	4.3	0.0	0.0	0.0	0.0	0.0	0.0	**1.8**	
HC %	0.0	2.3	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.9**	
Spit %	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	100	0.0	0.0	**0.9**	
LCR %	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.4**	
FU %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	**0.4**	

CBEU = Cytobacteriological Examination of Urine; VS = Vaginal Sampling; PL = Lumber Puncture; CS = Cervical Sampling; HC = Hemoculture; LCR = Liquide Cephalo-Rachidien; FU = Frottis Urethral.

Table 3 shows the percentages of MDR strains which were sensitive to standard discs of antibiotics used in the bacteriologic laboratories of the three hospitals examined. For example, out of 76 analysis of EC against ciprofloxacin (CIP), 75% were resistant and only 25% of strains were sensitive to; 41% of 49 tests to méropénem (MEM) were sensitive and 59% resistant; 28% of isolates were sensitive to chloramphenicol, and 27% were sensitive to nitrofurantoin. Almost all strains were resistant to amoxicillin (AMC) and ampicillin. For SA, 80% were sensitive to chloramphenicol and 40% sensitive to ciprofloxacin. For *EB*, only 27% were sensitive to amikacin and 21% sensitive to méropénem. For SP, 33% were sensitive to ciprofloxacin, 33% to amikacine and 67% sensitive to méropénem.

3.2. *In Vitro* Antimicrobial Susceptibility Findings

Table 4 shows the mean ± SDV inhibition zone (mm) of some MDR strains

Table 3. Percentage of MDR isolates to common antibiotics.

ABT		EC	EB	SA	SE	PM	KB	NG	SP	PA	CB	ET	MN	Total
CIP	TN	76	40	10	7	14	39	1	3	4	1	1	1	197
	R%	75.0	82.5	60.0	57.1	78.6	87.2	100	66.7	100	100	100	100	78.7
SXT	TN	68	33	4	8	12	33	1	2	2	1	1	1	166
	R%	73.5	81.8	100	100	100	100	100	50.0	100	100	0.0	100	84.3
AMC	TN	54	34	5	4	11	36		3		1	1	1	150
	R%	90.7	91.2	100	100	100	100		100		100	100	100	94.7
AK	TN	58	33	9	7	9	38		3	2		1	1	161
	R%	91.2	72.7	88.9	57.1	100	100		66.7	100		0.0	100	87.5
CRO	TN	69	36	11	7	13	44	1	3	4		1	1	190
	R%	95.7	100	81.8	85.7	100	93.2	100	100	100		100	100	95.3
MEM	TN	49	33	8	8	14	44		3	2	1	1	1	164
	R%	59.2	78.8	75.0	75.0	92.9	95.5		33.3	100	100	0.0	100	77.4
AZM	TN	68	38	7	6	12	40		2	3		1	1	178
	R%	88.2	84.2	100	100	100	92.5		100	100		100	100	90.4
AMP	TN	15	15	3	1	10	28			2	1	1	1	77
	R%	100	100	100	100	100	100			100	100	100	100	100
AM	TN	6	5	2	1	1	1		1					17
	R%	100	100	100	100	100	100		100					100
P	TN	9	11	2	2	9	25			2				60
	R%	100	100	100	100	100	100			100				100
OB	TN	7	14	2	2	9	24		1	2			1	62
	R%	100	100	100	100	77.8	100		100	100			100	96.8
NOR	TN	8	3	2	2	1	4		1	1			22	44
	R%	87.5	100	100	100	100	100		100	100			95.5	95.5
OX	TN	6	4	2	2	2	8			1				25
	R%	100	100	100	100	100	100			100				100
C	TN	18	17	5	5	9	33		1	2				90
	R%	72.2	100	20.0	60.0	88.9	93.9		100	100				84.4
TE	TN	1	1	1									1	4
	R%	100	100	100									0.0	75.0
F	TN	11	6		1		15							33
	R%	72.7	100		100		100							90.9
AKN	TN	1												1
	R%	100												100
CAZ	TN	1												1
	R%	100												100

Continued

NAL	TN	5	2		2	1					10
	R%	60.0	100		100	100					80.0
GN	TN	5	2		1			2			10
	R%	40.0	100		100			100			70.0
CXT	TN	2	3				1			1	7
	R%	100	100				100			100	100
AZT	TN	3	1	1				1			6
	R%	66.7	100	0.0				100			66.7
DO	TN	24	10			1		1			36
	R%	75.0	70.0			100		0.0			72.2
E	TN	9	8	1							18
	R%	100	100	100							100
CFM	TN	15	5								20
	R%	100	100								100.0
DA	TN	18		1		1			2	2	24
	R%	94.4		100		100			100	100	95.8
CX	TN	6	5								11
	R%	50.0	100								72.7
NA	TN	5	2				2				9
	R%	100	100				100				100
CIM	TN	2									2
	R%	50.0									50.0
CN	TN		1							1	2
	R%		100							100	100
IMP	TN		1					1			2
	R%		0					100			50.0
CR	TN		1								1
	R%		100								100
K	TN		1								1
	R%		0.0								0.0
LOM	TN		1								1
	R%		100								100

TN = Total Number of Isolates Tested; R% = Percentage of Resistant Strains; CIP = Ciprofloxacin; SXT = Trimethoprim Sulfamethoxazole; AMC = Amoxicillin Clavulanic Acid; AK = Amikacin; CRO = Ceftriaxone; MEM = Meropenem; AZM = Aztreonam; AMP = Ampiciline; AM = Ampicillin; P = Penicillin, NOR = Norfloxacine; OX = Oxacillin; CC = Clindamycin; TE = Tetracycline; FF = Fosfomycin; CAZ = Ceftazidime; NAL = Nalidixic Acid; GEN = Gentamicin; CTX = Cefotaxime; AZT = Azithromycin; DO = Doxycycline; E = Erythromycin; CFM = Cefuroxime; DA = Doxycycline; NA = Nalidixic Acid; CN = Cefalexin; IPM = Imipenem; CR = Cefpiroma; K = Kanamycin; LOM = Lomefloxacin.

Table 4. Mean ± SDV inhibition zone of MDR isolates and standard *E. coli* strain ATCC 8739.

Antibiotics	Disc	Brand	ATCC 8739	EC	PM	PA	SA	R	S
	Mcg		Mm	mm	Mm	Mm	Mm	mm	Mm
Gentamicin	10	G	-	18.3 ± 2.5	18.3 ± 2.5	5.3 ± 5.0	6.0 ± 0.0	<14	≥16
	10	S	17 ± 2.4	20.3 ± 1.5	27.3 ± 4.7	7.7 ± 2.9	6.3 ± 0.6	<14	≥16
Ceftriaxone	30	G	-	8.3 ± 2.1	23.7 ± 4.6	19.7 ± 2.1	-	<15	≥21
	30	S	21 ± 4.8	10.3 ± 2.5	29.7 ± 0.6	16.7 ± 5.9	-	<15	≥21
Chloramphenicol	50	G	-	30.3 ± 2.3	20.0 ± 11.8	9.3 ± 1.5	-	<19	≥23
	50	S	35 ± 5.3	22.7 ± 2.1	34.3 ± 2.5	7.3 ± 2.5	-	<19	≥23
Ampicillin	10	G	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	32.0 ± 2.7	<11	≥17
	10	S	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	34.7 ± 0.6	<11	≥17
Ciprofloxacin	5	G	-	10.7 ± 2.1	8.0 ± 0.0	6.0 ± 0.0	9.0 ± 1.0	<19	≥22
	5	S	-	11.7 ± 2.1	7.0 ± 0.0	8.3 ± 0.6	10.0 ± 1.7	<19	≥22

G = Disks of generic Antibiotic Prepared; S = Standard Disks Commercialized; EC = *Escherichia coli*, PA = *P. aeruginosa*; PM = *Proteus mirabilis*; SA = *Staphylococcus aureus*.

isolated and of *standard E. coli* strain ATCC 8739 to generics antibiotics from local market compared to standard disks. *E. coli* ATCC 8739 which is not MDR strain was sensitive to gentamicin, chloramphenicol and ceftriaxone. However EC isolated is sensitive to gentamicin and chloramphenicol only and not to ceftriaxone, ampicillin and ciprofloxacin. PM cultivated strain was sensitive to gentamicin, ceftriaxone and chloramphenicol but resistant to ampicillin and ciprofloxacin. SA cultivated strain responded to ampicillin while PA responded only to ceftriaxone. The inhibition zones obtained with discs prepared from market antibiotics were comparable to the zones obtained with standard discs.

4. Discussion

This study found MDR pathogens prevalence of 31.4% in 3 hospitals during one year period of data collection. In general, enterobacteria represented more than 3/4 of all isolated MDR organisms (*E. coli* with 38.8% and *K. pneumoniae* with 20.98%) followed by the group of Gram positive bacteria (*S. aureus* with 5.80%). The majority of persons carrying those MDR strains were adult women.

That is, the high percentage in women may be due to anatomical causes (proximity to anal and vaginal orifices), poor hygiene habits, intercourse, pregnancy, etc. [22]. This observation is in accordance with the data in the literature [23]. Some strains are specific to adults and not found in children like *N. gonorrhea*, *Streptococcus*, *P. aeruginosa*, *Citobacter*, *Enteroccus* and *M. norgali* which are rare even in adults. Moroccan studies also reported a prevalence ranging from 22.2% to 30.4% of MDR strains [24]. Anderson *et al.* [3], found a multicenter study in community hospitals 23% of MDR pathogens, and overall, the three most common pathogens were *S. aureus* (28%), *E. coli* (24%), coagulase-negative Staphylococci (10%), though type of infecting organism varied by location of

acquisition (e.g. community-acquired). Inappropriate empiric antimicrobial therapy was given to 38% patients. The work of Romli *et al.* [25] reported that *K. Pneumoniae* was the main MDR strains with 59.7% of isolates. Basak *et al.* [26], found in a study 37.1%) MDR bacterial, 13.8% XDR strains and no PDR were isolated. Gram negative bacterial strains were sensitive to colistin whereas all (100%) Gram positive bacterial strains were sensitive to vancomycin.

In recent years, the production of Extended-Spectrum Beta-Lactamases (ESBL) and carbapenemases by enterobacteria has increased considerably worldwide [27]. The Alert, Investigation and Surveillance Network on Nosocomial Infection (RAISIN) also reported an increase in the incidence of ESBL Enterobacteria (ESBLE) and a decrease in the incidence of *S. aureus* methicillin resistant (MRSA) (RAISIN, 2012). ESBL production is a real and urgent global threat in developing countries, where the burden of infectious diseases is high and cost constraints prevent the widespread application of new and more expensive agents [28].

Based on data from bacterial resistance monitoring networks in enterobacteria, the distribution of 3rd Generation Cephalosporin Resistant Enterobacterial species (EB RC3G) has been significantly altered with the onset and increase in resistance. This resistance is mainly ensured by the production of EBSL and, to a lesser extent, plasma cephalosporinases (AmpC). The resistance of *K. pneumoniae* to the third-generation cephalosporin is also important and widespread in the WHO Region of the Americas, the Western Pacific in the Eastern Mediterranean and the WHO European Region [5].

In our case gentamicin has been found effective against *E. coli* and *P. mirabilis* but resistant against *P. aeruginosa*, *K. pneumoniae* and *Salmonella*. The results obtained by Holmes *et al.* [29] have documented the resistance of *P. aeruginosa* to gentamicin and related aminoglycoside antibiotics. In the Maghreb, Tiouit *et al.* [30] obtained 98.93% gentamicin-sensitive *E. coli* and Bathily Diarra [31] in Bamako found 84% of *E. coli* susceptible to gentamicin. The partially preserved efficacy of aminoglycosides could be explained by the fact that these parenteral molecules limit their frequent use [32].

All MDR strains studied here were resistant to ciprofloxacin. The literature indicates however a very good efficacy of fluoroquinolones (norfloxacin and nalidixic acid) against Enterobacteriaceae. This is the case of the Tiouit *et al.*'s [30] study which revealed that 91.62% of *E. coli* strains were sensitive to fluoroquinolones. Waterer and Wunderink [33] reported that some Gram-negative microorganisms pose a particular problem of resistance to nosocomial infections, including *Enterobacteriaceae*, *P. aeruginosa*, *S. aureus*, *Acinetobacter* spp. Some of these strains show high resistance to aminoglycosides, β-lactams and quinolones [34]. Resistance to quinolones is frequently reported in Asia and Africa [35].

This study found that meropenem was sensitive to 41% EC, 21% EB, 25% SA, 25% SE, 67% SP and very resistant to other strains. Widespread of infections from the community with these organisms is likely to lead to a dramatic increase in empiric carbapenem use. Carbapenemase-producing Enterobacteriaceae Carba-

penem-Resistant Enterobacteriaceae (CRE) represents an immediate public health threat that requires urgent and aggressive action. Nevertheless, most clinicians consider a carbapenem the drug of choice for serious infections caused by ESBL producing enterobacteriaceae. The resistance of *P. aeruginosa* to carbapenems is the most typical and frequent example of so-called membrane impermeability [36]. Enzymatic inactivation of carbapenems is the most common mechanism of resistance of *A. baumannii* [5]. In a multicenter, prospective US study over a one year period in 2009-2010, 4% of *E. coli* community-onset isolates were ESBL producers [37]. *E. coli* ST131 is a globally disseminated MDR clone, and is characterized by resistance to fluoroquinolones in addition to production of CTX-M type ESBL [38]. In Asia, the Middle East, South America and some parts of Europe, community-onset infection with ESBL-producing *E. coli* is extraordinarily frequent. A significant problem in Asia is disseminated infection with hypervirulent Klebsiella pneumoniae strains. These "hypermucoviscous" strains have a propensity to cause community-onset pyogenic liver abscess and sometimes metastatic infections, including meningitis [39].

In a review paper by Leopold *et al.* [2], Median Prevalence (MP) of resistance to chloramphenicol in Enterobacteriaceae, isolated from patients with a febrile illness, ranged between 31.0% and 94.2%, whilst MP of resistance to third-generation cephalosporins ranged between 0.0% and 46.5%. MP of resistance to nalidixic acid in Salmonella enterica Typhi ranged between 15.4% and 43.2% in pathogens isolated from patients with a respiratory tract infection, meningitis, urinary tract infection or hospital-acquired infection suggested high prevalence of resistance to chloramphenicol, trimethoprim/sulfamethoxazole and tetracycline and low prevalence to third-generation cephalosporins and fluoroquinolones.

To consolidate the hospital retrospective data, we isolated and cultured some strains on specific media. The sensitivity of those cultured isolates was tested with standard antibiotics commercialized disks. The result revealed that the percentage of sensitivity was really low. We also tested the susceptibility of the cultured isolates to generic antibiotics sold in local pharmacies after verification of their pharmaceutical quality. The relative content of each product was deduced from the ratios in the zones of inhibition relative to the standard antibiotic disks selected as references. The result showed that the inhibition zones obtained with discs prepared from market antibiotics were comparable to the zones obtained with standard discs even though the zones of some generic products of chloramphenicol were smaller than those of standard chloramphenicol disks for the inhibition of *P. mirabilis* and *E. coli* strains.

The case of ampicillin and amoxicillin is striking. This study shows that they are totally ineffective against almost all strains. However, they are currently still widely prescribed in many cases and also used in self-medication. Previously, ampicillin and amoxicillin were the most used molecules in the treatment of infections caused by *E. coli*. Multidrug resistance (penicillin + two other classes) in Africa is 25%; in Latin America, 20%; in Eastern Europe, 12%; in Western Europe, 18%; in the United States of America, 26% [35]. Resistance of *N. gonorr-*

hoae to penicillin and tetracycline ranges from 9% to 90% in Asia and exceeds 35% in Sub-Saharan Africa and the Caribbean. According to the literature, *S. aureus* has become increasingly resistant to penicillins. In this case, the treatment failure is effectively linked to the resistance of bacteria and not to the pharmaceutical quality of antibiotics prescribed. We do not absolutely rule out the presence of counterfeit brands and poor-quality products in the Bukavu market [13].

5. Conclusion

Both data from hospital findings and *in vitro* post-testing confirmed the ongoing elevated prevalence of multi-resistant bacteria strains in Bukavu. Since still there is no national network for monitoring bacterial resistance to antibiotics in DRC, the rate found may be even underestimated. Risk factors for antimicrobial resistance are insufficient infection control in hospitals, public health systems inadequate for antimicrobial management, insufficient knowledge among prescribers and users, advertising and influence of pharmaceutical laboratories. These factors should be scrutinized in the prospective of establishing an effective control system against this threat to overall health. As recognized by WHO and CDCP, there is importance in studying the emergence and risk factors of resistance and establishing strategies for its control. The results of this work on MDR profiles have various implications for the management of infectious diseases. It provides indicators for the surveillance of antimicrobial resistance, practical guidelines for antibiotic susceptibility testing in biomedical laboratories, and guidance for antibiotic therapy. One of the limitations of the study is that we did not test for antimicrobial susceptibility to commercially available and standard antibiotics for all the bacterial strains listed in the 3 hospitals. Also, based on our results, further research will be able to genetically characterize multi-resistant strains.

Ethics and Consent to Participate

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the research committee of the Faculty of Medicine (UOB). As a retrospective study, informed consent was waived by institutional ethics review board of the Official University of Bukavu.

Availability of Data and Material

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Authors' Contributions

C.A.I., F.B. and P.B.M. designed the protocol, collected data, and did the analysis. F.M.K. and P.W. and A.L. did the literature search and wrote the first draft. J.N.K. validated the protocol, revised data analysis, and wrote the final draft. Y.C. reviewed the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

AG: Aminoglycosides
CB: Citrobacter
CDCP: Centers for Disease Control and Prevention
CRE: Carbapenem-Resistant Enterobacteriaceae
CSL: Saint Luc Clinic
DRC: Democratic Republic of the Congo
EB: *Enterobacter* spp.
EB RC3G: Enterobacteriaceae Resistant Cephalosporin 3rd Generation
EC: *Escherichia coli*
EF: *Enterococcus faecalis*
ESBLE: Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae
FAO: Food and Agriculture Organization of the United Nations
GLASS: Global Antimicrobial Surveillance System
HBP: BIO-PHARM Hospital
HGP: Hospital General de Panzi
KP: *Klebsiella pneumoniae*
MDR: Multidrug Resistant
MM: *Morganella morganii*
NG: *Neisseria gonorrhea*
NMDR: Non-Multidrug Resistant
PA: *Pseudomonas aeruginosa*
PDR: Pandrug-Resistant
PM: *Proteus mirabilis*
SA: *Staphylococcus aureus*
SE: *Salmonella enterica*
SP: *Streptococcus pyogenes*
STI: Sexually Transmitted Infections
TB: Tuberculosis
WHO: World Health Organization
WOAH: World Organization for Animal Health
XDR: Extended Drug-Resistant