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## **Bacteriological Profile of Nosocomial Infections in Visceral Surgery at the CNHU-HKM of Cotonou in Republic of Benin**

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### **Authors' contributions**

*This work was carried out in collaboration among all the authors. Authors AN, AAT and DSC designed the study. Author AN wrote the manuscript. Authors CFA, SA, EAP and RAL managed the statistical analyzes of the study. All authors have read and approved the final manuscript.*

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### **ABSTRACT**

Wound supuration is the formation and accumulation of pus in the soft tissue of the wound. As a rule, the natural inflammatory reaction disappears within three to five days and later the wound heals without any character. During this time, in some cases the inflammatory reaction in the wound exceeds the limits of the physiological norm due to the external supply of bacterial germs,

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which leads to the formation of pus, exaggeratedly prolonging the healing process and in turn leading to serious consequences. This study aims to identify the bacteria involved in these suppurations, to study their antibiotic resistance profiles and to review the role of the nurses taking care of these patients. The study was conducted at the Menontin zone hospital from May 15 to June 21, 2013. Fifty-one patients of all ages and sexes with surgical and traumatic discharge wounds were sampled and 30 hand samples were taken from the nurses' hands before and after dressing. From the results obtained, 76 bacteria were identified, including 35 cocci and 41 bacilli. 29% of the bacteria found were *Pseudomonas aeruginosa*, 28% *Staphylococcus aureus*, 14% *Escherichia coli* and others. Hand samples of 10 bacteria were isolated including 4 *Pseudomonas aeruginosa*, 3 *Staphylococcus* DNase-negative 2 *Klebsiella spp* and 1 *Staphylococcus aureus*. The strains isolated were particularly resistant to the antibiotics tested more specifically to  $\beta$ -lactam, Gentamycin Erythromycin and Colistin. The majority of strains have good sensitivity to ciprofloxacin and Fosfomycin. In view of these results, it is necessary to draw the attention of patients and nurses to the reality of the existence of these bacteria, the adequate intake of antibiotics preferably after an antibiotic test, hand washing and the use of appropriate sterile materials before and after any dressing.

**Keywords:** Nosocomials; bacterial strains; bacteriological profile; antibiotic; sensitivity; resistance.

## 1. INTRODUCTION

Bacteria are microscopic microorganisms that populate our environment, providing for some beneficial actions and for others harmful effects on the human organism depending on their location and pathogenicity [1]. In hospitals or clinics there are many sources of infectious germs responsible for diseases whose frequency varies according to the nature and structure of the hospital on the one hand and the activity of the hospital units and the quality of the health care team on the other [2] without forgetting the quality of the hygiene of the operating rooms and the medical equipment used [3]. The irrational use of antibiotics by patients themselves, combined with the lack of respect for hygiene rules, are all factors in the resurgence of these hospital infections. The problems they cause are in terms of morbidity, mortality and additional costs [4]. It is estimated that 60% of hospital infections worldwide are due to multi-resistant bacteria [5], which calls for more stringent hospital infection control measures. In Benin, for example, during our final training courses at the Menontin hospital laboratory, pus analyses carried out showed that the antibiotics prescribed by the clinician as a preventive measure, without an antibiotic susceptibility test, are mostly ineffective in preventing suppurative infections of open wounds related to traumatic accidents or surgical procedures. We have noticed that the dressing department of this hospital is overwhelmed by the management of traffic accidents, caesarean sections and other wounds, which have greatly contributed to the

increase in the number of dressings to be performed by the nurses. Are they still taking precautions to limit the transfer of germs, when hygiene and infection control must be carefully monitored?

## 2. STUDY FRAMEWORK AND METHOD

### 2.1 Study Framework

For this study, we chose the Menontin hospital located in the 9th district of the urban commune of Cotonou, in the M nontin lot 2130A neighborhood, not far from RNIE 2.

### 2.2 Study Method

51 pus samples were collected from any patient admitted to Menontin Hospital of any age and sex after their consent was obtained. These patients had a skin wound (superficial or deep) with suppurating pus, with two sterile swabs, one of which will be used for direct examination (fresh) and the other put in sterile peptone water and used to make the culture.

30 hand samples were taken from the nurses' hands before and after they had applied three dressings. The sample was collected with a sterile swab (moistened with sterile peptone water) applied to the palms of the hands and interdigital spaces.

The analysis of one sample took 72 hours over 3 days:

1<sup>st</sup> day: Fresh state, gram staining and culture according to the germ found on the gram slide. According to the present germ, were sown:

- For gram-positive cocci: chapman agar, fresh sheep blood agar 5% and D-cocosal agar.
- For gram negative bacilli: EMB agar, Schaedler agar enriched with fresh sheep blood 5%.
- The boxes were incubated at 37°C in the oven for 24 hours, those with blood were incubated anaerobically at 37°C for 24 hours.

2<sup>nd</sup> day: If there is a culture, whatever the colonies, a gram control has been done as well as the following tests:

- For gram-positive cocci: search for catalase and if positive, search for respiratory type and then search for DNase.
- For gram-negative bacilli: search for oxidase and if the latter is positive a seeding of the colony is done on MH agar and on Basal Medium plus glucose for the identification of pseudomonas. For gram-negative bacilli with negative oxidase, a mini biochemical gallery was inoculated on Urea-indole, Kligler, Manitol-Mobility, Simmons Citrate and then on VP-RM medium.

Still for this J2, the antibiogram was carried out with :

- Gram-positive cocci, the following TBA discs: Ampicilin, Amoxicillin + Clavulanic acid, Carbenicillin, Oxacillin, Cefotaxime, Ceftriaxone, Gentamycin, Erytromycin, Nitroxoline, Ciprofloxacin, Sulfadoxin, and Fosfomycin.
- Gram-negative bacilli: Beta-Lactamines (Ampicillin, Amoxicillin + Clavulanic acid, lipenem, Aztreonam, Cefalotin, Cefotaxime, Ceftriaxone); Aminocyclitol (Gentamycin); Quinolones (Ciprofloxacin); Sulfonamides (Sulfadoxin); Polypeptides (Colistin); and Fosfomycin.

All media were incubated at 37° in an oven for 24 hours.

3<sup>rd</sup> day: reading of inoculated media from J2 with finalization of identification tests including DNase, Indole, TDA; VP and MR as well as TBA

reading by measuring antibiotic inhibition diameters and comparing them to the provided reading scale.

### 3. RESULTS AND DISCUSSION

#### 3.1 Result

##### 3.1.1 Samples taken with positivity cases

There are 51 suppurative wounds collected from patients and 30 are hand samples taken, 22 of which were taken before dressing and 18 after dressing. Fig. 1 shows the cases of positivity of these samples.

##### 3.1.2 Survey results

From the individual patient information sheets we noticed that more than half of them (29/51) were already under antibiotic treatment (Beta-Lactamines, Aminocyclitol and Quinolones) and even with imidazoles. Combinations of treatment are sometimes performed.

##### 3.1.3 Isolated germs and their prevalence

The germs isolated in wound healing during our study totalled 76, of which 35 (46%) were gram-positive cocci and 41 (54%) gram-negative bacilli.

For the hand samples 10 germs were isolated, 6 before dressing and 4 after dressing. Their identities and numbers are shown in the Table 1.

##### 3.1.4 The resistance profile of these germs

Regarding the resistance profile of the bacteria isolated from the bacterial species showed strong resistance to most of the antibiotics tested apart from Fosfomycin which is active on almost all strains.

#### 3.2 Discussion

The purpose of this study is first to identify the bacteria involved in wound healing, to establish the resistance profile of these bacteria and then to examine more closely the likely role of nurses in this fact. We had 51 suppurative wound samples, 50 of which were infected with bacteria, to support the fact that pus is generally the result of an inflammatory process due to an interaction between tissue and pyogenic germs [6]. From the nurses' hand samples, cases positive for

germs have been recorded, including 6 before dressing and 4 after, which may be linked to poor hand hygiene, in line with Cooper's statement that "the human body is not sterile", and aseptic hand washing could reduce or even eliminate residential flora for a period of time [7,8].

76 bacteria were isolated from suppurative wounds with a predominance of gram-negative bacilli (54%) versus 46% of gram-positive Cocci, a result similar to that obtained by Rao et al. [9]. Of the bacteria identified in our study, *Pseudomonas aeruginosa* was the most common of the bacilli (29%) and *Staphylococcus aureus* of the cocci (28%), which is similar to the study by Slekovec et al. [10] and Hani et al. [11]. For these authors, these two bacteria are the most isolated from suppurative wounds, with Mohammed et al. showing a good inclination towards *Staphylococcus aureus* [12].

From the hands of the nurses we isolated *Pseudomonas*, *Staphylococci* and *Enterobacteriaceae* before and after the dressing. This shows that nurses are likely to contaminate patients' wounds, as Khoury says. The same author adds that the work environment can also be a source of infection [13]. Jeurissen et al. have shown that the quality of the bandage may promote the penetration of germs, leading to wound healing [14].

As for the antibiotic resistance profiles of these germs, we will start with *Pseudomonas*. The strains isolated were highly resistant to  $\beta$ -lactam antibiotics but highly susceptible to Ciprofloxacin, Ceftriaxone and Fosfomycin. These results are similar to those obtained by Hani and AL [11]. Mohammed et al. [12], as in our study, found that *Pseudomonas* isolated from wounds were sensitive to Ciproflocacin. Although it is reported that *Pseudomonas* are frequently isolated in wound infections and are highly resistant [15]; the emergence of these resistant strains, especially to antibiotics of last resort such as Colistin and Imipenem, should be of concern. Indeed, all strains isolated have been resistant to these antibiotics, but Auajjar et al. and Mesaros et al. have shown that *Pseudomonas* are highly susceptible to these antibiotics [15,16]. According to Rao et al. and Etok et al. [9,17], Imipenem is the most active. But according to Randrianirina et al. less than 50% of the strains would be sensitive to it [18]. *Pseudomonas* are bacteria that accumulate numerous mechanisms of antibiotic resistance. This would explain our results regarding the high resistance of the strains to Imipenem (13/22) and Colistin (22/22).

Next comes *Staphylococcus aureus*, which is generally the most common cause of surgical and traumatic wound infections [19]. The emergence of multi-resistant strains is thought to be one of the main causes of delayed and difficult healing of suppurative wounds. In our study, all strains of staphylococci isolated are resistant to Oxacillin (1 $\mu$ g) and there is a high resistance to sulfonamides. Our results are similar to those obtained by Sani et al. [19], Etok et al. [20] and Ogounshe et al. [21] in terms of the proportions of MRSA. Sani et al. have also shown in studies in Niger that staphylococci are highly resistant to sulfonamides. In contrast, Anas [22] obtained 50% methi-R pathogenic *Staphylococci* and 25% sulfadoxin-resistant *Staphylococci*. Sina et al. [23] in Benin isolated pus, only 42.85% MRSA and 51.42% of the sulfadoxin-resistant strains. The high resistance observed to Sulfonamides, Macrolides and Aminocyclitol antibiotics in streptococci, the second most common cocci involved in wound infections after staphylococci [24], was also demonstrated by Lopardo and Sood et al. [24,25] but in our study they showed a high sensitivity to Ampicillin. The high sensitivity of cocci to Fosfomycin is not surprising given that many studies in recent years have shown that this antibiotic is effective on more than 80% of cocci [26,27,28,29].

*Enterobacteriaceae* make up a quarter (25%) of the bacteria isolated. The high resistance extended to almost all families of antibiotics is similar to that observed by Randrianirina et al and Sani et al. [18,19]. The majority of the *Enterobacteriaceae* isolated from our samples are sensitive to Ciprofloxacin, Sani et al; Tumane and Wasnik [18,30] obtained similar results while Mohammed et al obtained high resistance to this antibiotic.

We noticed that in vitro, the antibiotics prescribed to patients were virtually inactive on the strains taken in isolation. This resistance of the different strains studied could be related to the fragile state of the patients sampled or even due to wound infection by nosocomial strains [31] accidentally colonizing the wounds through work equipment, gowns, bed sheets, the nurse's hands, to name but a few reasons. In addition, it could be natural [31] for some strains or acquired [31,32,33] for others due to the duration of treatment or inappropriate antibiotic prescriptions [34]. Hence the need for judicious use of antibiotics to reduce hospital-acquired infections in general and in the management of wound infections in particular [35].

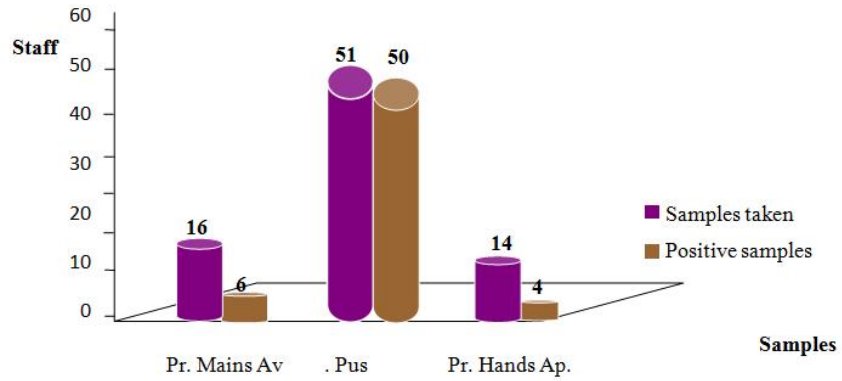


Fig. 1. Number of positive samples as a function of samples taken

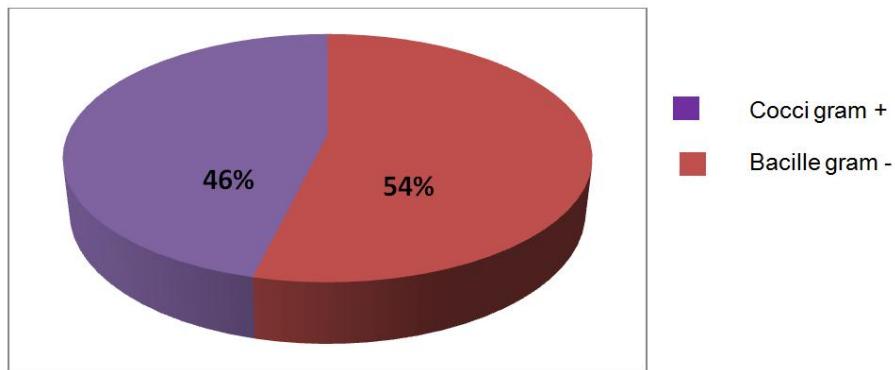
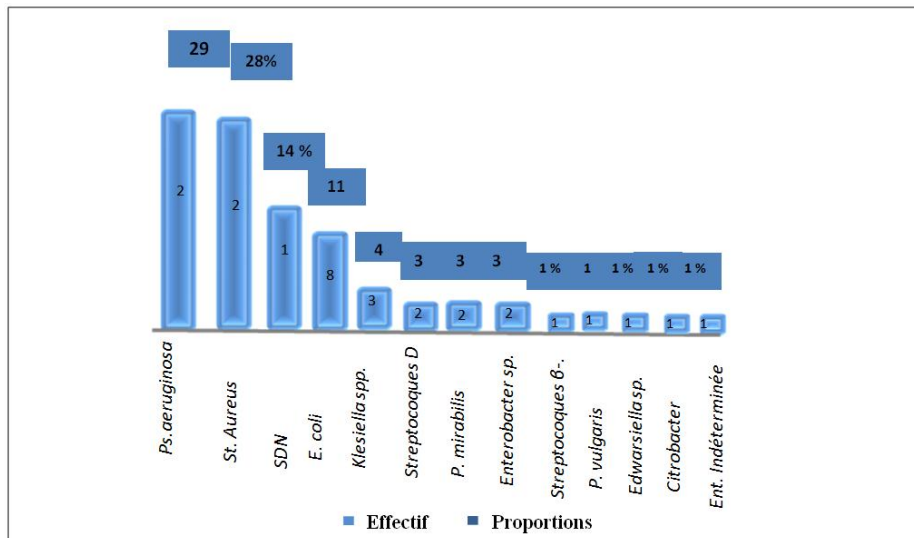


Fig. 2. Distribution of isolated germs as a function of Gram and Fig. 3 reveals the identity of its germs and their prevalences



Legend: SDN: Staphylococcus with DNase Negative - *Ent*: Enterobacterium

Fig. 3. Proportions of Bacteria Isolated from Suppurative Wounds

**Table 1. Hand samples**

| Isolated bacteria |                | Hand samples    |                |       |
|-------------------|----------------|-----------------|----------------|-------|
|                   |                | Before dressing | After dressing | Total |
| No Pathogen       | SDN            | 02              | 01             | 03    |
| Pathogen          | St. Aureus     | 01              | 00             | 01    |
|                   | K. pneumoneae  | 01              | 01             | 02    |
|                   | Ps. Aeruginosa | 02              | 02             | 04    |
| Total             |                | 06              | 04             | 10    |

#### 4. CONCLUSION

At the end of our study and in view of the results obtained we can say that the following bacteria : *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *DNase-negative Staphylococci*, *Escherichia coli*, *Klebsiella (K. oxytoca, K. pneumoniae)*, *Streptococci*, *Proteus*, *Enterobacter*, and *Citrobacter* are those involved in the suppuration of skin wounds and most of these germs were found on the hands of the nurses who dress these wounds. This underlines the need to promote hygiene measures, with aseptic hand washing being at the forefront of these measures, without overlooking the fact that the dressing kit is single-use. Isolated bacteria have shown strong resistance to several tested and commonly prescribed antibiotics including  $\beta$ -lactam antibiotics, aminoglycosides, macrolides and polypeptides. Only fosfomycin and quinolone antibiotics were active on the majority of the strains isolated. It is therefore necessary to develop a program to fight against nosocomial infections and against multiple bacterial resistances involving the entire health care chain: Ministry of Health, clinicians, nurses, biologists; and pharmacists. The promotion of our natural essences would be, above all, a great help in the fight against multi-resistant germs.

#### CONSENT

51 pus samples were collected from any patient admitted to Menontin Hospital of any age and sex after their consent was obtained.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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