Trends of antibacterial interactions in multidrug – resistant isolates: exploring resistance phenotypes in the Ndé division, West-Cameroon

ABSTRACT

Background: Reliable pieces of information concerning bacterial-antibiotic interactions are key assets for therapeutic management of bacterial diseases. **Objective:** The present study aimed at detecting phenotypic characteristics of bacterial resistance in multidrug-resistant isolates recovered from clinical specimens at the "Université des Montagnes" Teaching Hospital. **Methods:** The total of 226 isolates (142 Gram-negative rods and 84 Gram-positive cocci) were subjected to phenotypic screening of resistance mechanisms. All procedural steps were conducted according to standard protocols on bacterial susceptibility to antibiotics. **Results:** Primary pieces of information revealed high rates of resistant isolates, especially with beta-lactams and Trimethoprim/Sulfamethoxazole while Nitrofurantoin and Imipenem were most effective. Amongst Gram-negative rods, 56% expressed one enzymatic resistance mechanism and 12% expressed twoagainst beta-lactams. Also, with extended spectrum beta-lactamases, high level cephalosporinases and inducible cephalosporinases most commonly observed. About 62% and 14% of Gram-positive cocci expressed constitutive and Clindamycin-inducible resistance, respectively. Decreased susceptibility to Ceftriaxone and Penicillin G was also recorded in suspect mutant isolates selected by these antibiotics. Potential synergetic and other antagonistic interactions were evenly detected. **Conclusion:** Overall, the data could represent reliable clue for advocacy about personalized combination therapy, then capacity building for routine affordable susceptibility tests in caretaking.

Keywords: Antibiotics combination, Bacteria, Multidrug-resistant, Resistance mechanisms

1. INTRODUCTION

Detected for the first time in the 1945s, bacterial resistance to antibiotics has become one the major challenges for all health systems across the globe with permanent impact exacerbation. In fact, antimicrobial resistance (AMR) emerged as a serious threat to human health, animal health and environmental health a few decades ago [1-5]. Steady growth of tolerance to antibiotics reflects the great ability known in prokaryotes in general, owing to their cellular organization, their diversity and phylogenetic relatedness that contribute to genome flexibility and fitness [2]. These enabling characteristics are further potentiated by the role of mobile genetic elements from which genes composition can hardly be predicted with diverse conducive environmental variables that cannot be accurately assessed. A few enabling factors relate to selection engine like antimicrobial substances and dissemination facilitators like poor sanitation. Accordingly, the inappropriate use of antimicrobials and microbial exposure to heavy metals for instance, accelerate co-selection and/or cross-resistance that exacerbate the stochastic amplitude of resistance selection in vulnerable ecological systems [4-8].

Antimicrobial resistance represents therefore, a real burden at individual and community levels in terms of therapeutic failures that lead to increased morbi-mortality, extended hospital stays, economic losses, and risks of re-emergence of infections that go beyond control with available therapeutic arsenals [9]. Projection in the 2050s estimates the number of related deaths at about 10 million [1]. In addition to prolonged morbidity and increased mortality, some authors anticipate that exacerbated permanent genetic modifications might emerge as source of alteration in the genetic mapping that will cause significant changes in higher forms of life like those currently known in some metabolic disorders.

As global efforts to contain the AMR pan-threat develop, most research initiatives are centered on routine detection of classical resistant-intermediate-susceptible clinical categories for immediate therapeutic orientation in clinical settings in contexts of resource limitation; while very few use genomics and related tools in resource-enabling countries. These two extremes are endowed with inherent weaknesses that include the absence of guide for individual (personalized) therapeutic combinations for the first and the absence of guide for therapeutic combination and affordability for the second. The present survey targeted the intermediate strategy that relies on phenotypical drug interactions which could therefore orient combination drug-administration in resource-limited

contexts based on resistance mechanisms expressed by specific bacterial isolates. Previous studies in that frame revealed pieces of information on phenotypic diversity amongst isolates recovered from animal farms. It is therefore in the framework addressing bacterial susceptibility/resistance that it was initiate with focus on resistance phenotypes in multidrug-resistant isolates recovered from human in a health facility of West-Cameroon.

2. MATERIAL AND METHODS

2.1 Study design

The present work was a descriptive cross-sectional study conducted at "Université des Montagnes" Teaching Hospital (UdMTH) from September 2019 through June 2020 under the cover of the research authorization referenced N° 2019/052/AED/UdM/CUM and the ethical clearance referenced N° 2019/147/UdM/PR/CIE.

2.2Bacterial population and original specimens

Bacterial population subjected were consisted of isolates collected between September 2019 and May 2020, then cryopreserved in the bank of isolates (-20°C in brain-heart broth supplemented with 20% glycerol) at the UdMTH Laboratory of Microbiology. They consisted of 226 multi-drug resistant isolates recovered from clinical origin and included: *S. aureus*, *Enterobacter*, *E. coli*, *Serratia*, *Streptococcus*, *Klebsiella*, *Citrobacter*, coagulase-negative *Staphylococcus*, *Raoultella*, *Yersinia*, *Aeromonas*, *Pantoea*, *Pseudomonas*, *Enterococcus*, *Chryseomonas*, *Erwinia*, *Hafnia alvei*, *Kluyvera* and *Proteus*. They were recovered from different single-contaminated specimen namely: cerebrospinal fluid (0.4%), pleural fluid (1.3%), stool (2.2%), semen (3.1%), catheter tip (3.5%), blood (4.4%), purulent secretions (15.6%), urethral swab (11.9%), urine (28.8%), and cervical-vaginal swab (29.6%). All specimens were collected and processed according to standard guidelines [10].

2.3Bacterial revivification and antibiotic susceptibility test

Before tests, each cryopreserved isolates were streaked on nutrient agar and incubated 24 hours at 37°C.

Classical control of susceptibility profiles was performed according to standard protocol for disk diffusion on Mueller Hinton prior to specific phenotypic screening [11]. Antibacterial agents used consisted of Amoxicillin/clavulanic acid (20/10 µg), Amoxicillin (25 µg), Aztreonam (30 µg), Cephalothin (30 µg), Cefoxitin (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Erythromycin (15 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg), Oxacillin (5 µg), Penicillin G (10 U), Piperacillin (30 µg) and Trimethoprim/Sulfamethoxazole (1.75/23.25 µg).

2.4Bacterial revivification and antibiotic susceptibility test

A few enzymatic resistance mechanisms against beta-lactams and resistance mechanisms against Macrolide-Lincosamide-Streptogramin B (MLS_B) group were investigated using previous interpretative protocols [11-14] with slight modifications. The interpretive reading scheme was then summarized as presented in table 1.

2.5Other phenotypes

During the reading step of the susceptibility tests, other phenotypes not described in table 1 were observed. They consisted of negative interactions or antagonistic (D-zone shapes and interpreted similar to inducible resistance) and positive interactions or potential synergetic (extended inhibition rugby ball-shaped inhibitory zone between two antibiotic disks, or by an elongation of the inhibition zone of a disk towards another one).

2.6Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Based on previous assertions [15], some ceftriaxone or penicillin-resistant mutants were suspected through the growth of a few colonies around the antibiotic disks (within the inhibitory zone). Those colonies were isolated and resubjected to the minimum inhibitory and bactericidal concentrations tests beside the parental isolates. This essay was performed in liquid medium by macro-dilution for the minimal inhibitory concentration (MIC) and

in solid medium for the minimal bactericidal concentration (MBC) [10]. In each case, the intrinsic potential (bactericidal or bacteriostatic) of the antibiotic was clearly defined [16].

2.7Data analysis

Data were recorded in a Microsoft Excel 2013 spreadsheet program and processed with analysis tools provided by the IBM SPSS Statistics 20 software.

Table 1. Interpretative reading for phenotypic identification of a few mechanisms

Bacterial groups	Mechanisms	Related phenotypes
Enterobacteriaceae	LLPp	AX*; AMC*; CEF**
	HLPp	AX*; AMC*; PRL* CEF*
	IRPp (phenotype TEM)	AX*; AMC*; PRL*
	LLCp	AX*; AMC*; CEF*; FOX*
	HLCp	AX*; AMC*; PRL*; CEF*; FOX* CRO**; CAZ**; ATM**
	ESBLp	AX*; AMC*; PIPL*; CEF*; CRO*; CAZ*; ATM* (confirmed by the occurrence of synergy in a double disk synergy test with CAZ/ATM/CRO placed around AMC)
	Icp	Flattening of zone of inhibition around CAZ towards IMP disk producing a D-shaped during double disc Antagonism test (D-test) with CAZ and IMP
Non-fermentative Gram-negative rods	Penicillinase production	PRL*/**; CTX*/**; ATM*/**
	LLCp	CTX*
	HLCp	PIPL*; CRO*; ATM*; CAZ*
	ESBLp	AX*; AMC*; PRL*; CEF*; CRO*; CAZ*; ATM* (confirmed by the occurrence of synergy in a double disk synergy test with CAZ/ATM/CRO placed around AMC)
	ICp	Flattening of the inhibition zone around CAZ towards IMP disk producing a D-shaped during double disc Antagonism test (D-test) with CAZ and IMP
Gram-positive cocci (double disc antagonism test (D-test) with CL et ER)	Active efflux pump	MS phenotype = ER* and CL^+ (with a circular zone of inhibition around Clindamycin)
	Production of methylase enzymes by the bacteria in the presence of inducer-ER (the enzyme modifies the antibiotic target site on ribosome)	$iMLS_B$ phenotype = ER*, CL^+ (with flattening of zone of inhibition around Clindamycin towards Erythromycin disk producing a D-shaped)
		iL phenotype = ER^+, CL^+ (with flattening of zone of inhibition around Clindamycin towards Erythromycin disk producing a D-shaped)
	Expression of MLSB gene mutant resistant	$cMLS_B$ phenotype = ER*, CL* (with a circular zone of inhibition around Clindamycin)
	Expression of MLSB gene mutant resistant and methylase enzymes synthesized by the bacteria in the presence of inducer-ER	$cMLS_B$ + iL phenotype = ER*, CL* (with flattening of zone of inhibition around Clindamycin towards Erythromycin disk generating a D-shaped)

LLPp : Low level penicillinase production; HLPp : High level penicillinase production; IRPp : inhibitor resistant penicillinase production; LLCp: Low level cephalosporinase production ; HLCp: High level cephalosporinase production; ESBLp: Extended spectrum b-lactamase production; ICp: Inducible cephalosporinase production; MLS_B: Macrolide-Lincosamide-Streptogramin B; AX: Amoxicillin; AMC: Amoxicillin/clavulanic acid; CEF: Cephalothin; PRL: Piperacillin; FOX: Cefoxitin; CRO: Ceftriaxone; CAZ: Ceftazidime; ATM: Aztreonam; CL: Clindamycin; ER: Erythromycin; *: isolate is resistant to antibiotic; **: isolate is moderately resistant to antibiotic; */**: isolate is resistant or moderately resistant to antibiotic; $^+$: isolate is susceptible to antibiotics; i (iL, $iMLS_B$): inducible resistance; c ($cMLS_B$): constitutive resistance

3. RESULTS

3.1 Bacterial susceptibility to antibiotics

Overall view revealed very low susceptibility rates of the bacterial populations subjected to the antibiotics used and high frequency of multidrug-resistant bacteria. The distribution of clinical categories recovered was summarized as shown in table 2.

Table 2. Distribution of clinical categories per bacteria types

Antibiotics	*E. coli* (n=32)			*Enterobacter* spp. (n=34)			*Serratia* spp. (n=26)			*Klebsiella* spp. (n=18)			Others GNR (n=32)			*Staphylococcus* spp. (n=56)			*Streptococcus*spp. (n=28)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Amox./cla. acid (20/10 µg)	9	0	91	6	0	94	12	0	88	11	0	89	9	0	91	-	-	-	-	-	-
Amoxicillin (25 µg)	22	0	78	15	0	85	23	0	77	0	0	100	6	0	94	-	-	-	-	-	-
Aztreonam (30 µg)	31	6	63	32	3	65	27	0	73	22	17	61	38	6	56	-	-	-	-	-	-
Cephalothin (30 µg)	25	6	69	38	9	53	27	8	65	39	6	56	16	3	81	-	-	-	-	-	-
Cefoxitin (30 µg)	3	0	97	0	0	100	0	0	100	6	0	94	0	3	97	-	-	-	-	-	-
Ceftazidime (30 µg)	6	19	75	6	12	82	8	8	85	22	11	67	6	9	84	-	-	-	-	-	-
Ceftriaxone (30 µg)	19	28	53	41	3	56	35	19	46	22	11	67	38	13	50	-	-	-	-	-	-
Ciprofloxacin (5 µg)	34	3	63	18	6	76	35	15	50	28	11	61	59	6	34	-	-	-	-	-	-
Imipenem (10 µg)	53	38	9	59	24	18	77	4	19	67	22	11	59	13	28	-	-	-	-	-	-
Nitrofurantoin (300 µg)	72	0	28	71	3	26	54	0	46	56	11	33	63	3	34	-	-	-	-	-	-
Piperacillin (30 µg)	47	16	38	38	21	41	42	19	38	0	0	100	38	19	44	-	-	-	-	-	-
Gentamicin (10 µg)	56	6	38	41	12	47	46	0	54	50	6	44	66	3	31	54	0	46	46	0	54
Norfloxacin (10 µg)	41	16	44	21	3	76	46	15	38	44	0	56	63	3	34	61	0	39	50	0	50
Trim/Sulf (1.75/23.25 µg)	28	0	72	24	0	76	35	4	62	6	0	94	34	0	66	39	16	45	25	4	71
Clindamycin (2 µg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	20	64	12	0	88
Erythromycin (15 µg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	7	82	11	7	82
Levofloxacin (5 µg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	64	0	36	50	0	50
Oxacillin (5 µg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	95	7	0	93
Penicillin G (10 U)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	95	7	0	93

Amox./cla. Acid: Amoxicillin/clavulanic acid; Trim/Sulf: Trimethoprim/Sulfamethoxazole; GNR: Gram-negative rods; S: Frequencies of susceptible isolates (%); I: Frequencies of intermediate clinical category isolates or moderate resistant isolates; R: Frequencies of resistant isolates (%)

Amongst Gram-negative rods (GNR) in table 2, it was found that, for 5 antibiotics (approximately 36%), resistance rates were larger than 70% in each bacterial subgroup. The least effective agents included Cefoxitin, Amoxicillin, Amoxicillin/clavulanic acid, Ceftazidime and Trimethoprim/Sulfamethoxazole; while the most effective were Nitrofurantoin and Imipenem. Susceptible rates in Gram-positive cocci (GPC) were also very low with the salient example observed with Penicillin G, Oxacillin and Erythromycin.

3.2Resistance mechanisms in GPC with focus on the MLS_B family

All resistance mechanisms targeted in GPC concerning members of the MLS_B family were observed. The related phenotypic distribution is displayed in table 3.

Table 3. Resistance phenotype to the MLS_B family

Resistance phenotype to the MLS_B family	*S. aureus*	Non-*aureus Staphylococcus*	*Streptococcus* spp.	Total
iL phenotype	1	0	1	2
MS phenotype	3	1	2	6
$iMLS_B$ phenotype	1	0	0	1
$cMLS_B$ phenotype (without iL phenotype)	27	2	14	43
$cMLS_B$ + iL phenotype	5	0	4	9
Total	37	3	21	61
Total resistance phenotype frequency in each group	73%	60%	84%	74%

iL phenotype: susceptibility to Erythromycin and Clindamycin with inducible resistance to Clindamycin; MS phenotype: resistance to Erythromycin and susceptibility to Clindamycin; iMLSB phenotype: resistance to Erythromycin and susceptibility to Clindamycin with inducible resistance to Clindamycin; cMLSB phenotype: constitutive resistance to MLS_B family; cMLSB + iL phenotype: constitutive resistance to MLS_B family and inducible resistance to Clindamycin.

Overall picture from table 3 indicates that the majority of GPC expressed resistance to antibiotics belonging to the MLS_B family of drugs, and that constitutive resistance (62%) and inducible resistance to Clindamycin (14%) predominated.

3.3Resistance mechanisms in GNR with focus on members of the beta-lactam family

Among the GNR, 96 isolates (68%) expressed the investigated mechanisms. Out of these, a single mechanism was detected in 82% (table 4) and two in the others (table 5).

From tables 4 and 5, it appears that extended-spectrum beta-lactamases, high-level cephalosporinase, and inducible cephalosporinases expression were the most common enzymatic mechanisms in Gram-negative bacterial population.

3.4Potential synergistic combinations and other inducible resistance phenotypes

Thirty-six phenotypes reflected potential synergistic effects with antibiotic and 07 were associated to inducible resistance. In GPC subgroup, a synergistic potential was commonly recorded with Trimethoprim/Sulfamethoxazole-Erythromycin in 08 erythromycin-resistant isolates. Table 6 provides additional related details recorded in GNR.

According with table 6, the positive (potential synergetic) interactions rate is larger than the negative (inducible resistance). Overall view also reveals that a larger proportion of positive interactions involves combinations for which the isolates were either susceptible to both or resistant to both antibiotics on one hand, or susceptible to one and resistant to the other on the other hand.

Table 4. Distributions of GNR with single enzymatic mechanism of resistance against beta-lactams

Resistance Mechanisms	Subjected isolates														
	Aeromonas spp.	*Chryseomonas* spp.	*Citrobacter* spp.	*E. coli*	*Enterobacter* spp.	*Hafnia alvei*	*Klebsiella* spp.	*Kluyvera* spp.	*Pantoea* spp.	*Proteus* spp.	*Pseudomonas* spp.	*Raoultella* spp.	*Serratia* spp.	*Yersinia* spp.	Total
LLPp	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
IRPp	-	-	-	-	4	-	-	-	-	-	-	-	1	-	5
LLCp	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2
HLCp	-	-	2	4	3	1	-	1	1	-	-	2	5	-	19
ESBLp	1	1	2	6	9	-	6	-	-	1	2	1	7	-	36
Icp	-	-	-	4	5	-	2	-	1	-	-	-	3	1	16
Total	3	1	4	14	21	1	9	1	2	1	2	3	16	1	79

LLPp: Low level penicillinase expression; IRPp: inhibitor resistant penicillinase expression; LLCp: Low level cephalosporinase expression; HLCp: High level cephalosporinase expression; ESBLp: Extended spectrum b-lactamase expression; ICp: Inducible cephalosporinase expression; -: not observed

Table 5. Distributions of GNR with two enzymatic resistance mechanism of against beta-lactams

Resistance Mechanisms	Subjected isolates								
	Aeromonas spp.	*Citrobacter* spp.	*E. coli*	*Enterobacter* spp.	*Klebsiella* spp.	*Pseudomonas* spp.	*Raoultella* spp.	*Serratia* spp.	Total
ICp+LLPp	-	-	-	-	-	1	-	1	2
ICp+IRPp	-	-	1	-	-	-	-	-	1
ICp+LLCp	-	-	-	-	-	1	-	-	1
ICp+HLCp	-	1	2	-	-	-	-	1	4
ICp+ESBLp	1	-	3	1	2	-	1	1	9
Total	1	1	6	1	2	2	1	3	17

LLPp: Low level penicillinase expression; IRPp : inhibitor resistant penicillinase expression; LLCp: Low level cephalosporinase expression; HLCp: High level cephalosporinase expression; ESBLp: Extended spectrum b-lactamase expression; ICp: Inducible cephalosporinase expression; -: not observed

Table 6. Others antibiotic interactions against GNR

Interactions	Antibiotics	*Aeromonas* spp. (n = 1)	*Serratia* spp. (n = 2)	*Yersinia* spp. (n = 1)	*Citrobacter* spp. (n = 3)	*E. coli* (n = 8)	*Enterobacter* spp. (n = 5)	*Hafnia alvei* (n = 1)	*Klebsiella* spp. (n = 5)	Total of interactions
PSC	**CN$^{\alpha}$ and CIP$^{\beta}$**	-	-	$1^{a(+/*)}$	-	-	-	-	-	1
	AX$^{\alpha}$ and CAZ$^{\beta}$	-	-	-	-	-	$1^{(+/*)}$	-	$1^{(*/**)}$	2
	CAZ$^{\alpha}$ and IPM$^{\beta}$	-	-	-	-	$1^{(**/+)}$	$1^{(*/+)}$	-	-	2
	Trim/Sulf$^{\alpha}$ and FOX$^{\beta}$	$1^{a(*/*)}$	-	-	-	-	-	-	$1^{a(*/*)}$	2
	IMP$^{\alpha}$ and PRL$^{\beta}$	-	-	-	$1^{(+/*)}$	$1^{(**/*)}$	-	-	-	2
	PRL$^{\alpha}$ and ATM$^{\beta}$	-	-	-	-	-	$1^{(+/*)}$	-	$1^{c(*/*)}$	2
	CN$^{\alpha}$ and F$^{\beta}$	$1^{a(+/+)}$	-	-	-	$1^{a(+/+)}$	$1^{(+/+)}$	-	$1^{b(*/*)}$	4
	Trim/Sulf$^{\alpha}$ and CN$^{\beta}$	-	-	$1^{a(*/+)}$	-	$1^{a(+/+)}+1^{b(+/+)}$	-	-	$1^{(+/+)}+1^{a(*/*)}$	5
	Trim/Sulf$^{\alpha}$ and F$^{\beta}$	$1^{a(*/+)}$	$1^{(+/+)}$	-	-	$1^{(*/*)}+1^{(*/+)}+1^{(*/*)}+1^{b(+/+)}$	-	-	$1^{a(*/+)}+1^{b(*/*)}$	8
IR(&/@)	**CAZ$^{@}$ and AMC$^{\&}$**	-	-	-	-	$1^{(+/*)}$	-	-	-	1
	AMC$^{@}$ and PRL$^{\&}$	-	-	-	-	-	-	$1^{(*/+)}$	$1^{c(*/*)}$	2
	IMP$^{@}$ and CRO$^{\&}$	-	$1^{(**/+)}$	-	$2^{(*/**)}$	-	$1^{(*/*)}$	-	-	4
Total of interactions		3	2	2	3	10	5	1	9	35

PSC: Potential Synergistic Combination; IR(&/@): Inducible Resistance by @ to &; CN: Gentamicin; CIP: Ciprofloxacin; AX: Amoxicillin; CAZ: Ceftazidime; IPM: Imipenem; Trim/Sulf: Trimethoprim/Sulfamethoxazole; FOX: Cefoxitin; PRL: Piperacillin; ATM: Aztreonam; F: Nitrofurantoin; CRO: Ceftriaxone; AMC: Amoxicillin/clavulanic acid;

$^{(xx/xxx)}$: isolate clinical category against the first (α/@) antibiotic in interaction/isolate clinical category against the second (β/&) antibiotic in interaction;

*: isolate is resistant; **: isolate is moderately resistant; $^{+}$: isolate is susceptible; $^{a, b, c}$: indicate interactions observed in the same isolate

3.5MIC and MBC in suspected resistant mutant isolates

The MIC and MBC values recorded from alleged resistant mutant isolates revealed significant variations compared with those observed in the parental's. More related details were summarized and displayed in table 7.

Table 7. MIC, MBC and MBC/MIC in suspected antibiotic resistant mutant isolates and parental strains

Bacteria	Types	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC	Antibiotic effect
***Kluvera* spp.**	sRM	256	1024	4	Bacteriostatic
	P	32	16	2	Bactericidal
Raoultella omithinolytica	sRM	128	512	4	Bacteriostatic
	P	8	32	4	Bacteriostatic
Cytrobacter duversus	sRM	8	64	8	Bacteriostatic
	P	4	8	2	Bactericidal
Enterobacter cloacae	sRM	128	256	2	Bactericidal
	P	64	128	2	Bactericidal
***Enterobacter* spp.**	sRM	128	256	4	Bacteriostatic
	P	64	128	2	Bactericidal
Citrobacter youngae	sRM	128	512	4	Bacteriostatic
	P	64	512	8	Bacteriostatic
***Erwinia* spp.**	sRM	128	1024	8	Bacteriostatic
	P	32	64	2	Bactericidal
Proteus mirabilis	sRM	16	64	4	Bacteriostatic
	P	8	16	2	Bactericidal
E. coli	sRM	256	512	2	Bactericidal
	P	128	256	2	Bactericidal
Klebsiella oxytoca	sRM	128	512	4	Bacteriostatic
	P	32	64	2	Bactericidal
S. aureus	sRM	4	32	8	Bacteriostatic
	P	0.25	2	8	Bacteriostatic
S. aureus	sRM	4	16	4	Bacteriostatic
	P	2	16	8	Bacteriostatic
***Streptococcus* spp.**	sRM	2	16	8	Bacteriostatic
	P	1	2	2	Bactericidal

P: parental isolate; sRM: suspected resistant mutant isolate

Table 7 revealed great variations between the parental and resulting selectedresistant mutantssuspect. It was observed that the inhibitory potentials (MIC) of antibiotics were several times higher in suspectedresistant mutant isolates compared with values their parents. Overall, bactericidal potentials (MBC) of antibiotics were 2 to 64 times lower in these suspect isolates. From this table, it also appears that for the overwhelming proportion of resistant mutants, a bacteriostatic effect of antibiotics was observed instead of the bactericidal action globally recorded in original isolates.

4. DISCUSSION

Characteristics of bacterial resistance fluctuate in time and space on an ascending trend, demanding relentless efforts in the management policies to control bacterial diseases in plants, animals and humans [5]. The present survey on resistance mechanisms in multidrug-bacteria yielded valuable findings likely to orient and/or support antibiotic therapy policies.

Susceptibility/resistance-related profile control data analysis globally revealed high multidrug-resistance rates as predicted and could even be anticipated amongst clinical isolates in the setting. In fact, communities receiving care at the UdMTH are mostly West-residents where previous data consistently reported reduced susceptibility rates of bacterial in healthcare facilities [17-20]. The highest rates were recorded with beta-lactams and

Trimethoprim/Sulfamethoxazole, still consistent with previous findings [20,21]. For their availability and their affordability in fact, beta-lactams and Trimethoprim/Sulfamethoxazolewere found as the most common drugs used by the local populations [12,20,22]. Their route of administration (oral) further motivates the uncontrolled use trend instigated by their easy access without medical prescription [22]. To the healthcare view, these and other related factors promote the inappropriate undermining global efforts in the struggle against infectious diseases (IDs) caused by bacterial. Inappropriate use of antibiotics and other selection drivers is steadily on the rise and may cause sufficient pressure that exacerbates selection of resistant isolates beyond expectation [5-8,20,22,23] with the growing population and increased healthcare needs.

During the present investigation, Nitrofurantoin and Imipenem were found to be the most effective drugs. This effectiveness could be, at least partially due to the fact that these antibiotics are not common in caretaking like the others that fall into the first-line list of drugs as discussed above. In addition, not only they are more expensive, administration of Imipenem is also performed by parenteral route. Resistance expression against these antibiotics could thus be due to the emergence and spread of selected genetic determinants through co- and/or cross-selection processes caused by other drivers [5]. An overall decrease in drug effectiveness was also recorded. For these antibiotics, the general susceptibility trends was 63%, down from the 75%, reported during a similar study conducted for twelve months on GNR recovered from clinical specimens in the same healthcare facility in 2016 [20], consistent with the resistance increase assertions. This decrease effectiveness could also reflect an increase in the use of these and like agents in case of resistant to the above first-line drugs, or develop as consequence of bacteria host diversity which favors variation of stochastic genetic combinations in the microbial world [5-8]. Therefore, this finding primarily emerges as a warning signal on the use of members of the all above drugs' families in therapy. Otherwise, their use should be monitored as accurately as possible in order to preserve the inherent effectiveness in time and space.

More precisely in the local community context, these results could be understood with reference to the "One Health" paradigm which sustainably advocates permanent monitoring of interdependence between human health, animal health and environmental health [24]. Previous study disclosed anti-infective agent-driven flows of bacteria and their genes into the environment [2-4], in addition to the increased bacterial survival potential [5]. In the West region of Cameroon, animal husbandry and crop production were shown to contribute to resistance upsurge observed in bacteria [25-28].

Throughout the present investigation, it was also observed that close to 70% of GNR expressed enzymatic mechanisms of resistance to beta-lactams (largely high-level that included extended-spectrum beta-lactamases, high-level cephalosporinases and inducible cephalosporinases). Some GNR isolates further expressed inducible cephalosporinases and another anti-beta-lactam enzyme. In GPC, constitutive resistance was common against members of the Macrolide-Lincosamide-Streptogramin B antibiotics family, in addition to inducible resistances. Accordingly, therapeutic failure could be anticipated with about 62% of isolates expressing constitutive resistance and 14% expressing inducible resistance to Clindamycin; endowed with higher risk of mutation selection *in vivo.* Antibacterial agents belonging to the MLS_B family are frequent alternative in therapy for infections caused by GPC bacteria. But bacteria resistance against members of this group is reported to increase steadily [14,29]. Detection of the combined "constitutive resistanceand Clindamycin-inducible resistance" phenotype could therefore, justify the need for investigations through inducible resistance to Clindamycin in constitutive resistance isolates and strains. These outstanding findings represent additional evidence for acquisition of resistance traits upon exposure to selection drivers with respect to the inherent natural resistant phenotype expected.

Amongst the alleged resistant-mutant isolates, the inhibitory and bactericidal potentials of antibiotics (MIC and MBC, respectively) significantly decreased and matched with decreased antibacterial potential on subjected isolates [5,7], substantiating thereby, the fundamentals of selection processes which potentiate at lower concentrations. Beyond the current findings and for future needs, the presence of these suspected or confirmed mutants brings to light for clarification the use of antimicrobials that are contextually incriminated in their selection and the appropriate concentrations that should be used to cause lethal effect during case management; acknowledging that in infections caused by a resistant mutant, very high concentrations are required (theoretically). Practically, these concentrations are not known and can hardly (if ever) be predicted. If they were predicted, they would largely be beyond the acceptable threshold for therapeutic toxic doses. Otherwise, some authors admitted that exposed bacterial populations usually emerge in the mutant selection windows, with reference to a zone where sublethal concentrations of antibiotic results in increased resistance

risk [5-8,23]. Data from the present investigation are not only consistent with that assertion, they further display high level selection as well. Back to Nitrofurantoin and Imipenem potentials discussed above and acknowledging the multidrug-resistance profiles of subjected isolates, it could reasonably be anticipated that they act and inactivate several resistance mechanisms at once.

One of the therapeutic strategies against these multi-drug resistant bacterial infections is the use of drug combinations for therapy [30,31]. Positive interactions between antibiotics were observed. Most commonly they included Gentamicin-Nitrofurantoin, Trimethoprim/Sulfamethoxazole-Gentamicin and Nitrofurantoin-Trimethoprim/Sulfamethoxazole. Conclusion from the present survey could guide orientation of contextual combination therapy instead of the current probabilistic monotherapy orcombination therapies in force in healthcare governing bodies throughout most contexts, especially in resource-limited areas where biological arguments for diagnosis are not available or are hardly affordable. Combinations such as those reported in the present survey or combinations including only one of these antibiotics have shown positive or synergistic effects in other contexts. Some examples include Ciprofloxacin-Gentamicin that produced synergistic effects on Ciprofloxacin-resistant *Salmonella* Typhi [31]; aminoglycoside-based combinations on carbapenem-resistant *Enterobacteriaceae* [32] and Nitrofurantoin-Amikacin on multidrug-resistant uropathogenic *E. coli* [33]. Piperacillin-Aztreonam also proved effective and non-toxic as first-line for therapy in pediatric patients with malignant neoplasm and febrile conditions associated with neutropenia [34]. Further investigations are, however, required to determine the useful concentration of each member of the combination, with minimized risk of selection [5,35] to avoid exacerbation of the phenomenon in isolates that express moderate resistant phenotype to the antibiotic when it is tested alone.

The negative antibiotic interactions (inducible resistance) observed in some cases are also guides for orientation in drug administration during combination therapy. This does not rule out, however, explorations initiatives to better understand the phenomenon. Shortly and in other words, drug combination should be as much as possible personified based on arguments from phenotype-based susceptibility tests with expand beyond phenotypes that are currently known.

The overall related policy should include actions through identification of contextual determinants involved in the emergence and spread of resistant bacteria with the One Health perspective that advocates holistic view and stakeholders' permanent information sharing in controlling IDs at the preventive step; recognizing that about 90% of bacterial populations are recovered from frequently subjected specimens.

5. CONCLUSION

The present study on antibiotic interaction in antibacterial essays revealed that beta-lactams and Trimethoprim/Sulfamethoxazole combinations were the least effective on one hand, and that Nitrofurantoin and Imipenem were most effective on the other. Many GNR expressed extended-spectrum beta-lactamases, high-level cephalosporinases and inducible cephalosporinases which could undermine therapy with beta-lactams. Constitutive resistance against the Macrolide-Lincosamide-Streptogramin B group was also recorded in the overwhelming majority to GPC subjected. Overall, positive and negative interaction evenly reported represent reliable clue to advocate personalized combination therapy, then capacity building for routine affordable susceptibility tests in caretaking.

DATA AVAILABILITY

Data associated with this work were not deposited into a publicly available repository. All the data of this work are present in this paper.

Ethical Approval:ethical clearance referenced N° 2019/147/UdM/PR/CIE.

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