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Research Article

## Pattern of antibiotics resistance and phenotypic characterization of Multidrug resistant bacteria isolates in four hospitals of Littoral region, Cameroon

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### Abstract

**Objective:** To describe the pattern of antibiotics resistance and phenotypic characterization of Multidrug resistant bacteria isolates in four hospitals of Littoral region, Cameroon.

**Methods:** We conducted a descriptive hospital based cross-sectional study from December 2018 to May 2019. A simple random sampling was used to swap 10 selected equipment and 10 materials in the mornings after disinfection but before the start of work in seven units. After inoculation in four agar media consecutively (Eosine Metyleine blue, Cled, Manitol salt agar and blood agar ) and incubated in appropriate conditions, the Kirby-Bauer disk-diffusion method was used for antimicrobial susceptibility test.

**Results:** Among 50.4% (119/236) showed positive bacteria growth, a total of 89 (13 species), predominant bacteria and those more likely to cause nosocomial infections were selected and tested each one to 18 antibiotics. There was high level of resistance to *Penicillin* (amoxicillin (77.5%) and Oxacillin (76.4%)), followed by *3G Cephalosporine* (Ceftazidime (74.2%)) and *Monobactam* (Aztreonam (70.8%)). Although the least level of resistance was observed in *Carbapenem* (imipenem (5.6%)). The overall prevalence of MDRB was 62.9% (56/89). MRSA were the mostly detected 57.5 % (30/89), followed by ESBL 10.1% (9/89). Military hospital of Douala and Emergency unit was the MDRBs dominantly contaminated area respectively 39.3% (22/56) and 17.9% (10/56).

**Conclusion:** MDRB occurred to be a current public health problem as well as hospital surfaces are worrying reservoir that can be spread to patient, health professionals and visitors.

**Keywords:** Antibiotic resistance, susceptibility test, multidrug-resistant bacteria, Hospital facilities, Units, Littoral Region-Cameroon

### INTRODUCTION

Contaminated hospital environment has been recognized to be the most significant reservoir of bacteria causing nosocomial infections<sup>1-4</sup>. Nosocomial infections (NI) known as Health-care Associated Infections (HAIs) and also Hospital-Acquired Infections, are infections in a hospitalized patient not present or incubating on admission. It occurs after 48 hours or more of hospital admission or within 30 days after discharge<sup>5-7</sup>. These pathogens, present in air, hands and surface including equipment colonize patients, staff and visitors by dynamic transmission (to, from and between them)<sup>8,9</sup>. Several lines of evidence suggest that Nosocomial infections and resistant organisms as well as multidrug resistant bacteria (MDRB) often increase each other<sup>10,11</sup>. Thus, many compelling observations abound in the same direction that include: Antimicrobial resistance is more prevalent in nosocomial bacterial strains than in those from community-acquired, during outbreaks of nosocomial infection, patients infected with resistant strains are more

likely than control patients to have received prior antimicrobials, increasing length of hospital stay (LOS) of NI patient to hospital increases the likelihood of colonization with resistant organism, a quarter of HAIs in long-term acute care settings are caused by antibiotic-resistant bacteria and HAIs mostly caused by multi drug resistant (MDR) organisms<sup>12-15</sup>.

These organisms are those with acquired poor susceptibility to one, two at list three or more antimicrobial categories<sup>16,17</sup>. Antibiotic resistance is one of the biggest public health challenges of our time. The World Health Organization (WHO) considers Antimicrobial Resistance (AMR) to be one of the greatest threats to human health in the 21st Century. It is estimated that by 2050 if the AMR threat was not properly tackled, it would lead to 10 million people dying every year and a reduction of 2-3.5% in Gross Domestic Product (GDP) with an overall cost of 100 trillion USD<sup>18,19</sup>. Each year in the U.S., at least 2.8 million people get an antibiotic-resistant infection, and more than 35,000 people die<sup>20</sup>. This burden is expected to be relatively higher in sub-

Saharan Africa with a drop in GDP of US \$2895billion, representing 20% of the region's total economic output<sup>21</sup>. Furthermore, AMR remains a significant One Health problem, affecting humans, animals, and the environment<sup>20</sup>. Hence it is accessed that the selection of resistant organisms somewhere, worldwide, even within an animal may have long-term implication for vegetable and human health globally<sup>22, 23</sup>. In addition, the development of such infections causes patient's discomfort, delayed healing, anxiety, longer stays at hospitals and adds to cost of healthcare services significantly<sup>24</sup>. Dedicated prevention and infection control efforts worldwide are working to reduce the number of infections and deaths caused by antibiotic-resistant germs, but the number of people facing antibiotic resistance is still too high. In Cameroon despite the elaboration of national guidelines and directives on the prevention of nosocomial infections associated with surveillance and fight recommendations<sup>25</sup>, as CDC reported in 2019 in their antimicrobial resistance treat, preventing the spread of germs from the healthcare environment, incomplete adoption of the containment strategy, inconsistent implementation of some CDC recommendations, continued vigilance against serious threats like MDR remain challenges in healthcare facilities<sup>20</sup>. In addition, a study in Cameroon in 10 health facilities to five districts of Yaoundé with the aim to assess their compliance levels with recommendations on national NIs control programs, showed in the item of "infection outbreaks and surveillance of NIs" that all the health facilities had a score below 15% and based on previous research, demonstrated the need and follow-up of epidemiological surveillance of new pathogens and their antibiotic resistance<sup>8</sup>. More action is needed to fully protect people. While AMR cause substantial morbidity and mortality, the availability of timely and accurate epidemiological information in various hospitals surface reservoir is one of the essential approach to fight this priority public health problem. Several studies on AMR have been published in Cameroon in recent years. However, the data on AMR patterns in systematically collected hospital environment from multiple sources is limited. The aim of this study was to describe the pattern of antibiotics resistance and phenotypic characterization of Multidrug resistant bacteria isolates in four hospitals of Littoral region, Cameroon. This approach will be useful to the collection, analysis and sharing of data related to antimicrobial resistance at a global level to inform decision-making, drive local, national and regional action to apply suitable and effective intervention.

## MATERIALS AND METHODS

### Study site

The health facilities were selected using the following criteria: the different technical platform, attendance (social level and frequency), care offered (primary, secondary and central health care according to Cameroon health system) and units. Four hospital facilities (private and public sector) were selected: Saint Jean de Malte Hospital of Njombe (HSJMN), Military Hospital of Douala (MHD), New Bell District Hospital (NBDH) and Medical Health Center of Ndokoti (MHCN) were used for the study.

### Study design

A descriptive, cross-sectional hospital based study was performed. We collected swabs for culture from seven units (Pediatric unit, Medical unit, Operating Theatre, Laboratory, Surgical unit, Emergency and Maternity) specific environmental points (10 different equipment and 10 different materials) in the four hospitals between December

2018 to May 2019, for the selection expected reservoir sample.

### Sample collection and technique

In each health facility, simple random sampling technique was used to collect the sample. Consideration was given to equipment and material with constant hand contacts: 10 equipment : treatment tables, operating tables, delivery tables, office tables, anaesthesia equipment, surgical aspirators, oxygen concentrators, wheelchairs, patients and office chairs. 10 materials: fans, patient bedside tables, patients bed rails, trolleys, door handles, negatoscopes, baby scales, air conditioners, Antiseptic container boxes and Antiseptic container covers. Collection was daily done in the morning after disinfection by hospital workers, but before start of work in each unit. This was done by swabbing all selected equipment and materials using sterile cotton tipped applicators moistened in sterile normal saline. These swabs were placed in labelled caps with unit and type of equipment or material identification numbers and immediately transported to the microbiology laboratory for processing.

### Laboratory processing

#### Inoculation and identification of bacteria species

Specimens were inoculated directly according to Good Laboratory Practice Guidelines on four agar media consecutively (three sector petri plates with different media: Eosine Methylene blue (EMB), Cystine–lactose–electrolyte deficient agar (CLED), Mannitol salt agar and the blood agar medium in one segmented petri plates) Microexpress® ISO 9001 :2008 EN ISO 13485 :2012. After inoculation, the three sectors media were incubated overnight at 37°C aerobically for 18 - 24 hours and blood agar medium anaerobically up to a maximum of 48 hours and inspected for bacterial growths. Identification of bacteria to species level was done using colony characteristics, motility test, Gram's staining technique, different biochemical tests comprising of ; oxidase, catalase, coagulase, urease, indole and API Staph, API Strepto and API 20 E™ BioMerieux according to standard methods<sup>26</sup>.

#### Antibiotic Susceptibility Testing and MDRB

The Kirby-Bauer disk-diffusion method (on Mueller Hinton agar) was used for performing antimicrobial susceptibility patterns and reported according to Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>27</sup>. Base on it and also common locally antibiotic used, 18 antibiotics product of (BioMaxima S.A. (POLAND) and Oxoid (UK)) were included: Amoxicillin (25µg), Penicillin G (10µg), Cefoxitin (30µg), Ceftriaxone (30µg), Amoxicillin-clavulanic acid (20+10µg), Cefazidime (30µg), Aztreonam (30µg), Imipenem (10µg), Vancomycin (30µg), Oxacillin (1µg), Gentamycin (10µg), Amikacin (30µg), Erythromycin (15µg), Azithromycin (15µg), Ciprofloxacin (5µg), Trimethoprim-sulfamethoxazole (25µg), Tetracycline (10µg), Clindamycin (2 µg). Predominant bacteria and those more likely to cause nosocomial infections (a total of 89 isolated belonging to 13 species) were selected and tested each one with all the 18 antibiotics. Each inoculum was standardized to an optical density of 0.5 McFarland turbidity standard and inoculated by swabbing Mueller Hinton agar plates. The inoculated plates were allowed to stand at room temperature (for no more than 30 minutes) until the moisture in the inoculum spots was absorbed by the agar. The plates were inverted and incubated at 37 °C for 16 to 20 hr. The result of each antibiotic testing was determined in the cases where inhibition zone diameters of strains was within performance ranges according to those recommended by manufacturer's and CLSI guidelines, 2017<sup>27</sup>.

MDRB were as follows: extended-spectrum beta-lactamase (ESBL)-producing, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), vancomycin-resistant coagulase negative *Staphylococcus* (VRCoNS), (MDR) multidrug-resistant and extensive drug-resistant (XDR)

#### Determination of MDRB

- ESBL was performed using both initial screen test with ideal indicators 3G Cefalosporin discs (Ceftriaxone 30 µg, Cefoxitin 30 µg and Ceftazidime 30 µg) for all the resistant strains as potential ESBL producer and confirmatory test based on double disc synergy test; The phenotypic confirmation test was performed by using Cefotaxime 30 µg or Ceftazidime 30 µg both alone and in combination with clavulanic acid (10 µg) placed 25mm apart (center to center). Inhibition zone of  $\geq 5$  mm increase in diameter for antibiotics tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing isolate<sup>27-30</sup>.

- MRSA referred to all *S. aureus* strains resistant both to Oxacillin 1 µg and Cefoxitin 30 µg indicators discs<sup>31,32</sup>.

- VRSA and VRCoNS defined by *S. aureus* and coagulase negative *Staphylococcus* resistant to Vancomycin 30 µg.

VRE was reported for Vancomycin-resistant *Enterococcus*<sup>33,34</sup>

- MDR was noted for all the strains resistant to one or more antibiotics to a least two or more antibiotics class as  $\beta$ -lactams, fluoroquinolone and aminoglycoside except carbapenem<sup>35,36</sup>.

- XDR was indicated by all the strains resistant to two, three or more antibiotics classes including carbapenem<sup>36,37</sup>.

#### Quality control/ Quality assurance

Sterility of media was done by incubating one plate from each autoclaved of medium overnight at 35°–37°C and examine it for contaminants.

Control strains, *E. coli* ATCC 25922 (non ESBL-producer) and

*K. pneumoniae* 700603 (ESBL-producer) and *Staphylococcus aureus* ATCC 25923 (Methicillin sensitive *S. aureus*) were used to ensure both: Ability to support growth of the target organism(s), ability to produce appropriate biochemical reactions and adequate inhibition zone diameters.

#### Ethical consideration

An ethical clearance was obtained from Institutional Ethics committee for research on Human Health of the University of Douala: N°: 1646 IEC-UD/06/2018/T. Also, all afferent administrative authorizations to carry out the project was obtained from the Directors of the four hospitals (HSJMN, NBDH, MHD and MHCN).

#### Statistical analysis

Data were recorded in Microsoft Excel 2013 and later transferred to SPSS version 23 software. Categorical variables were summarized using counts, percentages and frequency distribution statistical analysis to reckon the results.

## RESULTS

### 3.1. Result of culture and forms of bacteria isolated

Out of 236 specimens collected from various hospitals, it was observed that majority 119 (50.4%) showed positive bacteria growth. Among them, 52.1% (62/119) were Gram positive cocci, 47.9% (57/119) Gram negative bacilli. 33 different species were found to belong to 5 Gram positive cocci and 28 Gram negative bacilli (13 Enterobacteriaceae and 15 non enterobacteriaceae). In general in Gram positive cocci the most prevalent identified were *Staphylococcus aureus* (37.8% (45/119)), while of Gram negative bacilli isolated, majority were non Enterobacteriaceae 26.1% (31/119) with a highest percentage of *Acinetobacter spp*, *Aeromonas hydrophilas* and *Pasteurella pneumotropica* (3.4% (4/119)), followed by Enterobacteriaceae family 21.9% (26 /119) where *E. coli* showed a highest percentage (5.0% (6/119)). The above explanation and other detail of bacterial isolates are illustrated in the table 1 below.

Table 1 : Prevalence of isolated bacteria

Bacteria	n	(%)	Bacteria	n	(%)	Bacteria	n	(%)	total
<b>Gram positive Cocci</b>	<b>62</b>	<b>(52.1)</b>	<b>Enterobacteriaceae</b>	<b>26</b>	<b>(21.9)</b>	<b>Non Enterobacteriaceae</b>	<b>31</b>	<b>(26.1)</b>	<b>119 (100)</b>
<i>Staphylococcus aureus</i>	45	(37.8)	<i>Serratia plymuthica</i>	2	(1.7)	<i>Pseudomonas putida</i>	2	(1.7)	
<i>Micrococcus spp</i>	3	(2.5)	<i>Serratia mubidaca</i>	3	(2.5)	<i>Pseudomonas fluorescens</i>	1	(0.8)	
<i>Staphylococcus epidermidis</i>	10	(8.4)	<i>serratia liquefasiens</i>	2	(1.7)	<i>Pseudomonas mallei</i>	1	(0.8)	
<i>Staphylococcus saprophyticus</i>	3	(2.5)	<i>Serratia cinetica</i>	1	(0.8)	<i>Acinetobacter spp</i>	4	(3.4)	
<i>Enterococcus faecalis</i>	1	(0.8)	<i>Erwinia spp</i>	2	(1.7)	<i>Acinetobacter bomannii</i>	3	(2.5)	
			<i>Escherichia coli</i>	6	(5.0)	<i>Areomonas hydrophilas</i>	4	(3.4)	
			<i>Enterobacter agglomerans</i>	3	(2.5)	<i>Pasteurella pneumotropica</i>	4	(3.4)	
			<i>Enterobacter sakazaki</i>	1	(0.8)	<i>Chryseomonas meningoseptica</i>	2	(1.7)	
			<i>Proteus mirabilis</i>	1	(0.8)	<i>Mannheimia haemolitica</i>	2	(1.7)	
			<i>Priovidencia stuartii</i>	1	(0.8)	<i>Xantomonas maltophila</i>	1	(0.8)	
			<i>Salmonella arizona</i>	1	(0.8)	<i>Plesiomonas shigelloides</i>	1	(0.8)	
			<i>Salmonella spp</i>	2	(1.7)	<i>Chyseomonas putida</i>	1	(0.8)	
			<i>Serratia odorifera</i>	1	(0.8)	<i>Alcaligenes spp</i>	2	(1.7)	
						<i>Pantoea spp3</i>	2	(1.7)	
						<i>Burkholderia cepctica</i>	1	(0.8)	

### Antibiotic resistance profile of the most prevalent bacterial isolates and those related to NIs

Among the 119 bacteria (33 different species) isolates, 89 (13 species), based on predominant bacteria and those more likely to cause nosocomial infections were selected and tested each to 18 antibiotics classified in four groups depending on their most target site and mechanism of action in bacteria structure (“**group 1**”: cell wall and/or cell membrane synthesis inhibitors, “**group 2**”: Protein synthesis inhibitors, “**group 3**”: Nucleic acid inhibitors and “**group 4**”: Folic acid synthesis inhibitor)<sup>38</sup>.

Regarding the global profile of resistance, there were high level of resistance to most of the antibiotics of “**group 1**” especially **Penicillin** (amoxicillin (77.5 %) and Oxacillin (76.4%)), followed by **3GCephalosporine** (Ceftazidime (74.2 %) and **Monobactam** (Aztreonam (70.8%)). Although the least level of resistance was observed in the same “**group 1**” concerning **Carbapenem** (imipenem (5.6 %)).

Looking at the most common prevalent bacteria tested, in Gram positif cocci, *S. aureus* was the only species that showed resistance to all antibiotic tested independently of the group, most predominant in group 1 to oxacillin 91.1%.

In general among Gram negative bacilli, all the most common isolated were all resistant (100%) to one or more tested antibiotics: *Acinetobacter spp* and *Aeromonas hydrophilas* both into “**group 1**” but two different class of antibiotics, especially to **Penicillin** (*Amoxicillin*) and **Glycopeptide** (*Vacomylin*). *Pasteurella pneumotropica* into “**group 1** and **2**” respectively to **3GCephalosporin** (*Ceftriaxone*) and **Macrolide** (*Azytromycin*). *E. coli* only into “**group 1**” to **2GCephalosporin** (*Cefoxitin*). Nevertheless, all were susceptible (100%) only into “**group 1**” as well as one class, particularly to **Carbapenem** (Imipenem), except *Pasteurella pneumotropica* with one strain resistant to **Carbapenem** (Imipenem). Details of these are depicted in Tables 2, 3 and 4 bellow.

Table 2: Antibiotic resistance patterns of major isolated bacteria species to different antibiotics of “**Group 1**” (Cell wall and or cell membrane synthesis inhibitors)

Name of bacteria	Total	Group 1									
		β-lactamin (cell wall and/ or cell membrane growth inhibitors)									
		AMX	PEN	FOX	CRO	AMC	CAZ	ATM	IMP	VA	OX
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<i>Staphylococcus aureus</i>	45	33(73.3)	36(80)	37(82.2)	32(71.1)	20(44.4)	40(88.9)	42(93.3)	4(8.9)	18(40)	41(91.1)
<i>Staphylococcus epidermidis</i>	10	9(90)	4(40)	5(50)	6(60)	4(40)	7(70)	7(70)	0	4(40)	7(70)
<i>Aeromonas hydrophilas</i>	4	4(100)	1(25)	1(25)	2(50)	0	2(50)	1(25)	0	4(100)	1(25)
<i>Serratia mubidaca</i>	3	2(66.7)	3(100)	2(66.7)	2(66.7)	2(66.7)	0	2(66.7)	0	1(33.4)	0
<i>Erwinia spp</i>	2	2(100)	0	0	2(100)	2(100)	2(100)	0	0	2(100)	1(50)
<i>Acinetobacter spp</i>	4	4(100)	4(100)	1(25)	1(25)	2(50)	2(50)	1(25)	0	4(100)	4(100)
<i>Escherichia coli</i>	6	4(66.7)	3(50)	4	1(16.7)	2(33.3)	2(33.3)	1(16.7)	0	3(50)	2(33.3)
<i>Pasteurella pneumotropica</i>	4	3(75)	1(25)	3(75)	4(100)	3(75)	3(75)	3(75)	1(25)	3(75)	3(75)
<i>Enterobacter agglomerans</i>	3	2(66.7)	2(66.7)	0	2(66.7)	0	2(66.7)	2(66.7)	0	2(66.7)	2(66.7)
<i>Salmonella spp</i>	2	0	1(50)	1(50)	1(50)	0	0	1(50)	0	0	0
<i>Acinetobacter bomanii</i>	3	3(100)	2(66.7)	2(66.7)	3(100)	2(66.7)	3(100)	2(66.7)	0	2(66.7)	3(100)
<i>Enterococcus faecalis</i>	1	1(100)	1(100)	1(100)	1(100)	0	1(100)	1(100)	0	1(100)	1(100)
<i>Pseudomonas putida</i>	2	2(100)	2(100)	2(100)	2(100)	0	2(100)	0	0	2(100)	2(100)
Total	89	69(77.5)	60(67.4)	59(66.3)	58(65.16)	37(41.6)	66(74.2)	63(70.8)	5(5.6)	46(51.7)	67(75.3)

AMX: Amoxicilin; PEN: Penicillin G; FOX: Cefoxitin; CRO: Ceftriaxone; AMC: Amoxicillin-clavulanic acid; CAZ: Ceftazidime; ATM: Aztreonam; IMP: Imipenem; VA: Vancomycin; OX: Oxacillin;

Table 3: Antibiotic resistance patterns of major isolated bacteria species to different antibiotics of "Group 2" (Protein synthesis inhibitors)

Name of bacteria	Total	Group 2				
		Protein synthesis inhibitors				
		CN n (%)	AK n (%)	E n (%)	AZT N (%)	CL n (%)
<i>Staphylococcus aureus</i>	45	14 (31.1)	13 (28.9)	22 (48.8)	37 (82.2)	10 (22.2)
<i>Staphylococcus epidermidis</i>	10	2 (20)	5 (50)	6 (60)	6 (60)	4 (40)
<i>Aeromonas hydrophilas</i>	4	0	0	1 (25)	1 (25)	1 (25)
<i>Serratia mubidaca</i>	3	1 (33.3)	1 (33.3)	1 (33.3)	1 (16.7)	1 (33.7)
<i>Erwinia spp</i>	2	0	0	1 (50)	0	1 (50)
<i>Acinetobacter spp</i>	4	0	0	1 (25)	1 (25)	1 (25)
<i>Escherichia coli</i>	6	0	2 (33.3)	3 (50)	1 (16.7)	1 (16.7)
<i>Pasteurella pneumotropica</i>	4	2 (50)	2 (50)	3 (75)	4 (100)	2 (50)
<i>Enterobacter agglomerans</i>	3	2 (66.7)	0	0	3 (100)	0
<i>Salmonella spp</i>	2	0	0	0	1 (50)	1 (50)
<i>Acinetobacter bomanii</i>	3	0	0	2 (66.7)	1 (33.3)	2 (66.7)
<i>Enterococcus faecalis</i>	1	0	0	0	1 (100)	1 (100)
<i>Pseudomonas putida</i>	2	1 (50)	1 (50)	2 (100)	2 (100)	2 (100)
Total	89	22 (24.7)	24 (26.9)	42 (47.2)	60 (67.4)	27 (30.3)

CN: Gentamycin; AK: Amikacin; E: Erythromycin; AZT: Azithromycin; CL: Clindamycin

Table 4: Antibiotic resistance patterns of major isolated bacteria species to different antibiotics of "Group 3 and 4" (nucleic acid synthesis inhibitors and folic acid synthesis inhibitor)

Name of bacteria	Total	Groupe 3		Groupe 4
		Nucleic acid synthesis inhibitors		Folic acid synthesis inhibitors
		CIP n (%)	TE n (%)	SXT n (%)
<i>Staphylococcus aureus</i>	45	14 (31.1)	14 (31.1)	22 (48.9)
<i>Staphylococcus epidermidis</i>	10	2 (20.0)	1 (10)	5 (50)
<i>Aeromonas hydrophilas</i>	4	2 (50)	2 (50)	4 (100)
<i>Serratia mubidaca</i>	3	1 (33.3)	1 (33.3)	1 (33.3)
<i>Erwinia spp</i>	2	0	0	1 (50)
<i>Acinetobacter spp</i>	4	1 (25)	2 (50)	2 (50)
<i>Escherichia coli</i>	6	1 (16.7)	3 (50)	3 (50)
<i>Pasteurella pneumotropica</i>	4	2 (50)	3 (75)	3 (75)
<i>Enterobacter agglomerans</i>	3	2 (66.7)	0	2 (66.7)
<i>Salmonella spp</i>	2	0	0	1 (50)
<i>Acinetobacter bomanii</i>	3	1 (33.3)	2 (66.7)	2 (66.7)
<i>Enterococcus faecalis</i>	1	0	1 (100)	1 (100)
<i>Pseudomonas putida</i>	2	2 (100)	2 (100)	2 (50)
Total	89	28 (31.5)	31 (34.8)	49 (55.1)

CIP: Ciprofloxacin; TE: Tetracycline; SXT: Trimethoprim-sulfamethoxazole;

#### Common MDRB detected

#### Common type of MDRB according to most common isolated bacteria and those at risk to causing NIs

A total of 119 bacteria (33 different species) were isolated in which 89 (13 species) were selected and tested each one to 18 antibiotics. Of all 89 strains, 11 different species showed more than the half 56 considered MDRB, giving the overall prevalence of 62.9%.

*Serratia mubidaca* (3 strains) and *salmonelle spp* (2 strains) did not show any type of MDRB.

Regarding the general profile of 89 tested strains, MRSA were the mostly detected 57.5 % (30/89), followed by ESBL 10.1% (9/89). The lowest percentage was recorded by VRE and XDR both with 1.1% (1/89). According to the type of MDRB in selected isolated bacteria, all the strains (100%) of 2 species, especially *A. hydrophilas*, and *E. faecalis* were characterized MDRB. However, *S. aureus* strains reported

significant rate of MDRB 84.4% (38/45) and recorded 4.4% (2/45) strains of ESBL. *P. pneumotropica* provided 75% (3/4), as well as the only Gram negative bacilli specie where one strain was resistant to more than three different class of

tested antibiotics included **Carbapenem** (imipenem) and consequently named XDR with 25% (1/4). Table 5 below show a detailed illustration of the above explanation.

Table 5: Common type MDRB profiles of tested bacteria isolates

Name of bacteria	Common type MDRB							Total	
	ESBL n (%)	MRSA n (%)	VRE n (%)	MDR n (%)	XDR n (%)	VRSA n (%)	VRCoNS n (%)	of MDRB n (%)	Isolated bacteria
<i>Staphylococcus aureus</i>	2(4.4)	30(66.7)	0	0	0	6(13.3)	0	38(84.4)	45
<i>Staphylococcus epidermidis</i>	0	0	0	0	0	0	3(30.0)	3(30.0)	10
<i>Aeromonas hydrophilas</i>	3(75.0)	0	0	1(25.0)	0	0	0	4(100.0)	4
<i>Erwinia spp</i>	1(50.0)	0	0	0	0	0	0	1(50)	2
<i>Acinetobacter spp</i>	0	0	0	1(25.0)	0	0	0	1(25.0)	4
<i>Escherichia coli</i>	1(16.7)	0	0	0	0	0	0	1(16.66)	6
<i>Pasteurella pneumotropica</i>	1(25.0)	0	0	1(25.0)	1(25.0)	0	0	3(75.0)	4
<i>Enterobacter agglomerans</i>	0	0	0	1(33.3)	0	0	0	1(33.33)	3
<i>Acinetobacter bomanii</i>	1(33.3)	0	0	1(33.3)	0	0	0	2(66.66)	3
<i>Enterococcus faecalis</i>	0	0	1(100.0)	0	0	0	0	1(100.0)	1
<i>Pseudomonas putida</i>	0	0	0	1(50.0)	0	0	0	1(50.0)	2
NA	0	0	0	0	0	0	0	0	5
Total	9(10.1)	30(33.7)	1(1.1)	6(6.7)	1(1.1)	6(6.7)	3(3.37)	56(62.92)	89

NA: none applicable (*Serratia mubidaca* and *salmonelle spp* without MDRB)

\* Frequency calculated based on type of MDRB detected in each species

ESBL: extended-spectrum beta-lactamase; MRSA: methicillin resistant *Staphylococcus aureus*

VRSA: vancomycin resistant *Staphylococcus aureus*; VRCoNS: vancomycin-resistant coagulase negative *Staphylococcus*

VRE: vancomycin-resistant *Enterococcus*

MDR: multidrug-resistant (to one or more antibiotics to a least two or more antibiotics class as  $\beta$ -lactamin, fluoroquinolone and aminoglycoside except carbapenem)

XDR: extensively drug-resistant (resistant to two or more antibiotics classes including carbapenem)

**Common type MDRB according to hospital facilities**

Out of 56 MDRB detected, MHD shelter the highest percentage 39.3% (22/56) as well as the only hospital where a single strains of XDR was isolated 1.8% (1/56). Followed by NBDH 28% (16/56), both mainly constituting of MRSA,

respectively 25% (14/56) and 12.5 % (7/56). In addition, it's necessary to notice that, ESBL, the most second MDRB type isolated, were observed in three hospitals (NBDH, MHCN and MHD) with equal level 5.4% (3/56). However, MRSA, the predominant MDRB type was detected in all the four hospitals facilities. Details are tabulated in **Table 6** below.

Table 6: Distribution of common MDRB per hospital facilities

Common MDRB	Hospital facilities				Total
	NBDH n (%)	HSJMN n (%)	MHCN n (%)	MHD n (%)	n (%)
ESBL	3 (5.4)	0	3 (5.4)	3 (5.4)	9 (16.1)
MRSA	7 (12.5)	7 (12.5)	2 (3.6)	14 (25)	30 (53.6)
VRE	1 (1.8)	0	0	0	1 (1.8)
MDR	0	3 (5.4)	1 (1.8)	2 (3.6)	6 (10.7)
XDR	0	0	0	1 (1.8)	1 (1.8)
VRSA	4 (7.1)	0	1 (1.8)	1 (1.8)	6 (10.7)
VRCoNS	1 (1.8)	1 (1.8)	0	1 (1.8)	3 (5.4)
Total	16 (28.6)	11 (19.6)	7 (12.5)	22 (39.3)	56 (100)

ESBL: extended-spectrum beta-lactamase;

MRSA: methicillin resistant *Staphylococcus aureus*

VRSA: vancomycin resistant *Staphylococcus aureus*; VRCoNS: vancomycin -resistant coagulase negative *Staphylococcus*

VRE: vancomycin -resistant *Enterococcus*

MDR: multidrug -resistant (to one or more antibiotics to a least two or more antibiotics class as  $\beta$ -lactamin, fluoroquinolone and aminoglycoside except carbapenem)

XDR: extensively drug-resistant (resistant to two or more antibiotics classes including carbapenem)

### Common type MDRB according to hospital units

Of all 56 MDRB detected, Emergency unit was more contaminated 17.9% (10/56) in which MRSA was overlooked 10.7% (6/56). This was followed by Pediatric unit, Medical unit and maternity all with equal level of 16.1%

(9/56). While laboratory reported the lowest with 8.9% (5/56). Likewise, in hospital facilities, MRSA was overlooked and present in all units. Nevertheless, the only one strain 1.8% (1/56) VRE was found in Medical unit the same as single strain 1.8% (1/56) of XDR recorded in Pediatric unit.

**Table 7:** Distribution of MDRB type per hospital units

Common	Pediatric		Operating		Emergency		Maternity	TOTAL
	unit	Medical unit	Theatre	Laboratory	Surgical unit	unit		
MDRB	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
ESBL	3 (5.4)	2 (3.6)	0	1 (1.8)	1 (1.8)	2 (3.6)	0	9 (16.1)
MRSA	4 (7.1)	4 (7.1)	5 (8.9)	3 (5.4)	3 (5.4)	6 (10.7)	5 (8.9)	30 (53.6)
VRE	0	1 (1.8)	0	0	0	0	0	1 (1.8)
MDR	1 (1.8)	0	2 (3.6)	1 (1.8)	1 (1.8)	1 (1.8)	0	6 (10.7)
XDR	1 (1.8)	0	0	0	0	0	0	1 (1.8)
VRSA	0	2 (3.6)	1 (1.8)	0	1 (1.8)	1 (1.8)	1 (1.8)	6 (10.7)
VRCoNS	0	0	0	0	0	0	3 (5.4)	3 (5.4)
Total	9 (16.1)	9 (16.1)	8 (14.3)	5 (8.9)	6 (10.7)	10 (17.9)	9 (16.1)	56 (100.0)

ESBL : extended-spectrum beta-lactamase ;

MRSA: methicillin resistant *Staphylococcus aureus*

VRSA : vancomycin resistant *Staphylococcus aureus* ;

VRCoNS: vancomycin -resistant coagulase negative *Staphylococcus*

VRE: vancomycin -resistant *Enterococcus*

MDR: multidrug-resistant (to one or more antibiotics to a least two or more antibiotics class as  $\beta$ -lactamin, fluoroquinolone and aminoglycoside except carbapenem)

XDR: extensively drug -resistant (resistant to two or more antibiotics classes including carbapenem)

## DISCUSSION

One of the major role of the clinical microbiology laboratory is to provide susceptibility testing data that can serve both as a guide to clinicians for effective therapy and however in the presence of multidrug-resistant organisms (MDROs), can be helpful to initiate early and appropriate investigation to prevent outbreak-related isolates. Moreover, effective targeted prevention has revolutionized the efforts to control the global crisis of NIs and identifies key factors influencing the emergence been of AMR patterns<sup>39- 41</sup>. In addition, as Center of Disease Control (CDC) reported in September 2019, to slow AMR, the Challenge resulted in more than 350 organizations across the globe<sup>42</sup>. Therefore, one critical area may require immediate attention for rational decision in the management of antimicrobial use and infection control policy.

### Prevalence of isolated bacteria

In this study, the overall prevalence of surface bacteria contamination was 50.4% (119/236). This is higher than 45.6% and 43.8% culture-positive bacteria from 57 surface sampled by Costa et al.(2019) in intensive care unit and Worku et al.(2018) from hospital devices of Mizan-Tepi University Teaching Hospital in Ethiopia, respectively<sup>43, 44</sup>. Nevertheless, 50.4% is significantly lower than what was recovered in several studies: Gonsu KH et al. Yaoundé-Cameroon 2015(98.6%), Fotsing et al. Baganté-Cameroon 2020 (95%), Bhatta et al. Pokhara-Nepal 2018 (78%), Aseer et al. Arba Minch-Ethiopia 2019 (71, 7 %), Ndu et al. Enugu-Nigeria 2019 (70.3%) and Afleet al. Abomey-Benin 2019 (65%)<sup>2, 45- 49</sup>. The justification of the difference might be related to various factors including: level of the target setting, target sample, sample size, decontamination systems gaps, but most likely to the type of bacteria isolated (Only Gram positive cocci and Gram negative rods in our study

versus all type of bacteria including Gram positive rods in those several studies. Looking at the type of bacteria found, the 52.1% (64/119) Gram positive cocci and 47.9% (57/119) Gram negative bacilli (Table 1), are consistent with those reported in recent studies<sup>2, 8, 44, 45</sup>. This higher prevalence of Gram-positive bacteria may be explained, by the chemical composition of this bacterial cell envelopes that provide resistance to harsher environmental conditions than Gram-negatives<sup>45</sup>. On the contrary, amongst culture positive infections, Gram-negative organisms predominated (13/16 (81.3%)), similar to Hearn et al. (2017), who prospectively determinate HAI incidence in a Cambodian pediatric referral hospital<sup>50</sup>. Our study also revealed that, *Staphylococcus aureus* was the predominant isolate bacteria 37.8% (45/119). This was similar to the findings of a several studies<sup>45, 46, 48, 51</sup>. Thus, *S. aureus* have relative resistance to common disinfectant and consequently predilection to persist in drying surface. This can be supported by the study of Suleyman et al. (2018). Regarding the survival times of nosocomial pathogens on environmental surfaces, *S. aureus* can resist and live up to 5 years in a dry inanimate surface<sup>52</sup>. Otherwise, our results are contrary to the findings of Agaba et al. (2017) and Nouri et al. (2020) whose predominant isolates was found to be *Klebsiella spp*<sup>53, 54</sup> and also at variance to the work of Gonsu et al.<sup>2</sup> in two referral hospitals Yaoundé-Cameroon. In General, as prominent isolated bacteria *S. aureus* was followed by *E. coli* (5.04% (6/119)) and *Acinetobacter spp* (3.36% (4/119)). A recent study (2018) had shown the predominance of *S. aureus*, *E. coli* and *Acinetobacter species*, in the bacteriological profile of frequently touched objects in a tertiary care hospital of Pokhara, Nepal<sup>46</sup>, as well as these bacteria are well-known and recognized like common nosocomial organisms<sup>53-55</sup>. However, *Staphylococcus* and *E. coli* are facultative aerobes, then ubiquitous, as well as *Acinetobacter spp*, Gram negative and non-fermenting coccobacilli are ubiquitous<sup>45, 56</sup>.

Furthermore, it had been demonstrated that the organisms from surface of frequent contact in hospital are likely to get transmitted to healthcare workers, patients and visitors<sup>9, 43, 46</sup>. In addition, as such, previously in our recent article we suggested that two of our major isolates (*S. aureus* and *Acinetobacter spp*), belongs to the three of the priority pathogens on the WHO list for Global Antimicrobial Resistance Surveillance System (GLASS) reporting, namely *Klebsiella pneumonia*, *Acinetobacter baumannii* and *Staphylococcus aureus* to cause HAIs<sup>57</sup>. Thus, recent studies have demonstrated that our prominent isolated bacteria (*S. aureus*, *Acinetobacter spp* and *E. coli*) propensity to cause outbreaks is facilitated by antimicrobial resistance and their ability to resist desiccation and survive for prolonged periods on multiple environmental surfaces<sup>52, 58-61</sup>.

#### Antibiotic resistance profile of the most prevalent bacterial isolates and those related to NIs

Regarding the global profile of resistance, over the 13 species (89 strains) tested through 18 antibiotics, there was a high level of resistance to most of the antibiotics of “group 1” especially **Penicillin** (amoxicillin (77.5%) and Oxacillin (76.4%)), followed by **3GCephalosporine** (Ceftazidime (74.2%) and **Monobactam** (Aztreonam (70.8%)). Although, observed in the same “group 1”, **Carbapenem** (imipenem (5.61%)) was the lowest except among *Pasteurella pneumotropica* with one strain resistant. Similar results are reported (2020) by the Canadian Nosocomial Infection Surveillance Program where the highest non-susceptibility antibiotics rates were in Penicillin class (ampicillin (43.0%)) and low than 92.8% resistant to Cephalosporine (Cefuroxime) reported by Makanjuola et al (2018), while showed all low resistance for Carbapenem (meropenem (0.4% ), Ertapenem (0.2%)) and despite low than meropenem 21.4% respectively)<sup>62,17</sup>. The main isolate of Enterobacteriaceae family, *E.coli* was 100% sensitive to **Carbapenem** (imipenem) as per Chelliah et al with 100% *E. coli*<sup>63</sup>.

#### Common type MDRB profile of different tested isolated bacteria

Looking at the type of MDRB, the overall prevalence was 62.9% (56/89) in which MRSA were predominantly 33.7% (30/89) detected in all hospital facilities and all units). Our results are higher than 58% obtained by Agada et al.<sup>53</sup> Looking for Nosocomial bacterial infections and their antimicrobial susceptibility patterns among patients in a Ugandan intensive care unit. This difference can be explained by various factors include the target site (single center versus multi center) and sample collection, (clinical & surface sample). However, it is known that environmental bacterial are more likely to develop resistance than those of clinical sample<sup>64</sup>. This can be supported by the study of Tan et al. where the target MDROs were recovered from 79 % of sampled surfaces, predominantly MRSA (74 % of all tested surfaces)<sup>65</sup>. Over 45 *S. aureus* isolates in this study, 66.7% (30/45) were MRSA. Our findings are high than those reported by Bhatta et al in a tertiary care hospital of Pokhara, Nepal, where, on 44 *S. aureus* isolates as predominant bacteria, less than the half 36.3% (16/44) were MRSA<sup>46</sup>. Additionally, likewise it is high compared to 20/47 (44.7%) MRSA detected by Chaoui et al<sup>64</sup> and 31.25% according to Chailliah et al.<sup>63</sup>. Otherwise in our study ESBL was the second predominant MRDB detected 9/56 (16.07%). Despite this, we did not confirm this producing enzyme within a MIC or genetic method, our results can be support by those reported by Teghonon et al. (February 2020), in a study looking for these enzymes in the hospitals of the Littoral region, Cameroon<sup>66</sup>. ESBL in *S. aureus* 4.4%

(2/45) strains are consistent with those of Olutola et al. in 2016, focused on “ESBL detection Antibiotic Susceptibility Profile of *Staphylococcus aureus* Strains Isolated from Surgical Wounds where ESBL phenotypically expressed in two isolates<sup>24</sup>. This can be support by the fact that, Extended Spectrum Beta-Lactamase (ESBL) capable of hydrolyze penicillins and are often located on plasmids that are transferable from strain to strain and between bacterial species<sup>28, 67</sup>. The role of selective pressures in the environment as well as the medical use of antimicrobials together with the interplay of various genetic mechanisms for horizontal gene transfer are considered and well demonstrated<sup>68</sup>. Additionally, a particular ESBL enzyme, called CTX-M, appears to be spreading in the United States and around the world. The CTX-M enzyme can be shared through DNA (genes) between different Enterobacteriaceae species<sup>30</sup>. XDR is a worrying crisis in the fact that Carbapenems are one of the few remaining antibiotics that can treat ESBL-producing germs<sup>30</sup>. As concerned *Salmonella arizona* isolates in this study, didn’t showed any MDR. On contrary, a review and a meta-analysis study carry out in Cameroon on one health perspective reported *Salmonella spp* in animals with a MDR rate of 46.2%<sup>41</sup>. This difference is evident due to the type of specimen (hospital environment & animal). Therefore, this high rate of MDR salmonella should be tackled with more consideration; consequently it can be shared within short or long-term to human. This is support by the fact that drug resistance in gram-negative bacterial hospital-acquired infections (GNB HAIs) has become ubiquitous in recent years<sup>28</sup>. The dry surface biofilms persist for long period and have been shown as a horizontal transmission driver of (MDROs)<sup>43</sup>.

#### Common types MDRB according to hospital facilities and units

MHD shelter the highest percentage 39.3% (22/56), followed by NBDH 28% (16/56), both mainly constituting of MRSA, respectively 25% (14/56) and 12.5% (7/56) as well as the only type present in all hospital facilities and units. Thus, in our previous publication as suggested to explain the distribution of *S. aureus* to all hospitals facilities and units, we expected to observe a higher percentage of MRSA in all hospitals and services due to an outbreak and spread, supported by a study that reported the presence and increase of MRSA in Many hospitals of several countries in Africa<sup>57</sup>. In addition, *S. aureus* have the ability to form biofilm (surface – attached communities of cells) facilitating their persistence in hospital devices and allowing their survival in high antimicrobial concentrations<sup>69</sup>. In fact, *S. aureus* are characterized by the best biofilm production adhesine that include: Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) and Polysaccharide Intercellular Adhesine (PIA) due to the presence of several genes: (Fibronectin-binding protein A (*fnbA*), intracellular adhesine (*icaA*, B, C, D) and accessory gene regulator (*agrI,II,III,IV*))<sup>70</sup>. Consequently, as a healthcare professional, this is the highest risk of horizontal transmission to NIs in risky population, hospitalize patients, healthcare workers, administration staff, and visitors that are expose within themselves<sup>9</sup>.

Comparing the units, the Emergency unit was the MDRBs dominantly contaminated area 17.9% (10/56). It is evident and largely known that many factors influencing the development of antimicrobial resistance in this unit include intensive care, immunosuppressive drugs, over consumption of antimicrobial and irrational use of antimicrobial therapy that contribute to selection pressure<sup>14, 71</sup>. Otherwise, Medical and Pediatric unit were the second contaminated with 16.1% (9/56), as well as the units where single strain of VRE

and XDR both 1.8% (1/56) were recorded. Prolongation stay of patient in these current hospitalization units, influencing selection pressure of antimicrobial resistance and can be explained by the emergence of new resistance strains that contributes to XDR within VRE. This can be supported by the study of Soltani *et al.* (2016) showing that the pediatric unit is the most contaminated area 52%<sup>72</sup>. However, our VRE percentage were low (1.8%) compared to 18.7% and 63% of Juarez *et al.* (2015) and Agegne *et al.* (2018) respectively. *Enterococci*, especially *faecalis* species, are well known antibiotic-resistant opportunistic pathogens commonly recovered from patients who received multiple courses of antibiotics and hospitalized for prolonged periods<sup>36, 73</sup>.

Finally, the results of our study could serve as a timely regional data of hospital surface epidemiological surveillance basis on which preventive strategy of HAIs and AMR should be built accompanied by active methods of supervisions aimed at improving the safety of health personnel, patients, and visitors. Worrying, such higher and various MDRB hospital surface contaminants area consequence of inadequate and ineffective containment and control measures (cleaning, disinfection and sterilization), in Low-and Middle -Income countries. Many factors account for this: waste disposal, poor infrastructure, insufficient equipment, understaffing, overcrowding, intensive and irrational use of antibiotics, lack or poor antimicrobial stewardship programs, paucity of procedure, briefs poor knowledge, attitude and practices of basic infection prevention control measures<sup>50, 74</sup>. Each hospital should be able to develop consistent and locally rapid detection means, recognize trends, report and publish susceptibility patterns to hospital leaders that can help to increase adherence to policies, especially regarding it specific barrier precautions. This will go a long way to reduce the AMR (including HAIs) rate, causing substantial increase in healthcare costs, morbidity and mortality<sup>62</sup>. Globally as it is recommended in the conference report (2017) of "Action on Antibiotic Resistance (ReAct) Africa Annual Conference, stop or slow the AMR crisis required several and combined actions that include: Frameworks, Raising Awareness, Surveillance, Antimicrobial Stewardship, Infection, Prevention, and Control, Monitoring and Evaluation, inventory and mobilization of resources, as well as strengthen one Health approach, national and international collaboration<sup>75</sup>.

## CONCLUSION

Paucity and/or poor data quality are a chronic problem for the flight of MDROs and NIs. Hence timely and accurate data for MDRB in hospital environment are essential for monitoring the spread of those crises and implementing preventive measures. MDRB are present in a significant level in hospital environment small to large, with or without currently perceived problems with antibiotic resistance. The present study highlights the high contamination of frequently touched Hospital surfaces and frequently used medical device by a variety of nosocomial bacteria associated to high distribution of resistant pattern, to commonly used antibiotics in humans, in Littoral region of Cameroon. MDRB occurred to be a current public health problem. All prevalent bacteria isolated were resistance to more than one drug selected in different or similar groups based on their target site and mode of action. Regrettably, in recognition of these burdens, it is essential that all health facilities periodically and carefully monitor and review emerging AMR patterns so as to prioritize adequate strategies, effective and realistic implementation of health facility level policies and guidelines. Also it is evident that dissemination of antibiotic-resistant pathogens in hospital environment can share with both outpatient setting and

animals ecosystem. Therefore, fight against AMR is a global health treat were all hospital personnel, zoologist, botanist and community have their great responsibility. Otherwise, we think it is necessary to pursue this work through studies that can help to understand the reason of the persistence of those various MDRB in various hospital surface by: verifying the contamination of health care givers by those microorganism, evaluating their knowledge and attitude on the infection prevention control and effective use of recommended disinfectant solution on hospital germs in the reduction and elimination of AMR bacteria and NI.

## CONTRIBUTIONS OF THE AUTHORS

MJWT, JCNA, AJ conceived and participated in the design of study. JMW collected, conducted laboratory assay, analyzed the data and drafted the primary manuscript. JCNA and AJ do interpretation and manuscript design. GOE, XFMK and MNN carried out field activities. All authors contributed to the review and approved the final version.

## CONFLICTS OF INTEREST

All the authors do not have any competing interest.

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