Microbiology Research Journal International

30(11): 1-11, 2020; Article no.MRJI.64443
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Antibiotic Resistance Pattern of *Staphylococcus aureus* and Associated Risk Factors in the Adamaoua and Far North Regions of Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MM, SRE, LA, AB and HGK designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Authors MM, EVA, DFB, JN, PDY and RN collect data, acquired analyzed, interpreted data, and authors MM, HGK, MCN and CM managed the analyses of the study, managed the literature searches, revised the article critically. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2020/v30i1130278
Editor(s):
(1) Dr. Lachhman Das Singla, Guru Angad Dev Veterinary and Animal Sciences University, India.
Reviewers:
(1) Natalia Formento Marín, Spain.
(2) Ana Carolina Lyra de Albuquerque, Federal University of Paraíba, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64443

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 31 December 2020

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ABSTRACT

Background: Infections due to *Staphylococcus aureus* (*S. aureus*) have been recognized as an important public health problem worldwide. Resistant *S. aureus* strains emerged and its prevalence has been on the rise. Many risk factors were identified as comorbidities and impact seriously the health state of infected individual by resistant *Staphylococcus aureus*. We focus our study on two of these risk factors (HIV-infection and diabetes) which may constitute high potential health conditions for emergence of resistant *S. aureus* strains.

Methods: A Cross sectional study was conducted during 19 months in five laboratories in the northern Cameroon. Collection of clinical samples from nine different types of specimens and first identifications were conducted in the various sites of study. Selected strains were transported in Yaoundé using standard procedure. Identification and drug susceptibility testing was performed using the disk diffusion method. Questionnaires were submitted to all the participants after an approved written and oral consent. HIV and Glycemia test were carried out on each patient in case their status was not known. During the study, 380 strains of *Staphylococcus aureus* were identified. Antimicrobial test was achieved using 16 different antibiotics.

Results: Result showed that 202/380 (53.2 %) of *Staphylococcus aureus* were resistant to at least three antibiotics (multiresistant) from three different families. Multiresistant strains displayed high resistance to cotrimoxal (76.7 %) followed by penicillin (66.8 %), tetracyclin (57.4 %) and oxacillin (51.5%). While high rate of sensitivity were obtained for rifampicin (78.7 %), fusidic acid (65.8 %), lincomycin (60.5 %) and minocyclin (58.9 %). Few participants 8.9 % were tested HIV positive and 7.9% were living with diabetes. Strains isolated from participants living with these diseases presented higher rate of resistance to antibiotics.

Conclusion: The rate of multiresistant *Staphylococcus aureus* are constantly in progress in northern Cameroon, most of the strains originated from community. These strains exhibited high resistance level to cotrimoxazol while displaying high sensitivity to rifampicine. People living with chronic diseases presented high resistance to antibiotic recommended for *S. aureus* infection treatment.

Keywords: Antibiotic resistance; Staphylococcus aureus; community acquired S. aureus; hospital acquired S. aureus.

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) has been recognized as an important public health problem worldwide [1-3]. This bacteria is still a major human pathogen which causes various clinical infections including: endocarditis, osteoarticular infection, skin and soft tissue infections, pleuropulmonary infections [4]. As for many bacterial infections, treatment of infections due to *S. aureus* are facing some challenges as treatment failure due to antibiotic resistance. Resistant *Staphylococcus aureus* strains emerged and its prevalence has been on the rise worldwide and is still heterogeneous [5]. Several deaths due to resistant bacteria are observed. Large number of deaths 25000 and 23000 are registered yearly in european countries and United States respectively [6]. Infections due to resistant bacteria has a major consequences, including prolongation of hospital stay, long term disability, and additional financial burden for health system, high costs for patients and their families [7]. High resistance (64%) of *S. aureus* infection was observed in Asian countries [5], while in north America, the rate varies from 36% to 62.6% [6-8]. In European countries resistance range from 20 to 50% [9-11]. Result from nine (9) African countries reported by their National data Institute showed high level of resistance between 12% to 80% with few countries exceeding 82% [12-14]. In east Africa for example, up to 31.5% to 42% were noted in Uganda [15,16]. While the rate varies from 31% to 82% in Rwanda [17,18]. In Cameroon the resistance varies from 25% to 50% depending on the studies and regions [19-21]. One of the documented *Staphylococcus aureus* resistance is induced by aquisition of *mec A* gene. Strains carrying this gene are named Methicillin resistance *Staphylococcus aureus* (MRSA). *mec A* gene encodes law affinity binding protein [21-23]. Recently *mec C* gene was also described. Previously, infections due to *Staphylococcus aureus* were only known to be healthcare associated infections with some associated risk factors like advanced age, surgical intervention and dialysis [24]. Those risk factors are identified

as comorbidities and impact seriously the health state of infected individual due to MRSA. Later on community acquired *S. aureus* were also registered. In this study, we are going to focus on evaluation of association between two diseases HIV and diabetes with *S. aureus* infection rate in the northern region of Cameroon. *Staphylococcus aureus* has been identified as responsible for mortality and morbidity among HIV patients with frequently acquire antibiotic resistant strains [25]. Furthermore diabetic patients were more frequently infected by *S. aureus* which is associated with most of the bacteriemia and diabetic foot ulcers cases [26-30]. Moreover we will mainly assess antibiotic resistance pattern of *S. aureus* isolated from the community and hospital environment.

2. MATERIELS AND METHODS

2.1 Study Design, Area and Period

A Cross-sectional study was conducted from April 2019 to October 2020 on patients received at five different laboratories in the Adamaoua and the Far north Regions of Cameroon. For the Adamaoua region the collection sites were: The Laboratories of the Regional Hospital and Protestant Hospital of Ngaoundere while in the Far north region samples were collected in the Regional Hospital, Socio medical health center of the University of Maroua and the pharmacy Laboratory of Far north. Collection and first identifications were conducted in the various sites of study. Then strains were inoculated in the nutrient agar, incubated at 37°C for 18 to 24 hours, removed and conserved at 4 to 8° C to avoid any other contamination. After 2 weeks at most, the strains were transported in Yaounde for further analysis.

2.2 Data Collection Method

Data were collected by questionnaires after an oral or written consent was obtained from the patients. Literature review was conducted to perform the questionnaire [31,32]. The questionnaire had four main parts: Sociodemographic informations, Output or input patients, immunologic status and sample name follow by the date of collection. Our samples came from 9 types of specimens: pus, urine, semen culture, blood culture, vaginal swab, urethral swab, stool culture, surgery wound and cerebrospinal fluid. After blood samples were collected, HIV and Diabetes test from each patient's were performed if their status were unknown.

2.3 Bacterial Identification

Re-identification of all the received strains were realized. Each sample was then inoculated on Chapman agar (Bio Rad France) and incubated at 37°C for 18 to 24 h. Identification of isolated bacteria were perform based on their morphology, microscopically (the Gram stain reaction), then followed by Biochemicals identification. The last test included catalase, mannitol fermentation, coagulase and Dnase (Oxoid UK) test. All cultured media were prepared following the manufacturer's instruction. Sterility test was checked by incubating 5% of the culture media prepared at 37°C for 24 h to observed microorganisms growth.

2.4 Antibiotic Susceptibility Testing

Sensivity test were performed using disc diffusion method on Muller Hinton Agar (Oxoid Ltd, UK) according to the instruction of Antibiotic Committee of France 2019 [33] using the Kirby Bauer disc diffusion method. Briefly, pure isolate (Four to five colonies) was added into tube containing 5 mL of sterile normal saline water and mixed gently with vortex until it forms homogeneous suspension. The turbidity of bacterial suspension was standardized by using 0.5 McFarland. A sterile coton swab was dipped into the suspension and the bacterial suspension was then inoculated over the surface of a Petri dish and left at the room temperature for 5 minutes. Antibiotics disc were placed using disc manual dispenser and incubated at 37°C for 18 to 24 h. Sixteen (16) antibiotics were selected according of Antibiotic Committee of France for *Staphylococcus aureus*, namely: novobiocin (5 µg; control of sensitivity), vanconicin (30 µg), oxacillin (5 µg), amoxicillin and clavulanic acid (30 µg), penicillin (10 µg), cefoxitin (30 µg), cotrimoxazol (75 µg), rifampicin (30 µg), fusidic acid (10 µg), gentanicin (10 µg), ofloxacin (15 µg), ciprofloxacin (5 µg), erythromycin (15 µg), lincomycin (15 µg), tetracyclin (30 µg) and minocyclin (30 µg). At the end of the incubation period, the diameter of growth inhibition area was measured by using a digital caliper. Growth inhibition zone was interpreted as susceptible (S), intermediate (I) or resistant (R).

2.5 Data Analysis

Excel inputed data were cleaned and exported in SPSS version 20.0 for analysis. The

graphs were performed using Graphpad prism 5 and excel. Descriptive statistics like mean, frequencies were performed on different variables. Categorical variables were tested for statistical significance of distribution using the chi square. P<0.05 was considered significant.

3. RESULTS

3.1 Prevalence of Multiresistant *Staphylococcus aureus*

During the nineteen (19) months of collection, 380 strains were identified as *Staphylococcus aureus* among which 201 (52.9%) originated from Adamaoua and 179 (47.1%) from far north. Results of antibiogram showed that 202/380 (53.2%) of *Staphylococcus aureus* were resistant to at least three antibiotics (multiresistant) from three different families. We focused our analysis on mulresistant bacteria. Among these resistant *Staphylococcus aureus,* 59.4% (n=120) were isolated at two laboratories of the Adamaoua region, 30% (n=36) were women while 70% (n=84) were men. Twenty (n=20) of the infected female gendered (55.6%) were pregnant. In the far north region 40.6% of resistant *S. aureus* strains (n=82) were identified. Women represented 35.4% (n=29) and men 64.6% (n=53) of the multiresistant *S. aureus* infected patients. Eleven of the twenty nine women 37.9 % were pregnant. The age group of our participant ranged from 0 to 85 years. The means age was 26.99 years. The most represented age group (33.2%) was the young people aged 15 to 30 years.

3.2 Antibiotic Susceptibility Testing

Sixteen (16) different antibiotics were tested on the strains of *Staphylococcus aureus* coming from nine (9) various specimens namely: pus, urine, urethral swab, vaginal swab, blood culture, semen culture, stool culture, surgery wound and cerebrospinal fluid. We registered one sample for cerebrospinal fluid but the strain displayed high sensitivity to antibiotics. The novobicin was use as control to complete identification of *Staphylococcus aureus.* All 380 strains of *Staphylococcus aureus* recorded were sensitive to novobiocin. High prevalence of *S. aureus* identified was registered for pus 25.7% (n=52) followed by urine, urethral swab and blood culture with 20.8%, 12.5% and 11.2% respectively.

High resistance to cotrimoxazol was observed 155/202 (76.7%) follow by penicillin 139/202 (66.8%), tetracyclin 115 /202 (57.4%), oxacillin 104/202 (51.5%) and cefoxitin (51.5%). While for the sensivity rate, high values were obtained for rifampicin 159/202 (78.7%), fusidic acid 133/202 (65.5%), lincomycin 123/202 (60.5%) and minocyclin 119/202 (58.9%). Same variability was observed in both regions with a few difference in the far north where we recorded the highest prevalence for cotrimoxazol (81.5%). Men were more infected with *Staphylococcus aureus* in this study and recorded high rate of resistance.

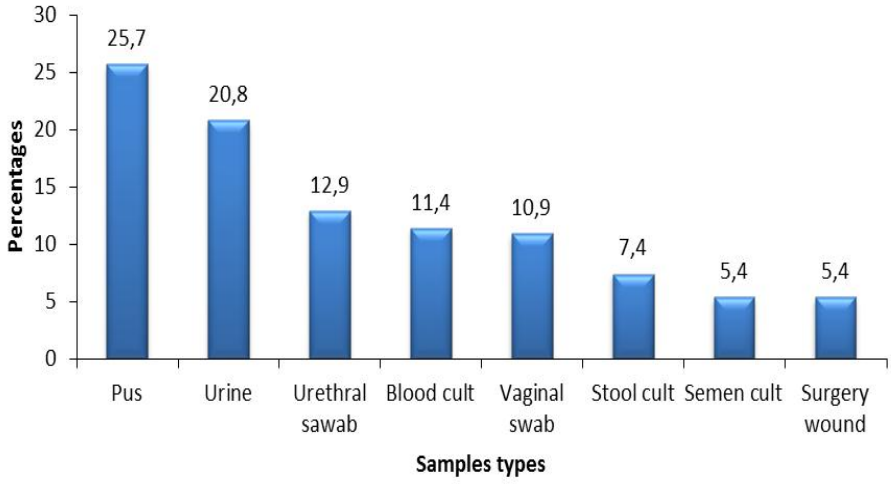

Fig. 1. *Staphylococcus aureus* isolated in various specimens of study

Table 1. Demographic data per region

Variables	Regions	Adamaoua(%)	Far north(%)	P value
Gender	F	36 (30.0)	29 (35.4)	0.423
	M	84 (70.0)	53 (64.6)	
Hospitalisation	Yes	35 (29.2)	38 (46.3)	0.013
	No	85 (70.8)	44 (53.7)	
Pregnant women	Yes	20 (55.6)	11 (37.9)	0.024
	No	16 (44.4)	18 (62.1)	
Age group (years)	[0-15[	30 (25)	27 (32.9)	0.023
	[15-30[	39 (32.5)	28 (34.1)	
	[30-45[	34 (28.3)	10 (12.2)	
	[45-60[	6 (5.0)	1 (1.2)	
	[60-75[	6 (5.0)	10 (12.2)	
	≥ 75	5 (4.2)	6 (7.3)	

3.3 Community or Hospitalized Based Patients

Out of all the multiresistant *S. aureus* isolated, 63.9% (129/202) originated from the community recognized as community acquired *S. aureus.* Whereas 36.1% (n=73/202) of them were from hospitals called hospital acquired *S. aureus*. The far north region had high rate (46.3 %) of hospital acquired *Staphylococcus aureus* compared to the Adamaoua (29.2%). Children in the age group ranged from 0-15 recorded the highest rate of hospitalization (32.9%; p=0.001). The majority (68) of hospitalized patients (93.2%) were in the common hospitalization ward. This may highly increase the risk of cross sectional contamination. Fifty two hospitalized patients (71.2%) were from wards harboring 8 to 12 beds. This factor may be of great cross transmission too. These results showed high prevalence of *S. aureus* from the community while the hospital acquired strains presented high rate of resistance. Few variability were observed with no statistical significance (p=0.6).

In both community and hospital acquired *S. aureus* strains, cotrimoxazol revealed the highest resistance rate (72.9% and 83.6% respectively), follow by penicillin (66.7% and 67.1 % respectively), tetracycline (58.9% and 54.8% respectively) and cefoxitin (49.6% and 54.8% respectively) as illustrated in the Fig. 3.

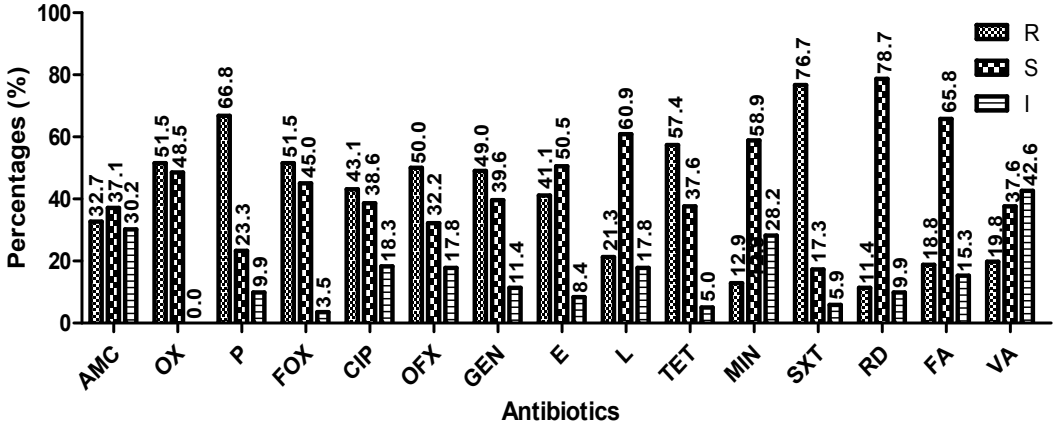

Fig. 2. Prevalence of *Staphylococcus aureus* and resistance profile

Amoxicilline+Clavulanic acid (AMC), Oxacillin (OX), Cefoxitin (FOX), Ciprofloxacin (CIP), Ofloxacin (OFX), Gentamicin (GEN), Erythromycin (E), Lincomycin (L), Tétrecyclin (TET) Cotrimoxazol (SXT), Rifampicin (RIF), Fusidic Acid (FA), Vancomycin (VA), Penicillin (P), Minociclin (MI)

R=Resistance ; S=Sensible ; I=Intermediaire

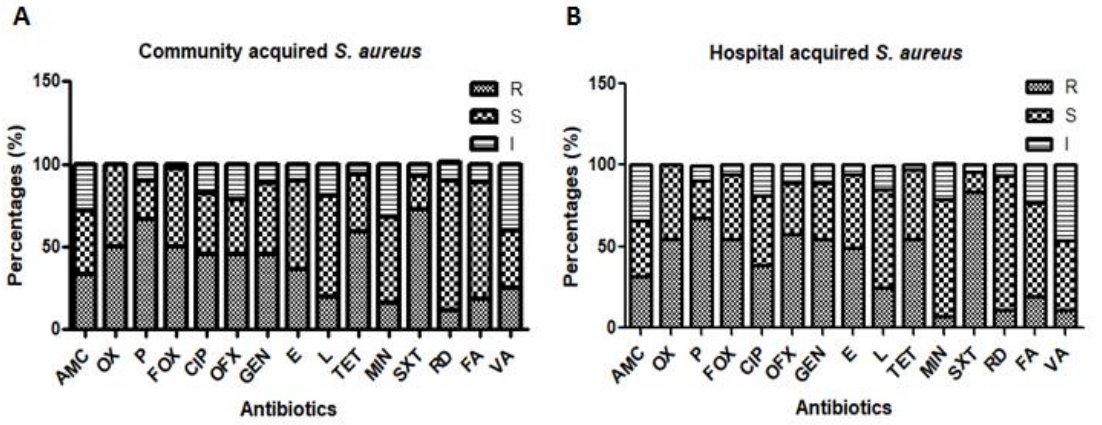

Fig. 3. Community and hospital based *S. aureus*

Amoxicilline+Clavulanic acid (AMC), Oxacillin (OX), Cefoxitin (FOX), Ciprofloxacin (CIP), Ofloxacin (OFX), Gentamicin (GEN), Erythromycin (E), Lincomycin (L), Tétrecyclin (TET) Cotrimoxazol (SXT), Rifampicin (RIF), Fusidic Acid (FA), Vancomycin (VA), Penicillin (P), Minocyclin (MI)
R=Resistance ; S=Sensible ; I=Intermediaire

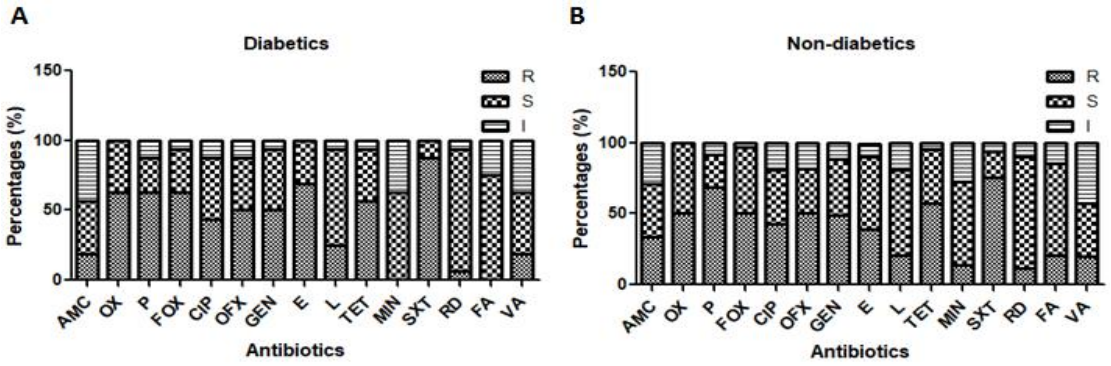

Fig. 4. Antibiotic resistance testing in diabetic and non-diabetic patients

Amoxicilline+Clavulanic acid (AMC), Oxacillin (OX), Cefoxitin (FOX), Ciprofloxacin (CIP), Ofloxacin (OFX), Gentamicin (GEN), Erythromycin (E), Lincomycin (L), Tétrecyclin (TET) Cotrimoxazol (SXT), Rifampicin (RIF), Fusidic Acid (FA), Vancomycin (VA), Penicillin (P), Minocyclin (MI)
R=Resistance ; S=Sensible ; I=Intermediaire

3.4 Diseases Recognized as Risk Factor of Resistance *S. aureus*: HIV and Diabetes

Many chronic disorders have been recognized as high associated risk factors for *Staphylococcus aureus* infection and complications. In this study we focus our attention on HIV-infection and Diabetes.

For diabetes, 7.9 % of our patients had elevated value of glycaemia and were recognized as diabetics. All of them were men. Men's age group from 30-45 years showed significant rate of *S. aureus* infection 9/16 (56.3 %). A Higher number of them were hospitalized 12/16 (75 %; p=0.001). The result of antimicrobial testing carried out showed considerable resistance rate for diabetic patients. Cotrimoxazol was still the antibiotic that have the most resistance patterns (87.5%), while rifampicin exhibited high sensitive count (87.5%) as illustrated below (Fig. 4).

4. DISCUSSION

Multiresistant *Staphylococcus aureus* are an important issue associated with high mortality and morbidity of many patients. They constitute a major public health concern worldwide. Infections due to resistant *Staphylococcus aureus* are still difficult to treat than ordinary infections [34]. These may prolonged the hospitalization of patients who are infected with

multiresistant bacteria with severe economic damages. The Northern regions of Cameroon are less developed and have too low indices of development in the national and international classification. Community acquired infections are constantly in progress in northern Cameroon. In fact we conducted a cross-sectional study in both of the public and private hospital laboratories in the northern part of Cameroon. Our study showed higher prevalence of *Staphylococcus aureus* (380/630) 60.3% in this part of Cameroon, approximately same prevalence 53.0% was obtained by Sinda et al. Garoy et al. in 2019 in Eritrea [36], while low level 28.5% was obtained in the economic region of Cameroon by Ebob et al. in 2016 [20]. This provides indication about resistance of *Staphylococcus aureus* which are constantly increasing in our country. The gender distribution showed that 64.6% of our participants were men, around same result 67.7% was observed in the studies of Tsige et al., 2020 in Ethiopia [37], while Kouemou et al., 2020 in Buea [35] and Hilaire et al., 2020 in Bardados [38] observed the reverse situation. In our case we explained the situation by the fact that in northern Cameroon and due to their socio cultural habits, men are in charge of activities out of the house and women are mostly housewife, this may reduce the risk factor of infection in women population. Our results showed that young adults age range from 15 to 29 years were more likely to develop resistance to this bacteria, same results was obtained in Ethiopia [37], unfortunately these age group constitute the main hand of our economic activity. So efforts must be done urgently to reduce and stop the progression of this issue. In this study, we recorded higher numbers (25.7 %) of *Staphylococcus aureus* isolated from pus. Others authors obtained same range, Garoy in Eritrea [36] and Ebob in Douala [20].

The antimicrobial testing results showed that most of our strains presented resistance to cotrimoxazol, penicillin, tetracyclin, oxacillin and cefexitin, approximately same results were obtained by several authors Zriouil *et al.* in Morocco [39], Schaumburg F. *et al.* in Africa [40], Kouemou *et al.* in Buea [35], Kengne *et al.* in Yaounde [41]. However, rifampicin, fusidic acid, lincomycin, minocyclin showed high rate of sensitivity, many authors also obtained the same results. However in the study we conducted in 2017 [42], to understand antibiotics use to treat infection due to *Staphylococcus* in absence of antibiogram result, we observed that amoxicillin and ceftriaxone were mostly use in this case. In the two regions that we conducted our study, most of the hospitals did not have technical equipments to carry out antibiogram, so patients were treated without precision. The population can buy antibiotic without any prescription and we found more illegal drug sellers in this part of Cameroon who administer antibiotic in any cases of illness. These reasons may rise up a great emergence of multidrug resistance in the community.

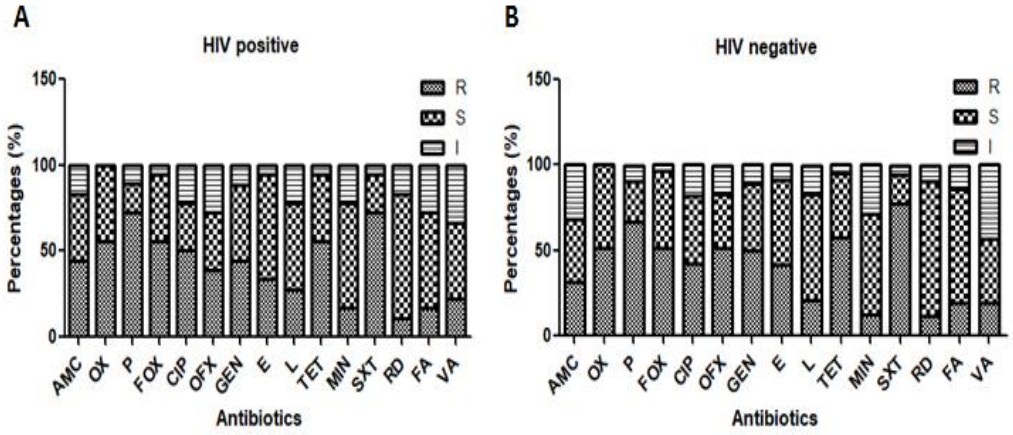

Fig. 5. Antibiotic resistance testing in HIV Positive and HIV negative patients

Amoxicilline+Clavulanic acid (AMC), Oxacillin (OX), Cefoxitin (FOX), Ciprofloxacin (CIP), Ofloxacin (OFX), Gentamicin (GEN), Erythromycin (E), Lincomycin (L), Tétrecyclin (TET) Cotrimoxazol (SXT), Rifampicin (RIF), Fusidic Acid (FA), Vancomycin (VA), Penicillin (P), Minocillin (MI)

R=Resistance ; S=Sensible ; I=Intermediaire

In our study, we registered 129 (63.9 %) community acquired *Staphylococcus aureus* while hospital acquired *S. aureus* were 73 (36.1 %). In fact many of samples collected from hospitalized patients showed sterile culture due to the fact that early antibiotic treatment on hospitalized patient without waiting for the result of analysis is constantly recurrent in these regions. This factor contributes mainly to the treatment failure, prolongation of hospitalisation stay and economic damage on patients. Other authors find high prevalence in hospital setting [43].

Living with HIV and diabetes has been recognized as greater risk factors associated to antimicrobial resistance [44]. Some of our participant (8.9 %) were tested positive to HIV and recorded high antimicrobial resistance as the finding of Kitara *et al.* [45] in Uganda (63.6 %). Besides, in our study, 44.4 % of HIV positive patients were outpatients, men were the most recorded HIV positive in this study, many other authors demonstrated the same finding. Of the antibiotics tested on our strains, *S. aureus* displayed highest resistance percentage to cotrimoxazol and penicillin 72.2 % followed by cefoxitin and oxacillin 55.6 %. This finding calls for concern because cotrimoxazol is the drug of choice for opportunist infections in HIV positive patients in Cameroon.

Patients living with diabetes are most likely (52.16 %) to be colonized by multiresistant *Staphylococcus aureus* [46]. *Staphylococcus aureus* still remains the primary bacteria implicated in diabetic foot osteomyelitis and can cause recurrent bone infection [47]. In our study, 7.9 % of the participants were diabetics, our results were closed to those obtained by Shettigar et al. in 2019 (9.20 %) [48]. All the diabetic patients were men and the aged range of 30 to 45 were most represented 9/16 (56.3 %); 75% of them were hospitalized patients. Diabetic patients presented high rate of multidrug resistance in this study compare to non-diabetics patients. Cotrimoxazol (87.5%) followed by penicillin, oxacillin and cefoxitin (62.5%) recorded the highest level of resistance. We can say that having diabetes highly increases the risk of developing resistance to antibiotic and consequently increases the mortality and morbidity of those patients.

5. CONCLUSION

Our study showed that *Staphylococcus aureus* is the highest prevalent Gram positive cocci isolated in Northern Cameroon. Multiresistant strains are constantly increasing and this was demonstrated by 53.2 % of multiresistant strains isolated in our study. This situation is so alarming by the fact that young adults aged from 15 to 30 years old were the most infected by multiresisant bacteria and several of them were men who are most dedicated to work in this part of the country. Community acquired *Staphylococcus aureus* were more dominant in our results, while the hospital acquired *S. aureus* presented the high rate of resistance. Strains isolated during our study were highly resistant to cotrimoxazol, penicillin, tetracyclin, oxacyllin and cefoxitin, while these same strains were mostly sensible to rifampicin, fusidic acid and minocyclin. In the absence of antibiogram results test in this area, we can recommend practitioners to use fusidic acid or minocyclin for patients treatment because these two antibiotics had the highest sensitivity when compared to the other antibiotic with the $p<0.05$. We also noticed that people living with chronic diseases such as HIV and diabetes had a higher risk to develop resistance due to antibiotics. This calls for concern. It is very worrying because the risk of mortality is really high in this case. To reverse this observed situation, it is imperative for the authorities in charge of public health to regulate the use of antibiotic in medical practice.

CONSENT AND ETHICAL APPROVAL

All participants provided informed consent. An ethical clearance was obtained at the National ethic committee of Cameroon (N°2017/12/958/CE/CNERSH/SP).Authorisations to conduct the study were also obtained from the health regional delegation and the directors of hospitals.

ACKNOWLEDGEMENT

We address our sincere gratitude to Pr. ESSAME OYONO Jean Louis the General Director of the Institute of Medical Research and Medicinal Plant Studies for granting the permission for us to go down to the field to carry out the study. We acknowledge Dr. FETESE TAMA Gerard, Dr. MAMOUDOU and Dr. Simon AROGA directors respectively for The Regional Hospital of Maroua, Ngaoundere and Protestant Hospital of Ngaoundere. We also thanks Dr. BABI EBAI Emmanuel head of pharmacy Laboratory of Far north and Dr. BAHANE consultant in the Bacteriogical laboratory of regional hospital of Maroua. We express many thanks to all the head

of hospitals services for their collaboration . Our acknowledges are also going to the two regional delegate of health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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