DOI: https://doi.org/10.53555/nnmhs.v8i5.1288 Publication URL: https://nnpub.org/index.php/MHS/article/view/1288

ANTIBIOTIC SUSCEPTIBILITY PROFILE OF GERMS RESPONSIBLE FOR NOSOCOMIAL INFECTIONS IN THE SURGICAL AND INTENSIVE CARE UNITS OF THE MILITARY HOSPITAL REGION N° 1, CAMEROON

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Abstract

Introduction: *Antimicrobial resistance is a real public health threat. The general objective of our study was to identify the germs associated with nosocomial infections and to study the antimicrobial susceptibility profile.* ***Methodology:*** *We conducted a cross-sectional analytical study in the surgical and intensive care units of the Number 1 Military Regional Hospital. The susceptibility study was done according to the CA-SFM guidelines. To determine the factors associated with nosocomial infection, a multivariate analysis was performed by logistic regression. The strength of the association was measured using the Odd Ratio (OR) and its 95% confidence interval (95% CI).* ***Results:*** *A total of 15 of 83 patients had a nosocomial infection (18.1%). ASA 3 (OR [95% CI]=15.65 [2.53-138.80]), ASA 1 (OR [95% CI]=0.11 [0.02-0.46]), dirty surgery (OR [95% CI]=2.88 [0.31-22.20]), absence of comorbidities (OR [95% CI]=0.23 [0.07-0.72]) and diabetes (OR [95% CI]=31.16 [3.87-805.55]) were associated with nosocomial infection ($p<0.05$). Escherichia coli (4; 23.5%) and Pseudomonas aeruginosa (3; 17.6%) were the most isolated. Strains showed better sensitivity to fosfomycin, chloramphenicol and levofloxacin. Candida sp strains were sensitive to the antifungal agents tested.* ***Conclusion:*** *A low rate of sensitivity to the tested antibiotics was observed. Regular monitoring of the sensitivity profile of germs responsible for nosocomial infections seems to be imperative in the fight against AMR.*

Key words: *antimicrobial resistance, prevalence, nosocomial infection, military hospital, Cameroon.*

INTRODUCTION

Nosocomial infections are infections acquired in a health care facility, they must not be present either during incubation or on admission of the patient [1]. The most frequent infections are pneumonia, urinary tract infection, bacteremia, meningitis and surgical site infection [2]. The main causative organisms are *Acinetobacter baumani*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [2,3]. In surgery, they occur later in the 30 days following surgery or within 365 days if an implant or prosthesis has been placed [4]. They can be caused by germs from the patient, the nursing staff or the hospital environment [5]. Nosocomial infection increases morbidity and mortality in patients. Its frequency, consequences and cost are sufficiently high and serious to be considered a real public health problem to be prevented [5]. In the USA, it is estimated that nosocomial infections are responsible for 9000 deaths per year, with an estimated global prevalence of 3 to 5% [6]. The European Centre for Disease Prevention and Control conducted a prevalence survey which revealed rates of 5.8% in France, 3.8% in the Netherlands, 3.6% in Germany and 2.9% in Lithuania [7]. In Mali, a study conducted in the pediatric surgery department of the Gabriel Touré University Hospital showed a prevalence of 15% [8]. In Cameroon, AR Ngono Monti et al, Monti N et al conducted studies that found a prevalence that varied from 9.9% to 18% [9, 10]. In the current context where antimicrobial resistance represents a real public health threat, the general objective of our study was to identify the germs associated with nosocomial infections and to study the antimicrobial susceptibility profile in order to improve management and reduce lethality in these services.

Methodology

Type, location and duration of the study

We conducted a cross-sectional analytical study in the surgical and intensive care units of the Number 1 Military Regional Hospital. It is located in downtown Yaounde and is the reference center for the care of military and civilian patients. The analyses of the specimens were performed in the bacteriology laboratory of the said Hospital. Our study was conducted from February 13 to April 27, 2020, i.e. 10 weeks.

Study population

These were patients seen in consultation at the HMR1, and hospitalized in the surgical and intensive care units. All patients who presented signs and symptoms of an infection after 48 hours of hospitalization and who gave their informed consent were included.

Procedure

The sociodemographic data of the participants were previously collected on a previously established questionnaire. The types of samples (urine, blood, skin and pus) and bacteriological analyses were performed according to the site of the suspected infection according to the classification of the Comité de Coordination de Lutte Contre l'Infection Nosocomiale 1996[11]. Pus from deep areas was collected either by aspiration during a surgical procedure or by puncture through the skin. Superficial infections were collected by swabbing after cleaning the surface with sterile physiological water or by aspiration with a needleless syringe. No transport medium was used; the samples were sent directly to the bacteriology laboratory and processed according to the specific procedure for the type of sample. Selective media were used according to the suspected germs for culture: Chapman for gram-positive cocci, Methylene Blue Eosin (MBE) for gram-negative bacilli and Sabouraud Chloramphenicol for yeasts. Antibiograms and antifungiograms were performed on Muller Hinton Agar using the agar diffusion technique according to the recommendations of the antibiogram committee of the French Society of Microbiology (CA-SFM) 2020 edition. The identification of isolated microorganisms was performed by classical fungal methods (fresh morphology, filamentation test) and bacteriology (fresh morphology and mobility, Gram staining, oxidase and catalase research as well as other biochemical characters (for enterobacteria) made with API 20E galleries. This is a standardized, manual system for bacterial identification. The sensitivity of the isolated bacteria was evaluated by successive and systematic tests on 17 antibiotic discs: beta-lactams (augmentin (2/1), oxacillin (1), piperacillin (30), cefuroxime (30), cefotaxime (5), ceftazidime (10), sulbactam/cefoperazone , aminoglycosides (gentamycin(10)), macrolides (azythromycin (15), clarythromycin (15), fluroquinolones (ciprofloxacin (5), levofloxacin (5), pefloxacin (5), cyclins (tetracycline (30), doxycycline (30)), chloramphenicol (30), fosfomycin (200). Interpretation was done using the manual of the Antibiogram Committee of the French Society of Microbiology 2020. The sensitivity rate of the isolated yeasts was evaluated by systematic antifungals on 6 discs of antifungals: Ketoconazole (10), Nystatin (100), Amphotericin B (100), Clotrimazole (10), Miconazole (10), Fluconazole (100). The search for the inhibition diameter giving the resistance or susceptibility profile was done following the guide proposed by the CA-SFM. S" was used for sensitive strains and "R" for those resistant to the tested molecules.

Statistical analysis

Data were recorded in CSPro version 6 and then exported to SPSS 23.0 for statistical analysis. Categorical variables were expressed by their numbers and frequencies and compared using the Chi-square or Fischer exact test depending on the application conditions. To determine the factors associated with nosocomial infection, multivariate analysis was performed using logistic regression. The strength of the association was measured using the Odd Ratio (OR) and its 95% confidence interval (95% CI). The difference was considered statistically significant for a p value less than 0.05.

Administrative Ethical Considerations
Administrative authorization from the HMR1 and ethical clearance from the Faculty of Medicine of Douala were obtained for this study. The study was carried out in accordance with bioethics laws, the law on information technology and freedom, as well as in accordance with good clinical practices and the Declaration of Helsinki. Informed consent was obtained from each participant before any involvement in the study and confidentiality related to the treatment of samples, data and results was respected.

Results
We selected 83 patients in our study, 62 in the surgical department and 21 in the intensive care unit.
Nosocomial infection: prevalence and associated factors
A total of 15 out of 83 patients had a nosocomial infection, i.e. a prevalence of 18.1%, distributed between the surgical (11/62; 17.7%) and intensive care (4/21; 19.1%) units. Among them were surgical site infections (9; 60%) and urinary tract infections (5; 33.3%). In addition, one case of ballistic wound was noted (1; 6.7%) (Table I).
The minimum hospital stay was 4 days and the maximum was 20 days in case of nosocomial infection.

Table I: Distribution of prevalence and sites of nosocomial infection in our study

Variables		Number	Percentage (%)
Nosocomial infection (N=83)		15	18,1
Site of infection (N=15)	Surgical site	9	60,0
	Urinary	5	33,3
	Ballistic wound	1	6,7

In bivariate analysis, the factors associated with nosocomial infection were the absence of comorbidities, diabetes, ASA classes 1 and 3, and dirty surgery (p<0.05) (Table II).
After multivariate analysis, ASA class 3 (OR [95% CI]=15.65 [2.53-138.80]; p= 0.001), dirty surgery (OR [95% CI]=2.88 [0.31-22.20]; p< 0.001), and diabetes (OR [95% CI]=31.16 [3.87-805.55]; p< 0.001) were risk factors for nosocomial infection occurrence. ASA class 1 (OR [95% CI]=0.11 [0.02-0.46]; p<0.001) and absence of comorbidities (OR [95% CI]=0.23 [0.07-0.72]; p<0.001) were protective factors for the occurrence of nosocomial infection (Table III).

Table II: Factors associated with nosocomial infection in our study

Variables		Nosocomial infection		p
		Yes n(%)	No n(%)	
Age				
	≤47	8 (53.3)	50 (73.5)	0.136
	>48	7 (46.7)	18 (26.5)	
Sex				
	Male	10 (66.7)	48 (70.6)	0.764
	Female	5 (33.3)	20 (29.4)	
Medical background				
	None	6 (40.0)	51 (75.0)	**0.008**
	Other	3 (20.0)	6 (8.8)	0.313
	Diabetes	4 (26.7)	1 (1.5)	**<0.001**
	Hypertension	2 (13.3)	11 (16.2)	1
Admission site				
	Surgery	11 (73.3)	51 (75.0)	0.964
	Reanimation	4 (26.7)	17 (25.0)	0.891
ASA Class				
	1	3 (27.3)	38 (74.5)	**<0.001**
	2	3 (27.3)	7 (13.7)	0.192
	3	5 (45.5)	3 (5.9)	**0.001**
	4	0 (0)	3 (5.9)	0.461
Type of surgery				

Clean	6 (54.5)	26 (51.0)	0.423
Clean contaminated	3 (27.3)	14 (27.5)	0.712
Contaminated	0 (0)	8 (15.7)	0.344
Dirty	2 (18.2)	3 (5.9)	**<0.001**

Table III: Multivariate analysis of factors associated with nosocomial infections in our study

Variables		**OR**	**CI (95%)**	**p**	**ORa**	**CI (95%)**	**p ajusté**
Medical background	None	0.22	0.07-0.72	< **0.001**	0.23	0.07-0.72	< **0.001**
	Diabetes	33.50	3.54-317.06	< **0.001**	31.16	3.87-805.55	< **0.001**
Type of surgery	Dirty surgery	2.96	0.43-20.41	< **0.001**	2.88	0.31-22.20	< **0.001**
ASA	ASA 1	0.10	0.02-0.45	< **0.001**	0.11	0.02-0.46	< **0.001**
	ASA 3	17.08	2.70-108.70	**0.001**	15.65	2.53-138.80	**0.001**

Microbiological profile of the nosocomial infection

Regarding the microbiological profile. 17 germs were isolated. including 9 enterobacteria (52.9%). 4 non-fermenting Gram-negative Bacilli (23.5%). 2 Gram-positive Cocci (11.8%) and 2 Yeasts (11.8%). The main species were *Escherichia coli* (4; 23.5%) followed by *Pseudomonas aeruginosa* (3; 17.6%). *Staphylococcus aureus* (2; 11.8%) and *Candida albicans* (2; 11.8%). The species mainly isolated at the surgical site were *Escherichia coli* (3; 27.3%). *Pseudomonas aeruginosa* (2; 18.2%). *Staphylococcus aureus* (2; 18.2%). In the urinary tract. the following germs were found in equal numbers: *Escherichia Coli*. *Klebsiella Oxytoca*. *Klebsiella sp*. *Enterobacter cloacae*. *Candida Albicans*. Moreover. in the case of ballistic wounds. the only nosocomial germ found was *Pseudomonas aeruginosa*. The isolated *Candida albicans* strains were sensitive to all the antifungal agents tested (Table IV).

Table IV: Distribution of isolated germs according to infection sites in our study

	Surgical site infection		**Urinary tract infection**		**Ballistic wound**		**Total**	
Germs	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Escherichia coli	3	27.3	1	20	0	0	4	23.5
Klebsiella Oxytoca	1	9.1	1	20	0	0	2	11.8
Klebsiella sp	0	0	1	20	0	0	1	5.9
Enterobacter cloacae	0	0	1	20	0	0	1	5.9
Citrobacter sp	1	9.1	0	0	0	0	1	5.9
Pseudomonas aeruginosa	2	18.2	0	0	1	100	3	17.6
Acinetobacter baumani	1	9.1	0	0	0	0	1	5.9
Staphylococcus aureus	2	18.2	0	0	0	0	2	11.8
Candida albicans	1	9.1	1	20	0	0	2	11.8

Antibiotic susceptibility profile

Isolated strains showed a better sensitivity to fosfomycin. chloramphenicol and levofloxacin. The majority of *Escherichia coli* strains were sensitive to fosfomycin (3/4) and gentamycin (25%); half of the *Klebsiella oxytoca* strains were sensitive to gentamicin. ciprofloxacin and levofloxacin. No antibiotic sensitivity was found for *Enterobacter cloacae* and Acinitobacter baumanii. *Citrobacter sp* showed 100% sensitivity to ciprofloxacin and levofloxacin. All strains of *Staphylococcus aureus* were sensitive to fosfomycin while 1 out of 3 strains of *Pseudomonas aeruginosa* were sensitive to fosfomycin (Table V). Candida sp strains were all susceptible to the antifungal agents tested (Ketoconazole. Nystatin. Amphotericin B. Clotrimazole. Miconazole. Fluconazole) (Table V).

Table V: Antibiotic susceptibility profile of the germs isolated in our study

Germs	Enterobacteria					Other		
Antibiotics	*E.coli* (4)	*K. oxytoca* (2)	*Klebsiella sp* (1)	*E.cloacae* (1)	*Citrobacter sp* (1)	*P. aeruginosa* (3)	*A. bumanii* (1)	*S. aureus* (2)
Gentamycin	25	50	100	00	00	00	00	50
Tetracycline	00	00	00	00	00	00	00	00
Doxycycline	nt	nt	nt	nt	nt	nt	nt	00
Oxacillin	nt	nt	nt	nt	nt	nt	nt	00
Cefuroxime	00	00	00	00	00	00	00	00
Augmentin	00	00	00	00	00	00	00	00
Piperacillin	00	00	00	00	00	00	00	00
Ceftazidim	00	00	00	00	00	00	00	00
Ciprofloxacin	00	50	00	00	100	00	00	50
Levofloxacin	00	50	100	00	100	33.3	00	50
Pefloxacin	00	00	00	00	00	00	00	00
Fosfomycin	75	00	100	00	00	33.3	00	100
Sulbactam/ Cefoperazone	00	00	100	00	00	00	00	00
Azythromycin	25	00	00	00	00	00	00	00
Clarythromycin	nt	nt	nt	nt	nt	nt	nt	00

Discussion

Our work is in line with the fight against antimicrobial resistance which has become a real public health threat. The aim was to identify the germs associated with nosocomial infections and to study the antimicrobial sensitivity profile in order to improve management and reduce lethality in these services. The overall frequency of nosocomial infections in our study was 18.1%. i.e. 17.7% in the surgical department and 19.1% in the intensive care unit. Our results are similar to those found by Rhazi K et al (2007) at the Hassan II University Hospital in Fez. Morocco. who found a higher rate in the intensive care unit. i.e. 25%. followed by surgery. 10.1% [12]. Njall C et al (2011) in Cameroon had had a nosocomial infection rate of 12% [9]. This infection rate was higher than that found by Samou F et al in the surgical department B of the point G hospital in Mali who had obtained a nosocomial infection rate of 6.7% [13]; Sekou B et al (2008) as for them had found an infection rate of 7.9% in the intensive care unit of the Gabriel Touré University Hospital [14]. the national survey of prevalence of nosocomial infections in France (2017) had obtained an overall rate of 5.2% [15]. This difference can be explained by the implementation in some countries of a national surveillance system for nosocomial infections. In our study. surgical site infections (60.0%) were the most frequent. followed by urinary tract infections (33.3%). Our results are similar to those found by Samou F et al (2005) in Mali who found a rate of 70% for surgical site infections and 30% for urinary tract infections [13]. Contrary to us. the national surveillance survey of nosocomial infections carried out in France (2017) showed that urinary tract infections were the most frequent. followed by other infections [15]; Njall C et al (2011) in Cameroon had a similar result. i.e. a urinary tract infection rate of 79.0% [9]. This could be explained by the target of our study which consisted of patients from surgical and intensive care units. ASA class 3. diabetes and dirty surgery had about 16. 31 and 3 times the risk of contracting a nosocomial infection respectively; on the other hand. the absence of comorbidities and ASA class 1 were protective factors. These results could help prevent nosocomial infections by setting up a targeted follow-up of patients with these warning signs.

Among the 15 nosocomial infections identified in our study. a total of 17 germs were identified. Enterobacteriaceae represented 53.0% of the isolated germs. followed by 23.4% of non-fermentative BGN and 11.8% of gram-positive cocci. E. coli was the most isolated germ followed by *Pseudomonas aeruginosa*. Our results corroborate with those found by Njall C et al (2011) in Cameroon who obtained 23.1% of E. coli followed by 15.4% of *Pseudomonas aeruginosa* [9]. On the other hand. Sanogo at Gabriel Touré University Hospital (2007) in Mali obtained Klebsiella pneumonia as the most isolated germ. i.e. 23.6% followed by *Staphylococcus aureus* 11.7% [16]. This disparate distribution of germs responsible for nosocomial infection points to the need for regular monitoring through public health programs.

A low sensitivity rate was observed for the different antibiotics tested in our study. due to the systematic administration of antibiotics to patients. The majority of *Escherichia coli* strains were sensitive to fosfomycin. Half of the *Klebsiella oxytoca* strains were sensitive to gentamicin. ciprofloxacin and levofloxacin. No antibiotic sensitivity was found for *Enterobacter cloacae* and Acinitobacter baumanii. *Citrobacter sp* showed 100% sensitivity to ciprofloxacin and levofloxacin. All strains of *Staphylococcus aureus* were sensitive to fosfomycin while 1 out of 3 strains of *Pseudomonas aeruginosa* was sensitive to the latter. Our results are similar to those found by Sekou B et al (2008) [14] who obtained a sensitivity to fosfomycin (100%) in gram positive cocci. This low sensitivity rate observed may be due to the prescription of antibiotics administered to each patient on admission or when the patient had episodes of fever. thus favoring the fluorescence of multi-resistant bacteria. The small size of our sample could explain the differences compared to other studies with larger sample sizes. It seems essential to continue the surveillance of nosocomial infections through a larger sample and in all hospital departments.

Conclusion

The frequency of nosocomial infections was 18.07%. ASA class 3. diabetes and dirty surgery were found to be risk factors for nosocomial infection; however. the absence of comorbidities and ASA class 1 were protective factors. Enterobacteriaceae were the most frequently isolated organisms. with *Escherichia coli* being the most frequent. A low rate of sensitivity to the antibiotics tested was observed. With the establishment of an AMR surveillance network. regular monitoring of the susceptibility profile of nosocomial infections appears to be imperative in the fight against AMR.

Acknowledgements

The research team would like to thank the Ministry of Public Health. the staff of the University of Douala. Yaounde I and especially all the participants in this study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Authors' contributions

The authors carried out the data collection, statistical analysis, drafting of the manuscript and its critical reading. All authors gave their approval for publication.

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