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RESEARCH ARTICLE

ANTIBIOTIC RESISTANCE PROFILE OF ISOLATED BACTERIA AT THE SANGMELIMA REFERENCE HOSPITAL, SOUTH CAMEROON REGION: A RETROSPECTIVE STUDY

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Abstract

Background and objective: Bacterial resistance to antibiotics is now one of the most serious threats to global health. Knowledge of the main bacterial species responsible for bacteremia and their antibiotic resistance profile makes possible to provide an objective basis for effective antibiotic therapy. However, in the South Cameroon region, microbiological documentation is not always available. The objective of current study was to determine the resistance profile of bacteria isolated at the Sangmélima Reference Hospital.
Methods: It was a retroprospective study on biological samples collected from interned and ambulatory patients seen at the Sangmélima Reference Hospital during the period from January 2021 to October 2021.The samples were cultured on specific media and the susceptibility was carried out on agar media using Kirby-Bauertechnique and interpretation according to the 2020 CASFM Guidelines.
Results: For the retrospective part, GNB were most represented (15/26; 57.7%,) with the *E. coli* (n=12/15) and only *Staphylococcus spp* for GPB (n=11/26). *Staphylococcus* resistance was particularly relevant with 81.81% to erythromycin, 63.63% to cefoxitin and 72.72% to Cotrimoxazole. *E. coli* showed a resistance of 66.66% for augmentin and 83.33% for cefuroxime. In the prospective part, GPB were only represented by *Staphylococcus epidermidis* (7/7; 50%), and GNB included *E. coli* (3/7; 21.42%), *K. pneumoniae* (2/7; 14.29%) and *A baumannii* (2/7; 14.29%). For all isolated bacteria strains, a high resistance to the majority of betalactams and penicillin was observed. However, bacteria with greater antibiotic resistance were *Staphylococcus* strains, highly resistant to beta-lactams, while *A. baumannii* strains showed higher resistance, and *E. coli* especially to penicillins and fluoroquinolones.
Conclusion: *E. coli* and *Staphylococcus spp* were predominant isolated bacteria with high rate of resistance to antibiotics frequently used in hospital. Strict adherence to infection prevention practices and judicious use of antibiotics are recommended to slow te spread of antimicrobial resistance (AMR).
Keywords: *A. baumannii*, Antibacterial resistance, *E. coli*, *Staphylococcus spp*., South Cameroon region.

INTRODUCTION

Bacterial infections are serious conditions, responsible for significant morbidity and mortality worldwide, and are among the most common care associated infections. In developing countries, Such infections remain currently a major public health problem[1]. Several studies conducted in Cameroon demonstrate the frequent involvement of *Staphylo-coccus aureus* in infections associated with a mortality of 15%-60%, a full resistance of *Escherichia coli* to amoxicillin and amoxicillin + clavulanic acid. Some lactose-fermenting coccobacilli such as *A. baumannii* and *Pseudomonas aeruginosa* show a recent increase in resistance to imipenem and piperacillin-tarzobactam[2,3]. Napa *et al.*, demonstrated that *P. aeruginosa* strains circulating in

the Center region have several enzymatic mechanisms of resistance to antibiotics associated with a high production of biofilm[4]. At the South Cameroon region, microbiological documenta-tion is not always present. We have therefore proposed to determine the the frequency of bacteria isolated within the Sangmélima Reference Hospital (HRS) and to evaluate their resistance to antibiotics in order to provide a database for future comparative studies, by analyzing hospital statistics over 6 months, in addition to a four-month bench job.

METHODS

Study design: It was a ten months period retro-prospective study carried out in the Microbiology Laboratory of the Sangmélima Reference Hospital (HRS), Sangmélima being a forest town located in the Southern Region of Cameroon, and knowing that hospital attendance has declined significantly during this covid-19 period. The retrospective part (January to June 2021) was carried out from the registers and antibiograms of the archives of the HRS Microbiology Laboratory. The prospective part was a 4-month bench work (July to October 2021) performed on clinical samples. All isolates came from diagnostic samples of patients hospitalized in the various HRS departments, ambulatory patients and samples from other health facilities in the region. Ethical clearance was obtained from The Yaoundé 1 University Institutional Ethics Committee, authorization to collect samples from HRS, and informed consent from all the study participants. Specimens were collected from both in and out patients as described by Rémic[5].

Retrospective part data collection : It consisted in extracting the results of the tests and antibiograms carried out from January to June 2021, from the bench registers of the Laboratory. The variables collected were: (a). Frequency of examinations requested, (b). Distribution by service, (c). Sex, (d). Sample type, (e). Isolated germs, (f). Antibiotic susceptibility.

Prospective part samples: Some samples were taken from the laboratory, others from the various departments of the hospital, and some sent from other health facilities in the southern region. These included urine and blood samples, pus and vaginal swabs, puncture fluids and semen.

Isolation and identification: Depending on the type of sample, a macroscopic examination was first carried out, then a stocking done by exhaustion on Chapman agar medium, chocolate +VCN and fresh blood agar, on EMB, CLED agar, Sabouraud agar, Columbia blood agar, Cooked blood agar + VCN. Each culture was followed by incubation from 18 to 24 hours incubation at 37°C. Colonies from positive primary cultures were stained using the Gram staining method. From the obtained results (Gram-negative bacteria-GNB bacteria and Gram-positive bacteria- GPB) biochemical tests were carried out using oxidase test, Catalase test, Coagulase test and DNase test. Identification of bacterial strian was made by API 20 NE (BioMérieux, France), following the manufacturer's instructions.

Antibiotic sensitivity test: The susceptibility test was performed using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The bacterial inoculum was obtained by using isolated colonies on nutrient agar and homogenized in 5 ml of sterile distilled water. This suspension was then adjusted in comparison to the McFarland 0.5 standard. The test was carried out in series of three copies according to the CLSI protocol M2-A9[7]. The antibacterial susceptibility of GPB and NGB was determined using the antibiotic discs listed below. Inhibition diameters measurement and interpretation (sensitive, intermediate, resistant) were made according to CASFM V1.2 2020[6]. BGPs were tested for Amoxicillin, Oxacillin, Cefoxitin, Cefotaxime, Chloramphenicol, Gentamicin, Erythromycin, Clindamycin, Norfloxacin, Fusidic acid, Cotrimoxazole, Rifampicin, Ciprofloxacin, Levofloxacin, Vancomycin, Kanamycin, Tobramycin, Netilmicin, Tetracycline, Minocycline, Tigecycline, Fosfomycin, Novobiocin. NGBs were tested for Amoxicillin, Augmentin, Piperacilline, Ticarcilline+Clavulanic sterile acidevillon, Cefoxitine, Cefotaxime, Cefuroxime, Ceftazidime, Cefepime, Cefixime, Imipenem, Amikacine, Gentamicin, Aztreonam, Nalidic acid, Levofloxacin, Ciprofloxacin, Fosfomycin, Cotrimoxazole, Colistine, Tobramycin, Tetracycline, Netilmycin, Ertapenema.

Detection of extended-spectrum beta lactamases: Extended-spectrum beta lactamases (ESBLs) are enzymes found in certain bacteria and are responsible for their resistance to antibiotics such as penicillins and cephalosporins. The double disc synergy was used to screen all the isolates for ESBLs production as recommended by CASFM[6]. Amoxicillin + clavulanic acid disc (30 µg/10 µg) was placed in the centre, equidistant from the ceftazidime disc (30 µg,) cefamin (30 µg) and cefotaxime (30 µg). A strain was ESBL positive if the production of inhibition zones in the form of champagne cork was observed between the amoxicillin + clavulanic acid discs and the ceftazidime, cefadepima, cefotaxime discs. Otherwise, the strain was negative ESBL.

Statistical analysis: Data was entered on Microsoft Excel 2016. Data analysis was performed with IBM SPSS Statistics Version 22.0. The descriptive data are presented in terms of frequency and percentages.

Ethical considerations: The study was performed after receiving an ethical clearance from the Joint Institutional Board for Animals and Human Bioethics (JIRB) of the University of Yaoundé 1 (Ref N° BTC-JIRB2022-019) and an approval from the Ethics Committee the approval of the Reference Hospital of Sangmelima(N°01/021/ARM/MINSANTE/SG/HRS/ CM *du 21 Juillet 2021*).

RESULTS

Retrospective epidemiological profile of samples : From the bench records of the laboratory the epidemiological profile of samples with identified bacteria could be established (Table 1). We recorded many samples, including urine (n=13; 50%), vaginal swabs (n=7; 26.92%), stool (n=3; 11.54%) and urethral

secretions (n=2 ; 7.69%) were the most represented. Samples from outpatients were the least represented (n=7; 26.92%) compared to 73.08% of inpatients (n= 19) shared majoritary between medical and emergency departments (15.38% and 23.07% respecti-vely).

Table 1: Retrospective characterization of samples.

Parameters	n (%)
Type of specimen	
Cervical spinal fluid	1 (3.85)
Stool	3(11.54)
Urethral secretions	2 (7.69)
Urine	13 (50)
Vaginal swab	7 (26.92)
Sample origin	
Outpatients	7 (26.92)
Inpatients	19 (73.08)
Sex	
Female	15 (57.7)
Male	11 (42.30)
Bacterial diversity in isolates	
GNB	15 (57.7)
GPB	11 (42.30)

GNB= Gram-negative bacteria; GPB= Gram-positive bacteria

Total 15 samples were recorded (57.7%) from women and 11 samples (42.30%) from men. The sample most represented in both males(n=7) and females (n=5) was urine, followed by stool. Gram-negative bacilli were most represented (n=15 ; 57.7%,) with the *E. coli*(n=12) and only *Staphylococcus spp* for Gram-positive bacilli (n=11). *E. coli* was the most isolated germ in the samples and was mainly present in urine and vaginal secretions. *Staphylococcus spp* strains were mostly isolated from urine and urethral secretions. *Staphylococcus* resistance was particularly relevant with 81.81% to erythromycin, 63.63% to cefoxitin and 72.72% to Cotrimoxazole. *E. coli* showed a resistance of 66.66% for augmentin and 83.33% for cefuroxime.

Prospective epidemiological profile of samples

In four months, fourteen participants were recruited for this study part, 12 men for 2 women, according to inclusion criteria. The average age of the sample donors was 29 years, ranging from 6 days to 78 years. Various samples were collected, the majority of which were urethral secre-tions and urine (n=4; 28.57% each) and at lower frequencies blood (n=1; 7.14%).

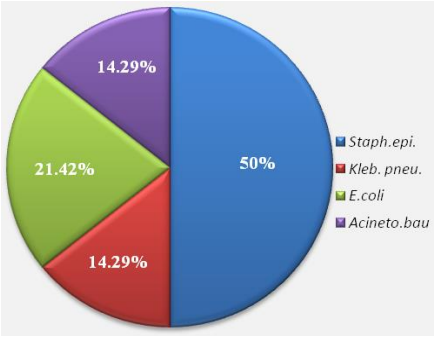

Figure 1: General frequency of isolated and identified bacteria.

Staph. epi.=S. epidermidis, Kleb. pneu.=K. pneumoniae, Acineto.bau=A. baumannii

The samples were from both hospital outpatients (50%, n=7) and in-patients (50%, n=7). For interned patients, the pediatrics department was in the lead with (n=4; 57.14%) of samples provided followed by the surgery department (n=2 ; 28.57%) (Table 2). Four bacterial species were isolated and identified. Half belonged to the Gram-positive coccis group represented by *S. epidermidis* (n=7; 50%), the other half belonged to the Gram-negative bacilli group and included *E. coli* species isolated at a frequency of (n=3; 21.42%), closely followed by *K. pneumoniae* (n=2; 14.29%) and *A. baumannii* (n=2; 14.29%), as shown in Table 3. Men were the most infected, mostly by *S. epidermidis* (58.33%, n=7/12).

Table 2: Prospective characterization of samples.

Parameters	n (%)
Type of specimen	
Blood	1 (7.14)
Urethral secretions	4 (28.57)
Urine	4 (28.57)
Vaginal swab	2 (14.29)
Pus	3 (21.43)
Sample origin	
Outpatients	7 (50)
Inpatients	7 (50)
Sex	
Female	2 (14.29)
Male	12 (85.71)

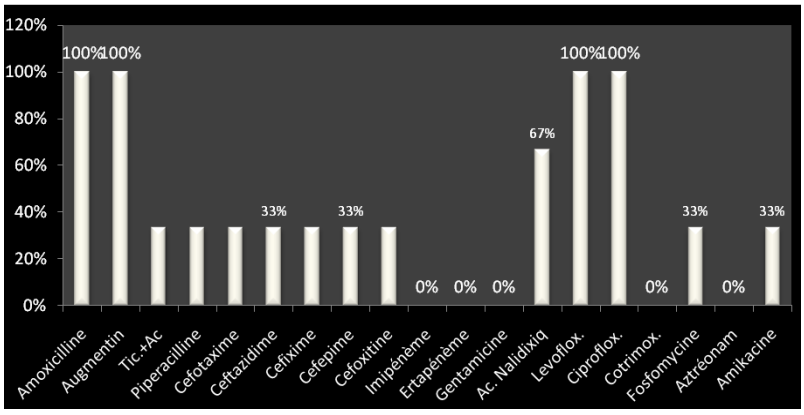

Figure 2: *E. coli* antibiotic resistance profile.

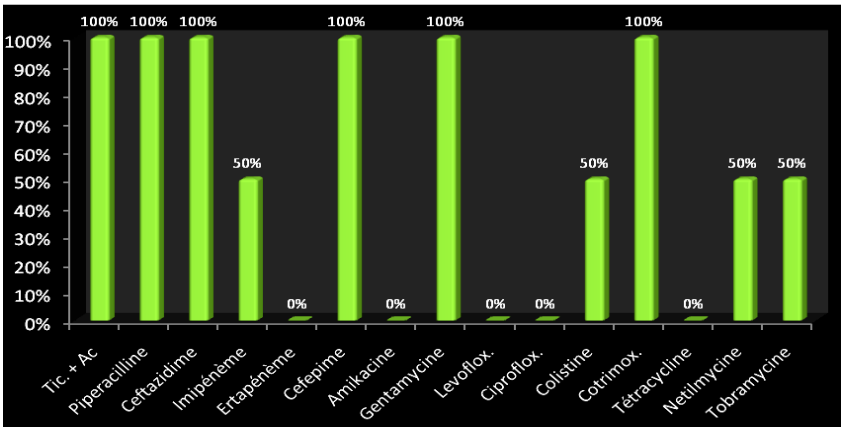

Figure 3: *A. baumannii* antibiotic resistance profile.

S. epidermidis was the bacterial species most isolated from urethral secretions (100%), pus (100%). *E. coli* was isolated more frequently in vaginal secretions and urine at frequencies of 100% (n=2), 50% (n=2) respectively.

Antibiotic sensitivity test : *E. coli* strains resistance to penicillins remains very high, 100% for amoxicillin and augmentin. The same is true for fluoroquinolones, particularly levofloxacin and ciprofloxacin, were innefficacious with 100% resistance each (Figure 1). All isolated strains of *A. baumanii* were resistant to all ß-lactam (100%) except ertapenem (Figure 2). Isolated *K. pneumoniae* strains were resistant to penicillins (100%) and fosfomycin (100%) (Figure 3). For *S. epidermidis*, many resistances were observed against all antibiotics classes and higher for ß-lactam (with a resistance of 71.42% to cefoxitin), and tigecyclin (100%) (Figure 4).

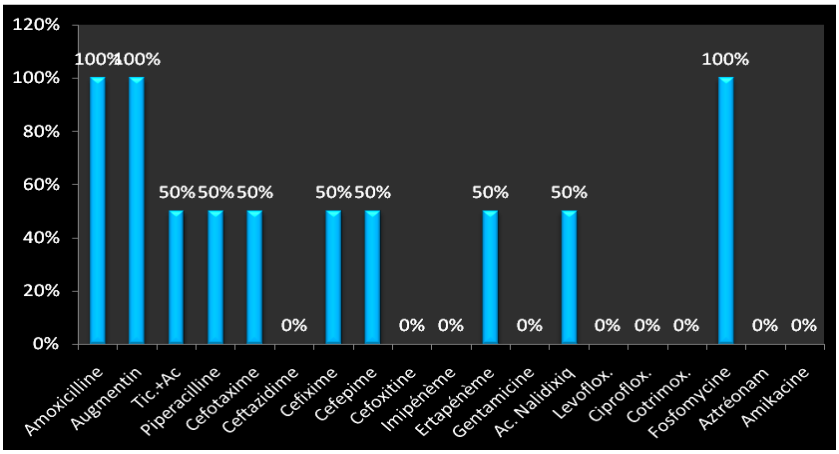

Figure 4: *K. pneumonia* antibiotic resistance profile.

DISCUSSION

For the retrospective fraction, GNB was more represented (57.7%) with the highest isolation frequency for *Escherichia coli*, followed by *Salmonella* spp and *Klebsiella* spp. and with GPB, only *Staphylococcus* spp. was isolated. These results, despite used small sample size, confirm the findings of Raed *et al*., in Jordan reported isolation frequencies of 29%, 14%, 7% and 3.5%, respectively, for *E. coli*, *K. pneumoniae*, *S. enterica*, and *S. epidermidis*[8].The frequency difference observed is due to the nature of the sample. Indeed, germs such as *E. coli* are usually isolated at lower frequencies in wounds than in urine or genital secretions as in current study. *Staphylococcus* resistance was particularly relevant with 81.81% to erythromycin, 63.63% to cefoxitin and 72.72% to cotrimoxazole. *E. coli* showed a resistance of 66.66% for augmentin and 83.33% for cefuroxime. These results are in agreement with the reports from Cameroon[2] and other African countries, as was the case in Ethiopia in 2017 where Mulu *et al*., revealed GPB and GNB resistance to cotrimoxazole and penicillin[9]. Indeed, these antibiotic families are widely prescribed in the first intension and HRS being a reference structure in the Southern Region, patients often come from other health structures where probabilistic treatments based on the use of these molecules have sometimes already been initiated. *S. epidermidis* (50%), as well as *E. coli* (21.42%), closely followed by *K. pneumoniae* and *A. baumannii* were successfully isolated and identified in this study. These trends are similar to previous works elsewhere in Cameroon by Ateudjeu *et al*., for who *E. coli* had a sample high prevalence while it was in stool, Kousseri city (Far North region)[3], Mbamyah *et al*., who revealed *K. pneumoniae* as the most prevalent species isolated from *Klebsiella* isolates identifiedin Yaoundé (Central Region)[10], and Okalla *et al*., who showed low rates of *A. baumannii*(3.8%) from clinical specimens in the city

of Douala (Littoral region)[11].This can be a proof that these strains are circulating in Cameroon. For all isolated bacteria strains, a high resistance to the majority of beta lactams and penicillin was observed. However, bacteria with greater antibiotic resistance were *Staphylococcus* strains, highly resistant to beta-lactams, while *A. baumannii* strains showed higher resistance, and *E. coli* especially to penicillins and fluoroquinolones.

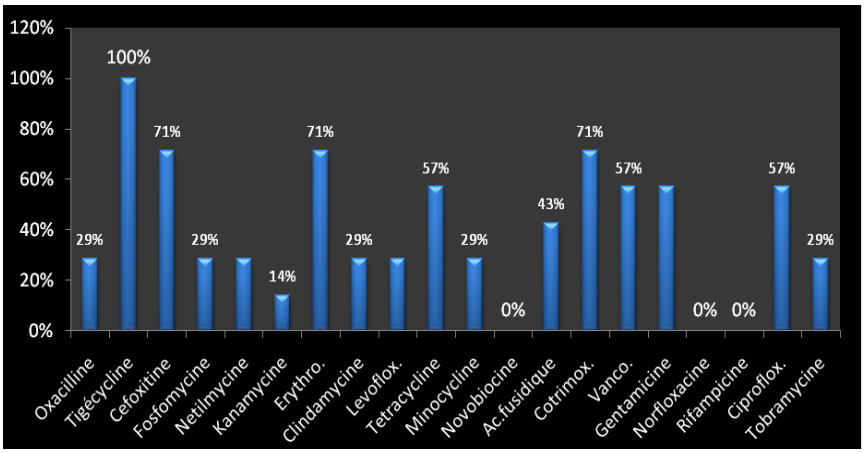

Figure 5: *S. epidermidis* antibiotic resistance profile.

This overall high rate of resistance of the isolates to the penicillins could be explained by the over use of these drugs in the treatment of common infections and ease of drug acquisition without prescription and even from road side vendors[10]. A High resistance to colistin was noted in current study, which complicates treatment because colistin is recommended for the treatment of *A. baumannii* infections[12].

Limitations

It was a monocentric study, done in Sangmélima a forest town located in the Southern Region of Cameroon, and knowing that hospital attendance has declined significantly during this covid-19 period. A small sample size of the study.

CONCLUSIONS

The purpose of our study was, on the one hand, to describe the epidemiological profile of the bacteria identified within the Sangmélima Reference Hospital. It emerged that the bacteria frequently involved were the GNB mainly represented by *E. coli* and the GPB only represented by the *Staphylococci*, and that some tests such as urine cultures were the most precribed. Resistance to frequently used antibiotics such as penicillins was very high, as well as a high level of resistance to beta-lactams were observed. Therefore, treatment of common bacterial infections in the study area needs to be guided by antibiotic susceptibility testings.

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AUTHOR'S CONTRIBUTION

Njiki Bikoï J: protocol design, writing original draft. **Moni Ndedi EDF:** protocol design, formal analysis. **Tsanga Manga BM:** protocol design, data collection tools development. **Makue Nguiffo E:** data collection tools development. **Membangbi AE:** data curation, investigation. **Koko-Ta SL:** investigation, conceptualization. **Mbaga DS:** data analysis and interpretation. **Mbongue Mikangue CA:** data analysis. **Ndongo Bela O:** critical review. **Njiki Bikoi AU:** data collection tools development. **Eone MM:** methodology, conceptualization. **Riwom Essama SH:** data analysis and interpretation. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest associated with this work.

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