Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 11(1): 131-143, February 2017

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Multicenter study on antibiotic susceptibility/resistance trends in the western region of Cameroon

Reine Flaviette TCHAPDIE NGASSAM[1,2], MEGNE TANTSE[1,2],
Pierre René FOTSING KWETCHE[1,2*], Dorice Pascale NOUKELA NOUMI[1,2],
Jonas KOUAMOUO[1,2], Josué SIMO LOUOKDOM[1,2,3],
Sandrine GAMWO DONGMO[1,2,3], Serge Honoré TCHOUKOUA[1,2],
Blandine Pulchérie TAMATCHO KWEYANG[4] and Kaba KOUROUMA[1,2]

[1] Higher Institute of Health Sciences, Université des Montagnes; Bangangté, Cameroon.
[2] Laboratory of Microbiology, Cliniques Universitaires des Montagnes; Bangangté, Cameroon.
[3] Faculty of Science, University of Dschang; Dschang, Cameroon.
[4] Faculty of Science, University of Yaoundé I; Yaoundé, Cameroon.
[*] Corresponding author; E-mail: prfotsingk@gmail.com / prfotsing@cum.aed-cm.org
Tel: +237 699619786 / +237 77018171

ABSTRACT

In the global frame aiming at assessing bacterial susceptibility for safer and cost-effective healthcare, the present survey was conducted in three hospitals: Bafoussam Regional Hospital (BRH), Bangwa Protestant Hospital (BPH) and Bangangté District Hospital (BDH). Sampling was performed by fingerprinting on culture media and swabbing of hospital devices or surfaces. Wards of interest included: Pediatrics, Medicine, Operating Theater, Intensive Care Unit, Maternity, and, in the BDH, Laboratory in addition. Culture, isolation, identification and susceptibility tests were conducted according to standard guidelines and assigned contamination rates. Seventeen antibacterial agents were chosen and included representatives of major families of antibiotics used in Cameroon. Analysis of 238 specimens revealed 90%, 86% and 92% contamination rates in the BRH, BPH, and BDH, respectively. On healthcare provider's hands, the respective rates were 63%, 100% and 91%. *Bacillus* and *Staphylococcus* were predominant bacteria types in all settings (BPH: 92%; BDH: 86%; BRH: 81%). Susceptibility profiles indicated high resistance rates and clonal distribution in all settings; and most reduced susceptibility with common drugs. Further investigations and previous works alleged drug use and basic hygiene as crucial in addressing resistance issues for safer care. This would be achieved with State support to public and private institutions.

Keywords: Hospital contamination, *Bacillus*, *Staphylococcus*, susceptibility profile, West Cameroon.

INTRODUCTION

Low-income and technologically advanced countries throughout the world currently face the double challenge of communicable and non communicable diseases that were previously overlooked in many areas. This became obvious thanks to advances in medical technologies and international

2868-IJBCS

DOI: http://dx.doi.org/10.4314/ijbcs.v11i1.11

cooperation. Though a global concern, the burdens associated are higher in developing countries due to several factors in connection with geography, demography, socio-economy, and related (Jasovsky et al., 2016; Laxminarayan and Chaudhury, 2016). Beyond the classic high mortality and morbidity rates recorded with communicable diseases in developing countries, the global burden in these areas actually encompasses the financial aspects related to prolong hospitalization and/or caretaking of Hospital Acquired Infections (HAIs) caused by resistant microbial strains (Bhutta et al., 2014; Jasovsky et al*.,* 2016). In fact, infectious diseases (IDs) impede human development in low-income communities and impact basic frames like education and health in general. In the pre-antibiotic era, IDs used to be serious threats because of lack or insufficient knowledge on microbial life and variants of host-microbe interactions.

Inspired by the findings of Louis Pasteur and Robert Koch in the 1860s and Fleming in the 1929s, better understanding was acquired to cope with bacteria. Improving knowledge on microbial life and host-microbe interactions became a permanent challenge for IDs investigators in connection with the stochastic feature of outcomes emerging as combinatory events between inherent and acquired attributes from both partners (genome flexibility, other forms of life and host defense, for instance). This interest in bacterial-host interactions has consistently shifted over time, extending from bacteria as "professional pathogens", "antibiotic resistant professional pathogen", "resistant opportunistic IDs etiologies" to the famous current "resistant environmental microbial flora" recognized as potent threats in healthcare facilities (Barber, 1961; Spinosa et al*.,* 2000; Tagoe et al*.,* 2011; Martínez and Baquero, 2014; Baquero et al*.,* 2015; Nurain et al*.,* 2015; Simo Louokdom et al*.,* 2016; Noukela Noumi et al*.,* 2016). Otherwise, it becomes clearer in the light of advances in mastering host-parasite interactions that persistent relationship and fitness are tributary to several constraints which interact to provide conditions for sustainable co-existence. In this, and in order to express the inherent ability enabling perpetuation, all organisms respond to ranges of environmental signals that might vary both in types and magnitudes (Baquero et al*.,* 2015; Teillant, 2015; Laxminarayan and Chaudhury, 2016). Disrupted harmony generally results from unusual signals that an organism receives from the surrounding multi-factorial systems. This type of interactions have been important in microbial evolution, in connection with the genome litheness that regularly adapt to changes and more specifically, to bacteria in which the clearer picture depicts a putative common "tool sets" from which all species might select the genetic traits they require for survival. In this, antibiotic resistance (AbR) is one of the most relevant features that enable materialization of adaptive genetic changes for better fitness in microbial life. AbR was first noticed by the end of the Second World War in the 1840s with resistance to Penicillin. Resistance to inhibitor-resistant Penicillin (Methicillin) which later emerged in the 1961s (Barber, 1961) further drew awareness on the reduced efficacy of previous potent antibacterial agents around the time. It was however, only by the 1980s that AbR, recognized as threat for global health, excited greater interests on bacteria and AbR. Nowadays, it is proven that this natural phenomenon is typically amplified by human activities through misuse of antimicrobials in human health, animal husbandry and crops production (Smith et al*.,* 2014; Islam, et al*.,* 2014; Teillant, 2015; Ramanan and Ranjit, 2016). The impact of chemicals like heavy metals from industries through untreated or partially treated wastes released in the environment could also be alleged (Matyar et al., 2008).

Mastering IDs has long been associated with control of known pathogenic types. Often omitted in AbR investigations, it is currently admitted that endogenous and environmental microbial flora might play significant roles in selecting, maintaining and spreading resistance traits (Spinosa et al., 2000; Tagoe et al., 2011; Oluyege et al., 2015; Simo Louokdom et al., 2016) undermining thereby, all infection prevention and control strategies in healthcare systems.

Next to previous works and within the global framework that aims at investigating novel approaches in tracking, tracing and controlling AbR in order to mitigate resistance effect in healthcare facilities, the present work was conducted in three hospitals in which bacterial flora from healthcare workers and hospital environmental items were studied. This investigation conducted on common opportunistic and environmental bacteria will serve as groundwork to initiate and implement a stewardship program with holistic database.

MATERIAL AND METHODS

Ethical consideration, study population and collection sites

Prior to field works, the go-ahead was obtained from the Université des Montagnes Ethical Committee under reference number 2015/074/UdM/PR/CAB/CIE. Authorizations were also obtained from the Regional Hospital of Bafoussam (high-ranked State Institution), the Protestant Hospital of Bangwa (Church property) and the District Hospital of Bangangté (low-ranked State Institution). Typically rural (Bangwa), semi-urban (Bangangé) and urban (Bafoussam), the general host populations share common traditional family and community values like assistance and physical presence by the side of a sick member. In these areas, traditional medicine practices and auto-medication with conventional drugs are so enrooted that most of the times only serious cases are taken to the hospital. Clean water resources are scarce and most people in these areas rely on wells and streams that also commonly serve as rubbish dump for local wastes. This scarcity extends to human resources for health and contrasts with fast growing populations accompanied by large networks of drugs from doubtful origins in all settings (unpublished). Specimens collected from inanimate items (surfaces and medical devices) and healthcare workers who consented to participate were processed at the Université des Montagnes Teaching Hospital's Infectious Diseases Laboratory. The number of collection sites per ward depended on the number of potential bacterial reservoirs that could be identified. Generally for this purpose, one item was selected out of three.

Sampling, culture, isolation, identification and susceptibility testing

Sampling

After all necessary ethical and administrative requirements were fulfilled; samples were collected as follows: first, healthcare workers fingers were printed on appropriate agar in Petri dishes and secondly, humidified sterile swabs were used to rub approximately 2.5x4 cm^2 surface areas on inanimate surfaces and hospital devices. All collected materials, preserved in brain-heart infusion agar and Petri dishes were kept in refrigerated containers (4-8 °C) and immediately conveyed to the Laboratory for analysis.

Culture, isolation and identification

Culture and isolation were conducted on MacConkey agar (Liofichem®) for Enterobacteriaceae, Columbia agar (Liofichem®) with 5% sheep blood supplement and chocolate agar for fastidious bacteria like *Streptococci*; and mannitol-salt agar (Liofichem®) for *Staphylococci*. For non fastidious bacteria, incubation was performed aerobically for 18-24 h at 37 °C. Fastidious bacteria were searched for after incubation for 24-48 h under 5% CO_2. When incubation was complete, the morphology of bacterial colonies was used for presumptive identification. Then, all suspected colonies were characterized on the basis of their biochemical properties, according to the standard guidelines (REMIC, 2007). A culture was regarded as positive (for high bacterial density on the item) when a total count of at least 8 CFU/cm^2 was obtained (modified Vandini et al., 2014). For identification and susceptibility tests, reference bacterial strains used in quality control were *S. aureus*: ATCC 29213, *S. aureus:* QC 1625, *E. faecalis:* ATCC 29212 and *E. coli:* ATCC 25922.

Susceptibility testing

This was carried out on 24 h bacterial pure culture. Prior to the test, bacterial isolates were streaked on fresh agar and incubated at 37 °C overnight. From the resulting bacterial population, a suspension to the density of a McFarland 0.5 turbidity standard in 0.9% saline was prepared and adjusted to the final opacity recommended for susceptibility tests by agar diffusion technique on Mueller Hinton or chocolate agar according to the "Comité de l'Antibiogramme de la Societé Française de Microbiologie, CA-SFM, EUCAST, 2014".

A total of seventeen conventional antibacterial agents, including representatives of major families of antibiotics used in Cameroon were chosen. These were: Penicillin (10 U), Cloxacillin (5 µg), Oxacillin (1 µg), Vancomycin (30 µg), Amoxicillin (30 µg), Amoxicillin/Clavulanic acid (20/10 µg), Cephalotin (30 µg), Cefoxitin (30 µg), Cefuroxime (30 µg), Ceftriaxon (30 µg), Ceftazidim (30 µg), Aztreonam (30 µg), Nalidixic acid (30 µg), Ciprofloxacin (30 µg), Nitrofurantoin (300 µg), Trimethoprim/sulfamethoxazole (1.25/23.75 µg) and Gentamicin (5 µg).

RESULTS

Overall, 109 items were targeted in the three hospitals. Hospital wards and inanimate materials of interest were summarized and displayed as shown in Table 1.

Overall picture showed that types and numbers of targets varied from one hospital to the other for similar wards and that, within the same hospital, inter-ward variations were observed. In this, the largest numbers of potential reservoirs were recorded in the District Hospital of Bangangté. When contamination rates were assessed based on culture results and the number of these potential reservoirs, data were grouped once again per hospital and summarized as displayed in Table 2.

It disclosed that a total of 238 samples were collected. In Bangwa all items' in the Operating Theater and the Maternity were contaminated above the assigned threshold, like all healthcare workers hands. Contamination due to Gram-negative rods ranged from 11- 20% on inanimate hospital items and 9 - 14% on healthcare workers'.

With regards to Gram type from healthcare environments, the total of 428 Gram-positive bacteria overwhelmed the isolations. Further details indicated that 92%, 86%, and 81% of bacterial isolates from Bangwa, Bangangté, and Bafoussam respectively, belonged to one of the genera *Staphylococcus* and *Bacillus*. In this, *Bacillus* spp. (≈ 62%) overwhelmed *Staphylococcus* spp. Otherwise and from specimens (collectively from hands and hospital inanimate items) 8%, 14% and 19% were other than Gram-positive bacteria in the respective healthcare institutions. In the BPH in addition, *Clostridium* spp. was isolated from the Operation Theater.

Susceptibility tests were therefore performed on major Gram categories of isolates according to their shapes and origins; then displayed in Table 3 and Table 4 as data summary from hospital devices and healthcare workers hands, respectively.

Most reduced susceptibility rates were obtained with beta-lactams headed by drugs from the penicillin sub-group (Penicillin and Oxacillin) third generation cephalosporins (Ceftriaxone, Ceftazidime) and Aztreonam regardless of healthcare facility. This reduced susceptibility was also recorded with Vancomycin (the Glycopeptide sub-group representative) and Nalidixic acid (first generation quinolone). In general, however, reduced susceptibility rates were relatively higher in *Bacillus* spp., while higher effectiveness was observed with Gentamicin and Ciprofloxacin; observed in both *Bacillus* spp., *Staphylococcus* spp. To a lesser extent, this effectiveness was recorded with Nitrofurantoin. The above tabled data further indicated varied levels of intermediate phenotypes.

A similar frame used to address caregiver's hands' resulted in the total of 95 isolates (*Staphylococcus* spp. 52% and *Bacillus* spp. 48%) recovered and used for susceptibility testing (Table 4).

Overall, reduced susceptibility was observed in both major bacterial types in all settings. The susceptibility rates in *Bacillus* spp., and *Staphylococcus* spp., were similar for Oxacillin and Ceftriaxon (<10%). Also, similar rates were recorded in *Bacillus* and *Staphylococcus* isolates recovered in Bafoussam for Penicillin; and Bangangté for Vancomycin and Cefoxitin. In the *Staphylococcus* sub-group especially, susceptibility rates lower than 50% was recorded for 87% of the antibiotics in Bangangté, 82% in Bangwa, and 75% in Bafoussam.

Table 1: Hospital wards and inanimate materials targeted per health facility.

		BANGANGTE			
Wards /Swab site	**Mt**	**Pd**	**Surg and O.T.**	**I.M.**	**Lab.**
Tap	√			√	√
Door handle	√	√	√	√	√
Bed stand	√	√	√	√	
Cot	√	√	√		
Examination Table	√		√		√
Scialytic		√	√		√
Infant scale	√	√			
Drip pole	√	√	√	√	
Pad lock	√	√	√		
Fridge Handle				√	√
Blood Pressure Cuff					
Bench Top	√	√	√	√	√
Computer Keyboard					√
Cart Handle	√		√		
Surgical Apron			√		√

Microscope Handle					ᵛ	
Spectrometer					ᵛ	
Total (44)	10	08	10	06	10	

BAFOUSSAM

Wards /Swab site	**Mt**	**Pd**	**O.T.**	**I.M.**	**ICU**	**Surg**
Radian Lamp	ᵛ					
Trolley		ᵛ		ᵛ	ᵛ	ᵛ
Coat			ᵛ			
Forceps			ᵛ			
Oxygen generator			ᵛ			
Air Filter			ᵛ			
Bed stand	ᵛ	ᵛ		ᵛ	ᵛ	ᵛ
Gallows	ᵛ				ᵛ	ᵛ
Oxygen container	ᵛ				ᵛ	
Oxygen machine	ᵛ		ᵛ		ᵛ	
Scialytic			ᵛ			
Bed-side lamp					ᵛ	
Tap	ᵛ				ᵛ	ᵛ
Examination table	ᵛ	ᵛ				ᵛ
Door Handle	ᵛ			ᵛ	ᵛ	ᵛ
Baby scale	ᵛ	ᵛ				
Total (35)	09	03	06	03	08	06

BANGWA

Wards /Swab site	**Mt**	**Pd**	**O.T.**	**I.M.**	**ICU**	**Surg**
Bed stand	ᵛ	ᵛ	ᵛ	ᵛ	ᵛ	ᵛ
Mattress				ᵛ	ᵛ	
Door handle	ᵛ	ᵛ	ᵛ	ᵛ	ᵛ	ᵛ
Gallows		ᵛ			ᵛ	
Oxygen machine		ᵛ	ᵛ		ᵛ	
Tap			ᵛ	ᵛ	ᵛ	ᵛ
Cupboard		ᵛ				
Stretcher				ᵛ		ᵛ
Baby scale		ᵛ				
Examination table			ᵛ			
Scialytic			ᵛ			
Aspirer			ᵛ			
Total (30)	02	06	07	05	06	04

Mt: Maternity; **O.T.:** Operating Theater; **Lab:** Laboratory; **Pd:** Pediatrics; **I.M.** Internal Medicine; **Surg**: Surgery; **ICU:** Intensive Care Unit; ᵛ: was sampled

Table 2: Contamination rates per hospital.

	Number of collection Sites /contamination rates (%)								Hosp		HcW		
Hospital	**ICU**	**Surg.**	**OT**	**IM**	**Pd**	**Mt**	**Lab**	**Total sp**	**GN (%)**	**Cont rate (%)**	**GN (%)**	**Cont. rate (%)**	**Resource origin**
BRH	26/92	20/60	6/17	8/50	7/86	19/42	NI	86	20	90	9	63	State
BPH	24/96	22/73	6/100	20/80	8/88	5/100	NI	85	16	86	12.5	100	Church
BDH	NI	16/75		10/90	11/88	18/94	12/67	67	11	92	14	91	State

NI : non investigated ; **ICU** : intensive care unit ; **Surg.**: Surgery ; **OT** : Operating Theater; **IM** : Internal medicine ; **Pd** : Pediatrics; **Mt**: Maternity; **GN**: Gram negative; **Cont. rate**: contamination rate; **Hosp**: Hospital; **HcW**: Healthcare worker. **BRH:** Bafoussam Regional Hospital**; BPH:** Bangwa Protestant Hospital**; BDH:** Bangangté District Hospital

Table 3: Antibiotic-related phenotypes (R/S) distribution in environmental isolates of *Staphylococcus* and *Bacillus*.

Antibiotics	***Staphylococcus* spp. (n=165)**						***Bacillus* spp. (n=263)**					
	S			**R**			**S**			**R**		
	BG	**BT**	**BF**	**BG**	**BT**	**BF**	**BG**	**BT**	**BF**	**BG**	**BT**	**BF**
Ceftriaxone 30 µg	12	3	0	83	92	89	9	11	5	78	83	95
Oxacillin (1 µg)	10	9	0	75	87	100	0	8	0	80	90	100
Amoxiclav (20/ 10 µg)	32	27	0	57	68	69	30	13	5	59	79	90
Aztréonam (30 µg)	0	1	0	93	99	100	0	/	25	0	/	52
Nitrofurantoin (300 µg)	43	18	68	47	65	17	34	26	15	62	54	80
Nalidixic Acid (30 µg)	8	4	0	83	88	96	0	/	25	2	/	60
Cefuroxime (30 µg)	30	29	33	67	68	42	25	14	0	72	75	95
Cotrimoxazole (25 µg)	18	5	25	75	93	75	35	15	5	60	83	95
Ciprofloxacine (30 µg)	33	72	56	60	26	33	/	/	71	/	/	29
Amoxicillin (30 µg)	35	25	17	57	74	83	19	9	5	81	89	95
Cefoxitin (30 µg)	28	10	0	55	87	100	15	7	32	81	91	68
Ceftazidime (30 µg)	20	8	8	77	88	92	32	18	21	67	74	79
Cephalotin (30 µg)	38	22	54	50	75	34	7	22	0	26	66	89
Gentamicin (120 µg)	68	70	77	10	21	15	81	63	73	10	28	22
Penicilline (10 µg)	23	8	0	72	91	100	20	5	0	76	92	100
Vancomycin (30 µg)	17	8	17	63	74	11	/	/	0	/	/	43

BG: Bangwa; **BT:** Bangangté; **BF:** Bafoussam; /: Not tested

Table 4: Antibiotic-related phenotypes (R/S) distribution in healthcare worker's *Staphylococcus* and *Bacillus.*

Antibiotics	***Staphylococcus* spp. (n=49)**						***Bacillus* spp. (n=46)**					
	S			**R**			**S**			**R**		
	BG	**BT**	**BF**	**BG**	**BT**	**BF**	**BG**	**BT**	**BF**	**BG**	**BT**	**BF**
Ceftriaxone (30 µg)	6	0	0	94	100	89	7	0	5	93	100	95
Oxacilline (1 µg)	0	5	0	100	100	100	13	0	0	87	100	100
Amoxiclav (20/ 10 µg)	3	16	0	47	84	69	20	7	5	80	93	90
Aztreonam (30 µg)	0	6	0	100	67	100	/	0	25	/	93	52
Nitrofurantoin (300 µg)	47	6	68	41	94	17	33	/	15	47	/	80
Nalidixic Acid (30 µg)	0	28	0	100	61	96	/	21	25	/	36	60
Cefuroxime (30 µg)	12	12	33	71	65	42	0	/	0	87	/	95
Cotrimoxazole (25 µg)	6	6	25	94	94	75	33	0	5	67	93	95
Ciprofloxacin (30 µg)	71	0	56	24	100	33	/	0	71	/	100	29
Amoxicillin (30 µg)	6	65	17	94	29	83	0	/	5	100	/	95
Cefoxitin (30 µg)	6	0	0	94	100	100	/	0	32	/	100	68
Ceftazidime (30 µg)	6	0	8	94	95	92	20	0	21	80	100	79
Cephalotin (30 µg)	59	/	54	35	/	34	/	0	0	/	100	89
Gentamicin (120 µg)	59	21	77	18	63	15	87	83	73	13	8	22
Penicillin (10 µg)	5	59	0	88	6	100	0	29	0	87	57	100
Vancomycin (30 µg)	12	0	17	53	100	11	/	0	0	/	100	43

BG: Bangwa; **BT:** Bangangté; **BF:** Bafoussam; /: Not tested

DISCUSSION

Data analysis from the present multicenter survey disclosed high isolation rates of *Bacillus* spp., and *Staphylococcus* spp. (>80%, collectively in all settings), in connection with the low rates of the Gram-negative bacteria which are most commonly alleged in HAIs and not stressed in the present discussion to minimize emphasis dilution. In a former study (Nagajothi et al*.*, 2015) the contamination rate estimate, 97.5%, was dominated by Gram-negative rods and coagulase-negative cocci. Reduced susceptibility was also observed in the major bacterial Gram types which overwhelmed the isolations in that survey.

These contamination rates were almost similar from one hospital to the other, and, within the same hospital amongst the investigated wards. Moreover, bacteria populations appeared similar in their proportions regardless of the location. The low isolation of Gram-negative rods could be associated with the conventional hygienic cleaning that might have been effective at a certain degree or, could be in connection with the large *Bacillus* populations. According to several authors in fact (Vandini et al., 2014; Martínez and Baquero et al., 2014; Amin et al., 2015), *Bacillus* can produce biocides which naturally and adversely affect the growth of Gram-negative bacteria. Further, and with regards to cultural values, hospital external environments and patient wards regularly and indiscriminately host visitors that likely reintroduce new germs soon after cleaning. This assertion is consistent and substantiated by data from the Pediatrics of all three hospitals where the contamination rates were found above 80%. Accordingly, the least contaminated wards were recorded at the Regional Hospital of Bafoussam (RHB). This public system-empowered healthcare facility is one of the high-ranked hospitals in Cameroon, before the BPH (a church property) and the BDH (the other public system hospital). Regarded as privileged amongst the three, it could also be observed that it is in the RHB's Operating Theater that the lowest contamination rate was recorded throughout; justifiable by the restricted in-and-out movements of people amongst other restrictive measures.

Admitting that the expected phenotype will only be delayed if the bacterial inoculum (number of individual bacteria per unit space or volume) is below a certain threshold, it can be understood how proper conventional cleaning that is more effective in some health facilities (especially resource-empowered ones) would reduce germ spread and mitigate HAIs. *Vice-versa*, reduced load would facilitate effective cleaning with conventional techniques. However, because conventional cleaning is relatively costly, it cannot be sustainably afforded by many communities, especially, those in the context of resource limitation like the ones concerned in the present works. One of the key points that deserved emphasis appeared, therefore, to be awareness and education on microbe-host interactions in connection with microbial loads for healthcare personnel, for beneficiaries of healthcare services and for their relatives as well as the role of hygiene.

Bacillus and *Staphylococcus* overwhelmed the isolations. Often omitted in general when addressing R/S in bacterial populations, the mastering of such parameters like population density, extreme flexibility (common in all bacteria communities) with regards to increasing numbers of immune-depressed human hosts (favored by advanced age, famine, malnutrition and other natural threats) make of these organisms, potent etiologies of human disorders and/or reservoirs for genetic elements dissemination (Spinosa et al., 2000; Yassin and Ahmad, 2012; Oluyege et al., 2015). This is consistent with the currently admitted concept of "bacteria tool set" that helps microbiologist communities to understand, at least partially, the unpredictable dynamics of bacterial genomes enabling the strain-adapting ability which is necessary for survival and explains the stochastic ranges of infections they can cause in their hosts. Moreover and with this dynamics, the Koch's Postulate used to identify IDs etiologies would prevail over the definition referring to specific genetic traits in "true or professional pathogens" when it comes to identify ID etiologies for several reasons two of which are: 1st, the genetic map may undergo changes that elicit new putative phenotypes which might be harmful to the host and 2nd, as not all known genetic elements are fully described, advent of *Bacillus* ssp., as causative agents of HAIs is likely, theoretically, beyond their acknowledged implication in human disorders such as anthrax and other food-borne infections, for their predominant populations in all settings (overall ≈62%). This allegation would be supported by the increasingly recognized role of strains from the related bacteria genus, *Clostridium,* in healthcare facilities (Dubberke et al*.,* 2009; Foster et al*.,* 2011) and the increasing numbers of susceptible hosts as stated above.

Resistance to several antibiotics was clearly highlighted in the present survey, independently of the locations. Acknowledged as facilitated by misuse of antimicrobial agents as discussed in previous works (Kouamouo et al*.,* 2013; Fotsing Kwetche et al*.,* 2015), positive selection and diversification promote fitness that results in emerging phenotypes with broaden host spectra, likely colonizers of new niches. This signal-dependent (type- and magnitude) natural phenomenon is known to occur in time and space scale throughout history and in all living systems (Planta, 2007*;* Martínez and Baquero, 2014; Laxminarayan and Chaudhury, 2016).

The terms "*Staphylococcus* spp." used in the present works actually encompassed coagulase-positive and coagulase-negative strains recognized as major agents of IDs (Anonynous, 2015a). One of the famous members, *S. aureus*, is common etiology of infections in almost all bodily systems. With a few exceptions (etiologies of anthrax and food-borne infections, for instance), members of the genus *Bacillus* have long been regarded as common hosts of the environment therefore, ignored in human disorders. In the light of advances, it becomes clearer that they might be relevant as causative agents or as engine for bacterial persistence within a niche for their role as providers of resistance genetic traits to all bacterial communities (Spinosa et al*.,* 2000; Tagoe et al*.,* 2011; Anonynous, 2015a; Noukela Noumi et al*.,* 2016). This assertion is consistent with the known role of members of the related *Clustridium* in case of immune-compromission (Dionisio et al*.,* 2002; Forster et al*.,* 2013; Singh et al*.,* 2013; Brouwer et al*.,* 2013; Shen et al*.,* 2013; Barbosa et al*.,* 2014; Martínez and Baquero, 2014; Anonynous, 2015a).

The R/S patterns that appeared invariably high in all settings were also consistent with the shared socio-cultural values and, undoubtedly, in connection with the purchasing power of the local populations. This special view needs to be addressed in order to redirect and redesign disease prevention and the healthcare delivery concomitant practices that mitigate HAIs. Necessary measures would include amongst others, the provision of human and financial resources approaching the required healthcare provider/healthcare needs ratio. Separate previous studies reported the alarming trends of resistance in the West Region of Cameroon (Kouamouo et al*.,* 2013; Simo Louokdom et al*.,* 216). The most affected antibiotics belonged to the large family of beta-lactams, and first generation of quinolones while, Ciprofloxacine, Gentamicin and, to a lesser extent, Nitrofurantoin could be used with the highest likelihood of therapeutic success. These authors blamed poor sanitation and misuse of antibiotics in humans; and alleged the impact from animal husbandry. The consistent blame on alleged poor sanitation was partially substantiated with data from the Ndé Division where only 25% of the daily needs in water are provided by the company in charge, and the in-charge population/medical doctor ratio estimated at 8000, according to the Bangangté Municipal council's report in 2015 (Anonymous, 2015b). With this resource scarcity it can be understood why healthcare in general and IDs prevention and management could be negatively affected, as most needy that cannot afford medical attention would refer to auto-medication. Still previous investigations reported that different resistance mechanisms also interplayed in local bacterial population (Fotsing Kwetche et al., 2015) and that bacterial strains could disseminate from one hospital area to the other (Noukela Noumi et al., 2016), as observed in each of the three hospitals targeted. R/S profile for *Staphylococcus* spp. and *Bacillus* spp. were similar in many cases. Whether strains actually carried the same genetic elements is likely, but yet to be fully addressed.

Conclusion

With a few exceptions, all settings were contaminated with high rates of *Staphylococcus* spp., and *Bacillus* spp. *Bacillus* (≈62%) overwhelmed the isolation while the susceptibility rates were similar in both bacteria groups and indiscriminately high, especially with antibiotics that are commonly used. Gentamicin, Cirpofloxacin and Nitrofurantoin proved to be most effective. Strains dissemination from one place to the other also appeared frequent in all settings. Consistent with previous works, these common bacterial types could sustainably be used in a cost-effective active R/S stewardship program. But the State support to public and private healthcare facilities is crucial for the HAIs mitigation to be achieved, especially in high-risk-patient's environments.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

RFTN, MT, PRFK and DPNN did the field and laboratory investigation. JK, JSL, SGD, SHT, BPTK and KK performed the data analysis and revising manuscript.

ACKNOWLEDGEMENTS

This work was performed thanks to the multivariate collaboration of the Heads of Hosting institutions. The Authors are so grateful to Drs M. BOUBA, G. FETSE TAMA and M. NANA of the District Hospital of Banganté, Regional Hospital of Bafoussam and Protestant Hospital of Bangwa, respectively for the invaluable support they provided during sample collection. Their Thankfulness is so firm because these and previous ones in their respective institutions have always provided opportunity for such studies. They highly appreciated this collaboration that will, undoubtedly, pave the ways for future steps and initiatives.

REFERENCES

Amin M, Rakhisi R, Ahmady AZ. 2015. Isolation and Identification of Bacillus Species From Soil and Evaluation of Their Antibacterial Properties. *Avicenna J. Clin. Microb. Infect*., 2(1): e23233. DOI: 10.17795/ajcmi-23233.

Anonymous. 2015a. *Clostridium difficile* Infections (CDI) and Multi-drug Resistant Organisms, in Hospital-Acquired Infections, by the New York State Department of Health, Albany, NY, 176.

Anonymous. 2015b. Elaboration du plan d'occupation des sols, Phase I : Analyse diagnostic de la situation actuelle, by the Bangangté Municipal Council, 2015: 283p

Baquero F, Lanza VF, Cantón R, Coque TM. 2015. Public Health evolutionary biology of antimicrobial resistance: Priorities for intervention. *Evolutionary Application,* **8**(3): 223-239. DOI: 10.1111/eva.12235

Barber M. 1961. Methicillin-resistant *Staphylococci*. *J. Clin. Pathol.,* **14**: 385-393.

Barbosa TM, Phelan RW, Leong D, Morrissey JP, Adams C. 2014. A Novel Erythromycin Resistance Plasmid from Bacillus Sp. Strain HS24, Isolated from the Marine Sponge Haliclona Simulans. *PLoS One*,

9(12): e115583. DOI: 10.1371/journal.pone.0115583.

Bhutta ZA, Sommerfeld J, Lassi ZS, Salam RA, Das JK. 2014. Global burden, distribution, and interventions for infectious diseases of poverty. *Infect. Dis. Poverty*, **3**: 21. DOI: 10. 1186/2049-9957-3-21.

Brouwer MS, Roberts AP, Hussain H, Williams RJ, Allan E. 2013. Horizontal gene transfer converts non-toxigenic *Clostridium difficile* strains in to toxin producers. *Nature Communications,* **4**: 2601. DOI: 10.1038/ncomms3601.

CA-SFM EUCAST. 2014. Comité de l'Antibiogramme de la Société Française de Microbiologie. Recommandations. Paris: 115.

Dionisio F, Matic I, Radman M, Rodrigues OR, Taddei F. 2002. Plasmids Spread Very Fast in Heterogeneous Bacterial Communities. *Genetics,* **162**: 1525–1532.

Dubberke ER, McMullen KM, Mayfield JL, Reske KA, Georgan to poulos P. 2009. Hospital-Associated *Clostridium difficile* Infection: Is It Necessary to Track Community-Onset Disease? *Infect. Control Hosp. Epidemiol.,* **30**(4): 332–337. DOI:10.1086/596604.

Forster AJ, Taljaard M, Oake N, Wilson K, Roth V, Walraven CV. 2011. The effect of hospital-acquired infection with *Clostridium difficile* on length of stay in hospital. *CMAJ.* DOI:10.1503 /cmaj.110543.

Fotsing Kwetche PR, Simo Louokdom J, Kamga C, Kourouma K, Kouamouo J. 2015. β-Lactamase–Associated Resistance Phenotypes amongst multidrug resistant bacteria isolated in a school hospital of West-Cameroon. *IJBRITISH.,* **2**(4): 1-70.

Islam S, Ahmed R, Rahman R, Hasan M, Khalil MR. 2014. Microbial load, antimicrobial sensitivity and plasmid profiles of *Vibrio cholerae* in fruit juice. *Int. J. Nat. Soc. Sci.,* **1**: 82-90.

Jasovsky D, Littmann J, Zorzet A, Cars O. 2016. Antimicrobial resistance a threat to the world's sustainable development. *Ups. J. Med Sci.,* **121**(3): 159-164.

Kouamouo J, Fotsing Kwetche PR, Yangoue D, Mbaya P, Simo Louokdom J, A. Nsangou. 2013. Female genital tract infection and engines of antibiotic resistance in fast-growing populations of Bangangté, West-Cameroon. *Int. J. Pharm. Biomed. Res.,* **4**(3): 181-186.

Laxminarayan R, Chaudhury RR, 2016. Antibiotic Resistance in India: Drivers and Opportunities for Action. *PLoS Med.,* **13**(3): e1001974. Doi: 10.1371/journal.pmed.1001974

Martínez JL, Baquero F. 2014. Emergence and spread of antibiotic resistance: setting a parameter space, *Ups. J. Med. Sci.,* **119**(2): 68-77.

Matyar A, Kaya A, Dinçer S. 2008.Antibacterial agents and heavy metal resistance in Gram-negativ bacteria isolated from sea water, shrimp and sediment in Iskenderun Bay. *Sci. Total Environ.,* **15**: 279-285.

Nagajothi J, Jeyakumari D, Vigneshwaran S, Kumar RP, Bharatwaj RS, Bagyalakshmi R. 2015. Study of Prevalence of Microbial Contamination with its Antibiotic Resistance Pattern in Automated Teller Machine in and around Puducherry, *India. Int. J. Earth Environ. Health Sci.*, **1**:27-31.

Noukela Noumi DP, Fotsing Kwetche PR, Kouamouo J, Simo Louokdom J, Gamwo Dongmo S. 2016. Bacillus spp. and Staphylococcus spp.:Potential Reservoirs of Resistance Traits in a Health care Facility? *J. Chem. Bio. Phy. Sci. Sec.,* **7**(1): 037-048.

Nurain AM, Bilal NE, Ibrahim ME. 2015. The frequency and antimicrobial resistance patterns of nosocomial pathogens recovered from cancer patients and hospital environments. *Asian Pac. J. Trop. Biomed.,* **5**(12): 1055-1059.

Oluyege JO, Oluwaniyi TT, Ijasan OC. 2015. Composition of Antibiotic Resistant Bacteria from Irrigated Vegetable Farmland. *J. Microbiol. Res*., **5**(5): 161-168. DOI:10.5923/j.microbiology.20150505.04

Planta, MB. 2007. The Role of Poverty in Antimicrobial Resistance. *J. Am. Board Fam. Med*., **20**: 533–539.

Ramanan L, Ranjit RC. 2016. Antibiotic Resistance in India: Drivers and Opportunities for Action. *PLoS Med*. DOI:10.1371/journal.pmed.1001974.

REMIC.2007. Référentiel en Microbiologie Clinique (Bactériologie et mycologie). SFM.

Shen J, Wang Y, Schwarz S. 2013. Presence and dissemination of the multiresistance gene cfr in Gram-positive and Gram-negative bacteria. *J. Antimicrob. Chemother.,* DOI:10.1093/jac/dkt092.

Simo Louokdom J, Fotsing Kwetche PR, Kouamouo J, Kengne Toam AL, Gamwo Dongmo S. 2016. High Antibiotic Resistance in Bacteria from a Healthcare Setting: Case in the surgery Wards of the Regional hospital of Bafoussam, West-Cameroun. *J. Chem. Bio. Sci*., **6**(4):1297-1307.

Singh PK, Ramachandran G, Ramos-Ruiz R, Peiro-Pastor R, Abia D. 2013. Mobility of the Native Bacillus subtilis Conjugative Plasmid pLS20 Is Regulated by Intercellular Signaling. *PLoS Genet.,* **9**(10): e1003892. DOI:10.1371/journal.pgen.1003892.

Smith S, Wang J, Fanning S, McMahon BJ. 2014. Antimicrobial resistant bacteria in wild mammals and birds: a coincidence or cause for concern? *Irish Vet. J.,* **67**:8.

Spinosa MR, Wallet F, Courcol RJ, Oggioni MR. 2000. The trouble in tracing opportunistic pathogens: cholangitis due to Bacillus in a French hospital caused by a strain related to an Italian probiotic? *Microbial Ecology in Health and Disease,* **12**: 99–101.

Tagoe NA, Baidoo SE, Dadzie I, Tengey D, Agede C. 2011.Potential sources of transmission of hospital acquired infections in the Volta Regional Hospital in Ghana. *Ghana Med. J.,* **45**(1): 22-26.

Teillant A. 2015. Costs and benefits of antimicrobial use in livestock; *AMR Control.,* 116-122.

Vandini A, Temmerman R, Frabetti A, Caselli E, Antonioli P, Balboni PG. 2014. Hard surface biocontrol in hospitals using microbial-based cleaning products. *PloS One*. **9**(9): 1-13. DOI: org/10.1371/journal.pone.0108598

Yassin NA, Ahmad AM. 2012. Incidence and resistotyping profiles of *Bacillus subtilis* isolated from Azadi Teaching Hospital in Duhokcinnty. *Iraq. Mat. Soc. Med.*, **24**(3): 194-199. DOI: 10.5455/msm.2012.24.194-197