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Hospitalization and colonization by methicillin-resistant *Staphylococcus* in the surgical department in 03 health facilities of the Ndé division, west-Cameroon

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Abstract

Background: Commensal flora colonization during hospitalization by bacteria is the first step for a nosocomial infections while antibiotic resistance reduces therapeutic options. In aim to control this phenomenon, we initiated this study to describe the impact of hospitalization on colonization by methicillin-resistant *Staphylococcus* in the surgical department in 03 health facilities of the Ndé division, west-Cameroon.

Methods: This quasi-experimental study was carried out on patients admitted for surgery in 03 health facilities of the Ndé division, west-Cameroon (District Hospital of Bangangté, Protestant Hospital of Bangwa and Cliniques Universitaires des Montagnes). After obtaining ethical clearance and authorizations, nasal swabs were performed at admission and discharge, with the aim of isolating bacteria and performing their antibiotic susceptibility tests. Information on each participant's antibiotic therapy was recorded. Laboratory investigations were carried out according to standard protocols (CASFM, 2019).

Results: The most commonly used antibiotics were β-lactam antibiotics. Of the 52 patients who agreed to participate in the study, 104 nasal swabs were performed. From the analysis, 110 (57 at admission versus 53 at discharge) *Staphylococcus* isolates were obtained. Overall, susceptibility testing showed that antibiotic resistance rates were higher at discharge than at admission; with significant differences between the susceptibility profiles obtained at admission and discharge for β-lactam antibiotics and not significant for fluoroquinolones and aminoglycosides. The nasal flora of 13 (25%) patients was colonized with methicillin-resistant *Staphylococcus* at admission versus 39 (75%) at discharge and 14 (24.56%) of the isolates obtained at admission were methicillin-resistant versus 37 (69.81%) at discharge. Of the variables studied, hospital stay remained the only variable significantly (p=0.0080) associated with colonization by methicillin-resistant *Staphylococcus*.

Conclusion: This work must be considered as an alarm bell regarding the role of health structures in the colonization and spread of multi-resistant bacteria in Ndé division. As a result, further investigations aimed at investigating the factors favouring this phenomenon should be carried out with an approach similar to that of the ONE HEALTH concept. Pending these investigations, we recommend strict compliance with hygiene rules, personalized antibiotic therapy (performing antibiotic susceptibility tests) and an updated antibiotic prophylaxis and probabilistic antibiotic therapy according to the studies carried out in the locality.

Background

Staphylococcus are Gram-positive cocci bacteria found on the surfaces of our environment, as well as on the skin and mucous membranes (nasal mucosa) of humans [1,2]. Mutualist bacteria in humans, they can be pathogenic when the balance is disrupted, in fact in the presence of a decrease in immunity/injury, these bacteria can cause a multitude of diseases (sepsis, impetigo, endocarditis etc....) more or less

severe while antibiotic resistance reduces therapeutic options. Methicillin resistance was observed in *Staphylococcus* strains in health facilities around 1961 after the introduction of methicillin, reflecting the role of health facilities in the selection and dissemination of antibiotic-resistant infectious agents [3]. This resistance results from the expression of the resistance genes such as the *mec A* gene, which induces resistance to almost all β-lactamins among *Staphylococcus* strains [3].

Studies on bacterial resistance in hospitals, more specifically on methicillin-resistant *Staphylococcus* (MRS) strains, have highlighted the acquisition of multi-resistant bacteria during hospital stay and the harmful consequences of these multi-resistant bacteria on patient's lives [4–6]; thus justifying the inclusion of the fight against bacterial resistance to antibiotics in global programs (Global Health Security Agenda, One Health) [7,8]. Also, several studies carried out throughout the world, in west Cameroon and more specifically in the hospitals of the Ndé division have shown that the hospital environment (mainly in surgery) is an environment conducive to the selection and dissemination of multi-resistant bacteria on one hand and to the exchange of genetic material between the bacteria in this environment and those in the human body on the other hand [9–13].

Management with antibiotics would become increasingly difficult as the length of hospital stay increases. Data useful for understanding this phenomenon of growing antibiotic resistance that threatens patient care are scarce around the world and particularly in developing countries where resources (human and financial) for research are limited. However, it is imperative to understand the evolution of these phenomena in order to address the morbidity and mortality that occur as a consequence of antibiotic-resistant bacterial infections. It is in this context and with the aim of making a modest contribution to global programs (Global Health Security Agenda, One Health) focused on the fight against antibiotic resistance that this work was initiated. In order to study the impact of hospitalization on colonization by MRS in patients interned in the surgical department of 03 care setting in the Ndé division. The results of this work will guide patient's antibiotic therapy (antibio-probabilistic) at admission and during hospitalization, and will provide a basis for implementing and evaluating strategies and policies to reduce the proportion of resistant bacteria among patients during their hospital stays and thus limit nosocomial infections. This will be done with a view to reducing the emergence of multi-resistant strains in hospitals and their spread from hospitals to the community.

Materials And Methods

Study site, populations and sampling

This study was a quasi-experimental study conducted from February to May 2019 in patients from admission to discharge in three health facilities of the Ndé division, west-Cameroon (District Hospital of Bangangté, Protestant Hospital of Bangwa and Cliniques Universitaires des Montagnes). When all administrative and ethical requirements were met, nasal swabs were performed by streaking both anterior nares with sterile moistened cotton swabs among patients whose consent was given. Nasal swabs were

placed in labelled tubes containing heart-brain broth, stored in an icebox (2-8 ^{o}C) and transported without delay to the Laboratory of Microbiology of Cliniques Universitaires des Montagne for processing.

Microbiological analysis

Bacterial isolation and identification

After seeding and 18-24 hours culture on selective culture media (Manitol salt agar), isolation and identification of *Staphylococcus* strains were based on cultural characteristics, Gram staining, catalase test, coagulase test and DNase test after performed sub-culture on nutritive agar.

Antimicrobial Susceptibility testing

The susceptibility testing of bacterial isolates against antibiotics was performed by the disk diffusion method (Kirby-Bauer method) on Mueller Hinton agar according to standard procedures recommended by "Comité d'Antibiogramme de la Société Française de Microbiologie (CASFM 2019)"[14]. After sub-culture on nutrient agar (during 24 hours at 37°C) of a colony isolated on selective media (Manitol salt agar), the bacterial pure culture obtained has been used to perform a suspension in 0.9% saline with density equal to that of the McFarland (0.5 turbidity) as recommended by CASFM 2019. The choice of antibiotics was based on those commonly used in the surgical department (after a survey of doctors in the health facilities) and according to the guidelines (CASFM 2019). A total of 10 antibiotics have been used: cefoxitin (30 µg), ciprofloxacin (5 µg), gentamicin (15 µg), norfloxacin (5 µg), penicillin G (10 µg), oxacillin (1 µg), erythromycin (15 µg), clindamycin (2 µg), fusidic acid (10 µg), co-trimoxazole (1.25/23.75 µg). Interpretations of antibiotic susceptibility results were performed according to the guidelines (CASFM 2019). Reference strains *Staphylococcus aureus* ATCC 29213 were used for quality control.

Detection of methicillin resistance Stahylococcus

The phenotypic method based on susceptibility of oxacillin (1µg) and cefoxitin (30µg). The tests were concurrently performed with susceptibility testing of each isolate. Reference strains *S. aureus* ATCC 700699 and ATCC 25923 were used as a positive and negative control, respectively.

Data analysis

The data collected in this study were recorded in Microsoft Excel 2016 software and analyzed using StatView5 software. Descriptive analysis were carried out on the study variables, which included computation of addition and frequencies. A chi-square test was used to compare the susceptibility profiles and frequency of methicillin resistance obtained at admission and at discharge of patients. Logistic regression analysis uni-variable and multi-variable was used to identify determinants of methicillin resistance by *Staphylococcus strains*. The analysis were performed using 95% as confidence interval and 5% as degree of significance.

Results

Characteristics of study participants

During the study period from February to May 2019, 52 patients participated in the study. The age of the latter ranged from 5 to 79 years with a mean of 40.77 years. Patients were hospitalized for 3 and 22 days with a mean of 7.58 days. The characteristics of this population are summarized and presented in **Table I**.

Table I. Characteristics of study participants

Characteristics	Number	Frequency (%)
Sex		
Men	37	71.15
Women	15	28.85
Type of surgery		
Visceral	38	73.08
Orthopedic	14	26.92
Antibiotics administered		
β-lactamine	**43**	**82.69**
Ampicillin	21	40.38
Ceftriaxone	29	55.77
Cefixime	9	17.31
Cefuroxime	2	3.85
Amoxicillin/Clavulanic Acid	4	7.69
Cloxacillin	1	1.92
Fluoroquinolone	**15**	**28.85**
Ciprofloxacin	15	28.85
Aminoside	**17**	**32.69**
Gentamicin	17	32.69
Nitro-5-imidazoles	**46**	**88.46**
Metronidazole	46	88.46
Macrolide	**1**	**1.92**
Clarithromycin	1	1.92

First of all, it appears that men accounted for almost 3/4 of the population. Similarly, visceral surgery was the most frequent type of surgery with a proportion of 3/4. Finally, the antibiotics most commonly administered to patients belonged to the families of nitro-5-imidazole and β-lactamines with proportions approximately equal to 4/5 and the antibiotics least administered to patients belonged to the family of macrolides with a proportion of 1/50.

Distribution of bacterial isolates

From admission to discharge from hospital, 104 samples were taken. From these analysis, 110 *Staphylococcus* isolates were obtained. Of these, 57 isolates were obtained at admission versus 53 at discharge. The distribution of *Staphylococcus* isolates is presented in **Table II**.

Table II. Distribution of *Staphylococcus* strains

	Hospitalization			
	Admission		Discharge	
	Number	Frequency (%)	Number	Frequency (%)
Staphylococcus aureus	**16**	**28.07**	**25**	**47.17**
Coagulase negative *Staphylococcus*	**41**	**71,93**	**28**	**52.83**

The results presented in **Table II** show a significant variation between *Staphylococcus aureus* strains and coagulase negative *Staphylococcus* strains isolated at admission and those isolated at discharge. Indeed, at admission *Staphylococcus aureus* represented practically 1/4 of the strains of *Staphylococcus* compared to approximately 1/2 at discharge.

Antibiotic susceptibility profiles of *Staphylococcus* isolates

The susceptibility profile of *Staphylococcus* isolates obtained at admission and at discharge is presented in **Table III**.

Table III. Antibiotic susceptibility profiles of *Staphylococcus* isolates

	$n_{admission}$ (%)	$n_{discharge}$(%)	*Chi-2*	*p-value*
Cefoxitin				
Susceptible	44(77.19)	14(26.42)	29.49	0.0001
Intermediate	2(3.51)	2(3.77)		
Resistant	11(19.30)	37(69.81)		
Gentamicin				
Susceptible	52(91.23)	46(86.79)	0.56	0.4559
Intermediate	0(0.00)	0(0.00)		
Resistant	5(8.77)	7(13.21)		
Erythromycin				
Susceptible	18(31.58)	19(35.85)	1.81	0.4051
Intermediate	9(15.79)	4(7.55)		
Resistant	30(52.63)	30(56.60)		
Clindamycin				
Susceptible	21(36.84)	26(49.06)	1.67	0.1957
Intermediate	0(0.00)	0(0.00)		
Resistant	36(63.16)	27(50.94)		
Norfloxacin				
Susceptible	52(91.23)	42(79.25)	3.17	0.0749
Intermediate	0(0.00)	0(0.00)		
Resistant	5(8.77)	11(20.75)		
Ciprofloxacin				
Susceptible	53(92.98)	43(81.13)	3.47	0.0624
Intermediate	0(0.00)	0(0.00)		
Resistant	4(7.02)	10(18.87)		
Fusidic acid				
Susceptible	10(17.54)	4(7.55)	2.47	0.1160
Intermediate	0(0.00)	0(0.00)		
Resistant	47(82.46)	49(92.45)		

Co-trimoxazole				
Susceptible	19(33.33)	17(32.08)	0.02	0.9878
Intermediate	3(5.26)	3(5.66)		
Resistant	35(61.41)	33(62.26)		
Penicillin G				
Susceptible	27(47.37)	3(5.66)	24.09	0.0001
Intermediate	0(0.00)	0(0.00)		
Resistant	30(52.63)	50(94.34)		
Oxacillin				
Susceptible	43(75.44)	16(30.19)	22.61	0.0001
Intermediate	0(0.00)	0(0.00)		
Resistant	14(24.56)	37(69.81)		

$n_{admission}$: number at admission ; $n_{discharge}$: number at discharge**.**

Table III shows that overall, the resistance rates obtained at discharge were higher than those obtained at admission. Significant and insignificant differences between the susceptibility profiles of the isolates obtained at admission and discharge were observed.

Significant differences were observed (p0.0001) for β-lactamines: cefoxitin, oxacillin and penicillin G in favour of a higher proportion of resistance to these antibiotics at discharge than at admission, resulting in resistance proportions respectively 4 times, 3 times and 2 times higher at discharge. In contrast, insignificant differences were mainly observed for fluoroquinolones (ciprofloxacin and norfloxacin with p=0.0624, p=0.0749 respectively) and cotrimoxazole (p=0.9878).

Distribution of methicillin-resistant *Staphylococcus* isolates

A systematic screening of methicillin-resistant *Staphylococcus* isolates was performed during the interpretive reading of the antibiotic susceptibility test. Overall 13 (25%) of patients were colonized by methicillin resistant Staphylococcus at admission versus 39 (75%) at discharge. The results of distribution of methicillin resistant *Staphylococcus* obtained at admission and at discharge are presented in **Table IV**.

Table IV. Distribution of methicillin-resistant *Staphylococcus* isolates

	$n_{admission}$(%)	$n_{discharge}$(%)	*Chi-2*	*p-value*
Staphylococcus strains				
MR+	14(24.56)	37(69.81)	22.61	0.0001
MR-	43(75.44)	16(30.19)		

$n_{admission}$: number at admission; n_{sortie} : number at discharge ; MR+: resistance to methicillin; MR- : no resistance to methicillin.

From **Table IV**, it appears that the proportion of methicillin-resistant *Staphylococcus* isolates obtained at discharge from hospital was almost three times that obtained at admission with a significant difference (p 0,0001).

Research of the factors associated to colonization by MRS

In order to determine the factors associated to colonization by MRS, variables were identified and univariate analyses were first performed. Those who had presented a $p \geq 0.20$ were selected for the multivariate analysis. The results of the latter are summarized and presented in **Table V**.

Table V. Research of factors associated to colonization by MRS

Variable	OR	OR 95%CI	p-value
Admission/discharge			
Admission	1		Réf
Discharge	0.03	[2.36x10^{-3}-0.40]	0.0080
Metronidazole			
No	1		Réf
Yes	3.54	[0.56-22.46]	0.1797
Duration of hospilization			
	0.79	[0.65-0.96]	0.0185
Ampicillin			
No	1		Réf
Yes	0.82	[0.19-3.50]	0.7879
Ceftriaxone			
No	1		Réf
Yes	0.23	[0.04-1.18]	0.0788
Cefixime			
No	1		Réf
Yes	10.24	[0.75-140.16]	0.0813
Type of surgery			
Orthopaedic	1		Réf
Visceral	1.87	[0.55-6.39]	0.3149
Gentamicin			
No	1		Réf
Yes	1.24	[0.30-5.03]	0.7684

It appears that in multi-variate analysis that hospitalization with an OR of 0.03 (95% CI; [2.36x10^{-3}-0.40]) and hospitalization time with an OR of 0.79 (95% CI; [0.65-0.96]) remained significantly associated to colonization by MRS with p=0.0080 and p=0.0185 respectively.

Discussion

The aim of this work, conducted between February and May 2019, was to evaluate the impact of hospitalization on colonization by methicillin resistant *Staphylococcus* in patients interned in the surgical department of a few health facilities.

The majority of patients were male (3/4). This could be justified by the fact that they are the most regularly involved in hazardous activities, requiring great physical effort and therefore more frequently victims of accidents of all kinds. These activities expose them to accidents that can only be remedied by invasive acts such as surgery. This view is shared by the proportion of visceral interventions for hernias. Other causes that are not clear from this work but which are most probably at the origin of internalizations in surgical departments include road accidents and those associated with other high-risk professions.

Data analysis indicated that more than 80% of the most commonly administered antibiotics to patients were nitro-5-imidazol and β-lactamines. Several reasons could explain this rate of use: 1. these antibiotics have a wide spectrum of action on several bacterial types, have good tissue diffusion and are the most available and accessible in hospital pharmacies; 2. the less frequent adverse reactions (toxicity) to their administration encourage their choice in the management of patients during prophylaxis and anti-infectious therapy [15,16]. This development is further supported in the present work by the rates of use of other antibiotics (macrolide), which are certainly lower in relation to their relatively limited spectrum of Gram-positive bacteria and high toxicity [15].

Different frequencies were recorded for *Staphylococcus aureus* and coagulase-negative *Staphylococcus* at admission and discharge. While *Staphylococcus aureus* represented 1/4 of *Staphylococcus* at admission, the proportion of *Staphylococcus aureus* had doubled at dicharge. This result could be justified, at least in part, by a deselection of coagulase-negative strains in favour of *Staphylococcus aureus* strains. But the mechanisms and conditions conducive to this selection are far from being clarified in the present work.

From the study of the susceptibility profiles of isolates to the antibiotics used and more specifically to methicillin (highlighted in the present work using oxacillin and cefoxitin), it appears that the overall resistance rates recorded at discharge were higher than those observed at admission. This result could be justified by the mobility of genetic factors, favoured both by the selection pressure imposed by broad spectrum antibacterial agents and the flexibility of the bacterial genome. These same factors could justify the multiple resistances regularly reported (Simo et al., 2015; Tchapdie et al., 2017; Noukela et al., 2017) in accordance with the results of this study [9,10,12,17–19].

Between admission and discharge, significant differences (p 0.0001) concerning susceptibility profiles had been observed for antibiotics belonging to the β-lactam family (cefoxitin, oxacillin and penicillin G). This significant difference was in favour of a 4 times, 3 times and 2 times respectively higher resistance at discharge. This result could be justified by the direct effect of the use of antibiotics belonging to the β-

lactam family (most commonly used antibiotics) in the selection and expression of resistance genes such as the *mec A* gene, which codes for methicillin resistance and induces resistance to practically all antibiotics in the β-lactamine family. [1,20–23].

These resistances are an alert as to the difficulty that would exist in managing a resistant infection and the need to use an antibiotic susceptibility test for therapeutic choice (personalization of management) with the antibiotics available and accessible in the target hospital settings in this work.

In univariate analysis, the factors significantly associated to colonization by MRS were: hospitalization (p<0.0001), metronidazole (p<0.0001), duration of hospitalization (p=0.0176), ampicillin (p=0.0045), ceftriaxone (p=0.0057) and cefixime (p=0.0276) while in multivariate analysis only hospitalization and duration remained significantly associated with p=0.0080 and p=0.0185 respectively. This result could be justified at least in part by a flaw in the respect of hygiene rules and the importance of the selection and dissemination of multi-resistant bacteria in the hospital environment.

Emergence and dissemination of bacteria resistant to antibiotics in hospitals as demonstrated in this investigation is an indirect indicator of the increasing additional cost to the patient. Given the standard of living and purchasing power, this evolution of resistance would be seen as a factor aggravating poverty through prolonged hospital stay and the cost of care. Taking into account the susceptibility profile at the patient's entry could be a major asset for drug management using antibiotics.

Conclusions

At the end of our investigation, the aim was to evaluate the impact of hospitalization on colonization by methicillin-resistant *Staphylococcus* in patients interned in the surgical department of 3 care setting in the Ndé division. From this, it appears that the rates of antibiotic resistance of *Staphylococcus* strains isolated at discharge were globally higher (mainly with regard to antibiotics belonging to the β-lactamine family, the most used family) than those obtained at admission and that colonization by methicillin-resistant *Staphylococcus* was more important at discharge than at admission. It should be noted that the search for factors that could be significantly associated with colonization by methicillin-resistant *Staphylococcus* in the present work was limited to factors directly related to patients and their management (surgery and antibiotics administered). Although this aspect can be considered as a limitation, it does not detract from the quality of this work, which must be considered as an alarm bell with regard to the emergence and spread of resistant bacteria in Ndé division. As a result, further investigations to find the factors that promote this phenomenon should be carried out with an approach similar to that of the ONE HEALTH concept.

Abbreviations

ATCC : American Type Culture Collection ; **CASFM** : Comité de l'Antibiogramme de la Société Française de Microbiologie ; **CI** : Confidence Interval ; **OR** : Odds Ratio ; **MRS** : Meticillin-resistant *Staphylococcus.*

Declarations

Ethical consideration

Ethical approval for the study was obtained from the Institutional Committee of Ethics of Université des Montagnes under reference number: Ref: 2019/236/UdM/PR/CIE. The authorizations of the health facilities directors were also obtained with informed written and signed consent was obtained from study participants.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors were involved in study design, data collection, analysis and interpretation of the data, as well manuscript writing. All authors read and approved the final manuscript.

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