(RESEARCH ARTICLE)

# Biochemical profile and resistance phenotype of bacteria isolated from the operating site departments of the National Reference University Hospital of N'Djamena

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## Abstract

The aim is to assess the level of contamination of wound bacteria in operated patients in the surgical departments of the National Reference University Hospital (CHURN) of N'Djamena.

From August 1, 2018 to August 1, 2019, an observational culture study on wound pus was carried out in patients operated on from the surgical services of the N'Djamena CHURN according to standard methods of medical microbiology.

Of the 1092 patients operated on, 565 patients were released within a normal period of hospitalization and 527 in contact with the pathogens were maintained. Significant differences were observed between the proportions of positive (86%) and sterile (14%) cultures; female (30.36%) and male (69.63%) operated subjects with probabilities of 0.02 and 0.001 respectively.

*Escherichia coli* were the most common germs (32.7%), followed by *Staphylococcus* spp (20.9%). The bacteria isolated were resistant to beta-lactam antibiotics at an average rate of 40%, only imipenem, a last-resort antibiotic, was very sensitive (99.5%).

In view of these results, we recommend that prescribers avoid prescribing antibiotics without laboratory evidence for fear of losing the beta-lactams permanently.

Keywords: Infection; Surgical site, Antibiotic; Resistance; N'Djamena.

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# 1. Introduction

Surgical Site Infections (SSI) are hospital infections. They are related to the pre and / or post-operative elements. The term "SSI" replaces that of surgical wound infection [17].

ISO is defined by the presence of pus from a wound in the following locations:

- Superficial part of the surgical incision (skin and subcutaneous tissue);
- Deep part of the surgical incision (soft tissue, deep below the fascia);
- Cavity or organ near or far from the surgical site but linked to the procedure. [17, 6].

An SSI is considered nosocomial when it is neither present nor incubating on entry; it occurs within 30 days of the operation. This period is extended to one year in the event of placement of artificial prosthetic material [6]. Nosocomial infections are a worrying public health problem due to their frequency, cost and severity. The World Health Organization (WHO) estimates that an average of 190 million people is hospitalized each year worldwide and that 9 million of them contract a nosocomial infection during this time [6].

Statistics relating to the frequency of postoperative infections rank that of the operative site second after urinary tract infections [2]. They are the leading cause of morbidity and mortality in surgery [3].

In France, in 2004, the number of people operated on was estimated at 6 million per year and 2 to 3% of them developed an ISO. This rate is confirmed by the result of the common database (RAISAIN) of the 5 investigation and surveillance networks for the fight against nosocomial infections (CCLIN): In 1998 and 1999, on three types of interventions combined, 2551 ISO were declared for 102228 acts [6].

ISO is the second most frequently reported type of nosocomial infection in the United States [14].

In sub-Saharan Africa the incidence of surgical site infections was 6.8 in Kenya, 11.4% (Ethiopia), 23.6% (Nigeria) and 26% in Tanzania [12].

In Chad, a survey carried out from January 28 to February 22018 at the N'Djamena National Reference Hospital-University Center (CHU-RN) reported that the prevalence of nosocomial infections was 16.8% [23].

ISOs increase significantly and patient mortality is due to microorganisms for which the study of antibiotic sensitivity is essential to guide antibiotic therapy and improve management. It is with this in mind that our study, the objective of which was to assess the level of contamination of bacterial agents involved in the ISOs of the General Surgery, Urology and Traumatology departments of the CHU-RN, falls within this perspective.

# 2. Material and methods

## 2.1. Framework, Type and period of study

Our study took place in the bacteriology unit in the CHURN laboratory. The pus samples were taken in the General Surgery, Urology and Traumatology Departments of the said hospital.

This was a prospective observational study that took place in N'Djamena over a period from August 01, 2018 to August 01, 2019 at CHURN.

## 2.2. Study population

Our study concerned patients operated on and hospitalized during this period in the departments of General Surgery, Urology and Traumatology.

## 2.3. Inclusion and non-inclusion criteria

Were included in our study, patients who were operated on and hospitalized for at least 72 hours and who were in contact with ISOs from August 01, 2018 to August 01, 2019 in the General Surgery, Urology and Traumatology departments.

Patients hospitalized in the departments concerned but not operated on and operated on and not having contact with the ISOs were excluded from our study.

## 2.4. Collecting samples

A search for SSIs was performed in 527 patients, aged 15 to 90 years, hospitalized in operative sites with informed consent.

Wound pus samples were collected in each hospital ward, by syringe puncture or by swabbing. The sample is then sent to the laboratory department with bulletins containing information about the patient (hospitalization department, last name, first name, age, sex, profession and address).

## 2.5. Data processing

Data was entered and analyzed using Microsoft Word and Excel 2013.

The chi-square test ($\chi^2$) was used for the comparison of qualitative variables with a significance level set at 0,05.

## 3. Microbiological analysis

### 3.1. Macroscopic examination of pus

*3.1.1. Macroscopic examination of pus consisted of noting:*

- The smell of pus: foul, excrement;
- The consistency of the pus: liquid, thick or sticky;
- The color of the pus: yellow-green, red-brown, whitish or greenish;
- Microscopic examination.
- Microscopic examination was performed before and after the culture:
- The morphology of the germs isolated was studied on a smear stained with Gram;
- The study of cultural characteristics focused on the appearance and color of the colonies.

### 3.2. Culture and antibiogram

*3.2.1. Culture of bacteria*

It consisted of inoculating pathological products on culture media (Hektoen, chapman, chocolate agar) by the streak method. It is always done near the flame of a Bunsen burner and the boxes with inverted lids have been incubated in an oven at 37 ° C for 18 to 24 hours.

*3.2.2. Biochemical identification*

The test for catalase was carried out after isolation on Chapman's medium or 24-hour chocolate agar, a distinct colony is taken and placed on a slide, a drop of hydrogen peroxide $H_2O_2$ will be added to the colony.

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide ($H_2O_2$) into H2O and ½ $O_2$ producing a foaming solution. *Staphylococcus* were tested catalase positive and *Streptococcus* catalase negative.

The coagulase search was carried out from a 24-hour culture on Chapman medium, a characteristic colony is suspended in physiological water, using a Pasteur pipette, 1 mL is transferred into 9 mL of (BHIB). At the end of an 18 h incubation at 37 ° C, if the medium is cloudy, 0.5 mL of the latter is taken and 0.5 mL of human plasma is added and placed in an oven at 37 ° C. The plasma coagulation is observed every 3 min and then after 30 minutes, 1 hour, 2h, 4h and 6h.

Oxidase test: the oxidase test is done on a fresh colony from Mueller-Hinton agar. In practice, a colony of germs to be studied is taken with the taper of a Pasteur pipette or a platinum loop, and it is crushed on a filter paper impregnated with a 1% solution of tetramethyl-para. phenylene diamine. Oxidase negative organisms will remain colorless and positive, turning purple after 10 seconds. Stains appearing beyond this time are not taken into account.

TDA (Tryptophan Deaminase) can also be detected in urea by adding 2 drops of TDA reagent, the spontaneous appearance of dark red brown color shows that TDA is positive.

ONPG (Ortho Nitro Phenyl Galactosidase), the ONPG disc is deposited in a bacterial suspension of 1 mL of distilled water, the yellow color release in the suspension shows its use by bacteria and attests to the demonstration of Bêtagalactosidase.

To better identify the bacteria isolated during this study, API galleries were used. API galleries are systems for the identification of Gram-negative bacteria and Gram-negative bacteria using standardized and miniaturized biochemical tests, in microtubules in dehydrated form. The principle is based on the inoculum of microtubules with a bacterial suspension which rehydrates the media. Incubation is carried out at 37 ° C for 24 hours during which biochemical reactions take place (decarboxylation, fermentation, and deamination.) Which result in spontaneous colored products revealed by the addition of reagents.

The identification of bacteria is obtained using the API catalog. The catalog provides the identification of a large number of profiles obtained on API, which confers great reliability in the interpretation of the results.

The API 20 E galleries (Bio Mérieux) were used to characterize Enterobacteriaceae, *Pseudomonas* and the API STAPH and API STREP (Bio Mérieux) galleries, for the detection of *Staphylococcus* and *Streptococcus*.

### 3.2.3. Antibiogram

Two methods were used to perform the antibiogram: the manual method and the automated method with the Vitek Compact:

### 3.2.4. Manual methods.

This method, by diffusion, uses discs whose active principal load is variable according to the antibiotics tested. The antibiotics were deposited on an agar culture medium, inoculated by flooding or swabbing. The antibiotic diffuses into the agar creating a zone of inhibition of growth of the germ around the disc, depending on the diameter of this zone of inhibition the strains can be classified as sensitive (no growth), intermediate (growth in the well weakly dosed in antibiotic) or resistant (grows in both cups).

### 3.2.5. Principle

The principle of this consisted in placing paper discs impregnated with the different antibiotics tested on the agar surface of a Petri dish, each antibiotic diffuses into the agar and its concentration is found inversely proportional to the distance from the disc. It is a simple agar diffusion method using discs to determine the susceptibility of bacteria to antibiotics.

The bacteria were cultured on Muller Hinton agar (Bio Mérieux) for 18 to 24 hours so that they were in the exponential phase of growth. From this culture, a few colonies were picked and emulsified in sodium chloride saline solution (0.9% NaCl). The turbidity of the inoculum was measured with Densimat (Bio Mérieux) adjusted to 0.5 of the Mac Farland scale in accordance with the recommendations of the CA-SFM (2018). Tenth (1/10) dilutions of these suspensions were made in physiological water.

### 3.2.6. Seeding

The inoculation was carried out from a pure young strain of 18-to-24-hour culture, isolated on MH agar. The bacterial inoculum was diluted in a sterile 5 mL hemolysis tube. This inoculum was adjusted to an optical density of 0.5 Mc Farland as recommended by CA-SFM 2018). Fifteen (15) minutes after adjustment, a swab was dipped into the inoculum. The soaked swab was pressed firmly against the bottom wall of the tube just above the level of the liquid while rotating it to remove the excess inoculum. This squeezed swab was rubbed three times on the agar surface, rotating the dish 60 ° each time, after strongly. This is to achieve an even distribution of the inoculum. The inoculated dishes were left in the microbiological safety hood for 15 minutes before the application of the antibiotic discs.

### 3.2.7. Choice of antibiotic discs

We have selected a total of 18 antibiotics from different families (Beta-lactams, Sulfonamides-Trimethoprims, Cyclines, Aminoglycosides, Fusidic acid, Macrolides, Glycopeptides, Quinolones and Fluoroquinolones). Antibiotic discs tested include: penicillin (PEN: 10 IU), oxacillin (OXA: 5 µg), amoxicillin / clavulanic acid (AMC) (20/10 µg), ceftriaxone (CRO) (30 µg) , amoxicillin (AMX) (30 µg), ceftazidime (CAZ) (30 µg), imipenem (IMP) (10 µg), gentamicin (CN) (10 µg), amikacin (AK) (30 µg), tobramycin (TOB) (10 µg), nalidixic acid (NA) (30 µg), ciprofloxacin (CIP) (5 µg), ofloxacin (OFX)

(5 µg) and trimethoprim-sulfamethoxazole (SXT) (1.25 / 23.75 µg), erythromycin (Ery) (15 IU), fusidic acid (FA) (10 µg), tetracycline (TE) (30 IU), vancomycin (VA) (30 µg).

### 3.2.8. Disposal of antibiotic impregnated discs

The discs are deposited firmly on the surface of the inoculated and dried agar. The number of discs deposited per box is limited due to the overlap of the zones of inhibition and to limit interference between the antibiotics. It is important that the diameters of the inhibition zones are measurable.

## 3.3. Seven discs were placed for the 90 mm diameter boxes.

### 3.3.1. Incubation of Petri dishes

The dishes were inverted within 15 min after depositing the discs and then incubated in a 37 ° C incubator for 18 to 24 hours. The stack of boxes in the oven was three boxes to avoid temperature differences related to the heating and ventilation system.

### 3.3.2. Measurement of zones of inhibition and clinical categorization

The border of the zone of inhibition was read with the naked eye and at the level of complete culture inhibition. Each diameter of the inhibition zones was read to the nearest millimeter using a graduated ruler. Clinical categorization was done in accordance with the recommendations of CASFM (2018) / EUCAST (2017). Intermediate category antibiotics were also counted as resistant.

### 3.3.3. Detection of Extended Spectrum Beta Lactamase (ESBL) production

The demonstration of ESBL was made on Muller-Hinton agar by the double disc synergy test method according to the procedure of Jarlier et al. (1988). The strains to be tested were inoculated on the agar by the standard antibiogram method. Cefotaxime (30 µg), cefixime (30 µg), cefoxitin (30 µg), and vancomycin (30 µg) discs were placed 20 to 30 mm (center to center) from an amoxicillin disc + clavulanic acid (20/10 µg) then incubated at 35 - 37 ° C. After 18 to 24 hours of incubation, the production of ESBL by the organism tested was based on the partial inhibition of ESBL by clavulanic acid. The existence of even a weak synergy between cefotaxime, cefixime, cefoxitin, vancomycin and clavulanic acid is characterized by a champagne cork-shaped image.

### 3.3.4. Automated method (VITEK)

The system consists of the VITEK® 2 Compact instrument, a computer (workstation) and a printer. The software supplied with the VITEK® 2 Compact system includes programs for analysis and data management. A two-way computer interface automatically transfers results to the User's Laboratory Information System (LIS) and to various product and patient reports. A quality control system is available to validate a VITEK® 2 Compact System test kit. An Advanced Expert System ™ (AES) (clinical use) is available, in order to allow systematic and online validation of the results and an interpretation of the resistance phenotypes that have been demonstrated by the antibiograms.

### 3.3.5. Principle

This technique makes it possible to determine the sensitivity of bacterial agents to antibiotics in a semi-liquid medium. The VITEK®2 card includes 64 reaction cups comprising 64 antibiotics at 64 different concentrations. The reading is taken at 660 nm. The growth rate analysis is performed every 15 minutes in kinetics. Then, for each antibiotic, a specific algorithm converts the raw values (RTU) into the calculated MIC. To calculate the MIC, the machine checks the filling of the wells, then checks the raw values. It eliminates raw outliers associated with background noise and difficult-to-suspend strains. Then, it determines the incubation time of the card. In the control cup, it evaluates the speed of growth of the germ. The reading will stop if there is sufficient growth in all wells. This will be considered the highest that can be measured for that antibiotic.

Finally, the MIC results are interpreted in S-I-R (sensitive-intermediate-resistant) according to the specific critical concentrations of the different committees.

Preparations of the inoculum for the isolation of bacterial agents were carried out according to the manufacturer's procedures and procedures. Using a dispenser, we distributed 3 mL of saline solution (Reference 1204, 500 mL, Nacl 0.45%) in the 5 mL tubes classified in a cassette. Then, using a Pasteur pipette, a colony of bacteria or yeasts was suspended in 3 mL of saline solution, well mixed and then the optical density was checked with DensiChek McFarland (0.5-0.63) McF for Gram (-) and Gram (+) bacteria.

For each suspension, there is a biochemical and antibiogram identification. A Gram (-) V1 221 pipette (0.5-250 µl) was used to distribute 145 µl of identification suspension in 3 ml of saline solution for antibiogram (GN = Gram (-) and AST = corresponding antibiotic) for each identification. A Gram (+) V1 222 pipette (100-1000 µl) was used to distribute 280 µl of identification suspension in 3 ml of saline solution for antibiogram (GP = Gram (+) and AST = corresponding antibiotic) for each identification. The biochemical identification cards, antibiogram were inserted in the suspensions arranged in the cassette and the whole was introduced into the VITEK2. Once the cassette has been inserted into the VITEK2, we launch the loading and the Vitek2 reads the bar codes of each card and then the sealing. At the end of the sealing, we remove the identification cassette and the VITEK2 proceeds to analyzes. Vitek2 gives the minimum inhibitory concentration of antimicrobials according to the European Antibiotic Committee (CAEU).

## 4. Results

### 4.1. Study population, laboratory and clinical parameters of patients

The study concerned 527 patients, aged 15 to 90 years, including 367 (70%) men or 3/4 and 160 (30%) women followed in the various surgical departments (General Surgery, Urology and Traumatology).

The average age of the patients was 52.5 years with the extremes of 15 to 90 years and 43% of them belonged to the age group of 20 to 30 years.

### 4.2. Prevalence of different germs isolated in pus

Table 1 shows the distribution in number of cases of opportunistic microorganisms isolated from the pus of different surgical departments.

Of the 527 cultures performed, 453 cultures were positive (86%) and 74 (14%) sterile cultures ($x^2$ = 5.731 dof = 1, $p$ = 0.02 significant difference for the positive culture).

### 4.3. Distribution of germs by services

Table 1 below shows the distribution of germs according to the services.

During the investigation, 425 (94%) bacterial agents were identified with a predominance of *Escherichia coli* 139 (32.7%) followed by *Staphylococcus* spp 89 (20.9%).

**Table 1** Distribution of germs according to services

| Bacterial agent | GRAM | | Services | | |
|---|---|---|---|---|---|
| | | NT (%) | Visceral surgery | Urology | Traumatology |
| *Escherichia coli* | BGN | 139 (32.7) | 31 | 61 | 47 |
| *Staphylococcus* spp | CGP | 89 (20.9) | 24 | 38 | 27 |
| *Streptococcus spp* | CGP | 52 (12.3) | 13 | 24 | 15 |
| *Staphylococcus aureus* | CGP | 51 (12) | 14 | 19 | 18 |
| *Proteus* spp | BGN | 23 (5,5) | 5 | 11 | 7 |
| *Pseudomonas aeruginosa* | BGN | 19 (4,6) | 2 | 9 | 8 |
| *Proteus mirabilis* | BGN | 16 (3,8) | 3 | 7 | 6 |
| *Pseudomonas* spp | BGN | 16 (3,8) | 1 | 9 | 6 |

BGN = *GRAM negative bacillus.*; CGP = GRAM positive Cocci; NT = Number tested; % = percentage

### 4.4. Distribution of patients by sex

Female subjects operated on were 30.36% (160/527) and 69.63% (367/527) patients were male ($x^2$ = 22.031> $x_0^2$> 3.84, $p$ = 0.001, dof = 1, significant difference in favor of the masculine gender).
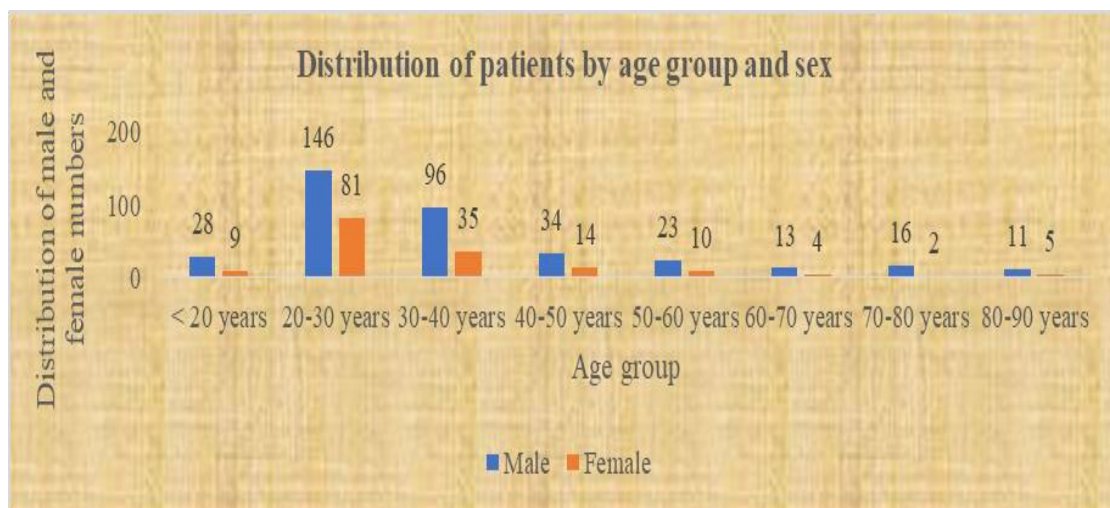
The male / female sex ratio is 2.29 (367/160) for operated subjects with positive cultures (Figure 1) and 3 (819/273) for all operated subjects.
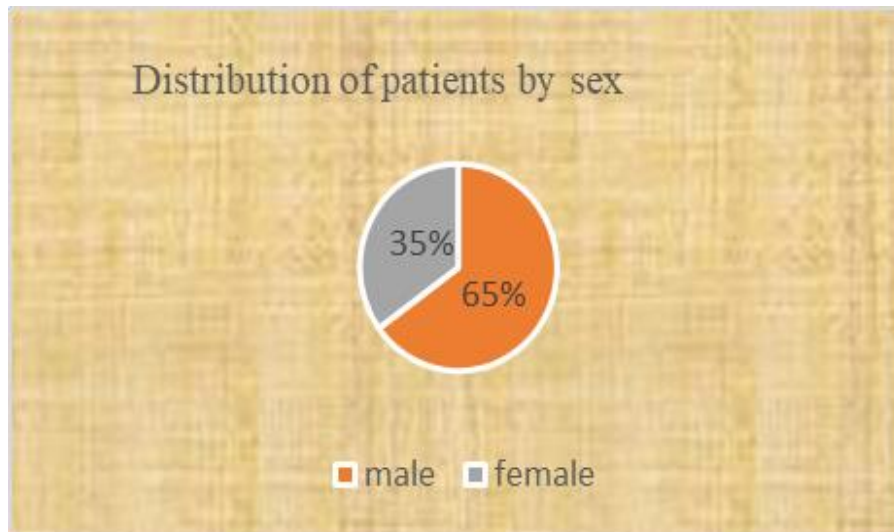


**Figure 1** Distribution of patients by sex

## 4.5. Distribution of patients by age group and sex

The following figure shows the distribution of patients by age group and sex



**Figure 2** Distribution of patients by age group and sex

## 4.6. Distribution of patients by profession

Table 2 below shows the distribution of patients by profession. During our study, the most represented profession was that of pupils and students 146 (27.8%).

**Table 2** Distribution of patients by profession

| Profession | Effective | % |
|---|---|---|
| Pupil / Student | 146 | 27.8 |
| teamster | 82 | 15.7 |
| Unemployed | 69 | 13.1 |
| Housewife | 63 | 11.9 |
| Functionary | 59 | 11.1 |
| Trader | 59 | 11.2 |
| Cultivator | 49 | 9.2 |
| **Total** | **527** | **100** |

## 4.7. Distribution of patients according to the sampling site

The abdomen was the most frequent harvest site with 284 (53.9%) followed by the lower limbs (29.5%).

**Table 3** Distribution of patients according to the sampling site

| Sampling site | Number | % |
|---|---|---|
| Abdomen | 284 | 53.9 |
| Trunk | 8 | 1.5 |
| Upper members | 80 | 15.1 |
| Legs | 155 | 29.5 |
| Total | 527 | 100 |

## 4.8. Morphological characteristics of isolated bacteria

After 24 hours of incubation, the *Staphylococcus aureus* degrading mannitol into lactic acid on Chapman, the modification of a pH indicator indicating the consumption of a sugar and the lowering of the pH causes the acidification of the medium and the appearance yellow in color. The whitish and greyish colonies in the same medium were characterized as groups of *Staphylococcus* spp.

Opaque yellow colonies in Hektoen medium were suspected to be *Escherichia coli*. In 24 to 48 hours, there is often a blue or greening of the culture media due to the diffusible pigments produced by the *Pseudomonas* colonies in the Hektoen medium. Grayish colonies in chocolate agar medium were suspected of *Streptococcus*.

## 4.9. Assessment of the sensitivity of 11 antibiotics to bacteria isolated from ISOs

Table 4 below shows the sensitivity of the 11 antibiotics to the different strains isolated during the investigation.

## 4.10. Beta-lactam susceptibility profile of bacterial agents

Table 4 below shows the distribution of the germs isolated according to their sensitivity to antibiotics.

## 4.11. Biochemical profile of bacterial agents isolated from the carbohydrates tested

Most of the isolated Staphylococci were: Catalase +, ADH (arginine hydrolase) +. All *Staphylococcus* aureus were coagulase positive. They ferment glucose and sucrose.

Most of the *Escherichia coli* identified were ONPG +, Indole + and fermented many sugars (carbohydrates).

The *Pseudomonas* isolated were oxidase +, possessing an oxidative type of carbohydrate metabolism or no action on sugars.

**Table 4** Sensitivity of Bacterial Agents to Beta-lactams

| Bacterial agents | Nbre T | PEN | | OXA | | CAZ | | CRO | | AMX | | AMP | | AMC | | IMP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R |
| *Staphylococcus aureus* | 51 | 15 | 36 | 23 | 28 | 20 | 31 | 26 | 25 | 24 | 27 | 13 | 37 | 24 | 27 | 51 | 0 |
| *Staphylococcus spp* | 89 | 30 | 59 | 28 | 61 | 27 | 52 | 46 | 43 | 49 | 40 | 58 | 31 | 60 | 29 | 88 | 1 |
| *Streptococcus agalactiae* | 8 | 3 | 5 | 2 | 6 | 4 | 4 | 5 | 3 | 2 | 6 | 1 | 7 | 5 | 3 | 8 | 0 |
| *Streptococcus faecalis* | 8 | 2 | 6 | 1 | 7 | 3 | 5 | 4 | 4 | 3 | 5 | 3 | 5 | 4 | 4 | 8 | 0 |
| *Streptococcus spp* | 52 | 22 | 30 | 11 | 41 | 25 | 27 | 29 | 33 | 24 | 26 | 18 | 34 | 34 | 18 | 52 | 0 |
| *Escherichia coli* | 139 | 37 | **102** | 41 | **98** | 52 | **87** | 79 | **60** | 75 | **64** | 39 | **100** | 89 | **50** | 139 | 0 |
| *Enterobacter cloacae* | 4 | 1 | 3 | 2 | 2 | NT | NT | 3 | 1 | 2 | 2 | 1 | 3 | 2 | 2 | 4 | 0 |
| *Proteus mirabilis* | 16 | 6 | 10 | 4 | 12 | 7 | 9 | 10 | 6 | NT | NT | 5 | 11 | 9 | 7 | 15 | 1 |
| *Proteus spp* | 23 | 8 | 15 | NT | NT | NT | NT | 13 | 10 | 6 | 17 | 5 | 18 | 14 | 9 | 23 | 0 |
| *Pseudomonas spp* | 16 | 5 | 11 | 3 | 13 | **10** | 6 | 7 | 9 | 6 | 10 | 5 | 11 | 7 | 9 | 16 | 0 |
| *Pseudomonas aeruginosa* | 19 | 6 | 13 | NT | NT | **13** | 6 | 8 | 11 | 6 | 13 | 5 | 14 | 9 | 10 | 19 | 0 |
| Total (%) | 425 (100) | 135 (31.7) | 290 (68.2) | 115 (27) | 268 (63) | 161 (37.8) | 227 (53.4) | 230 (54.1) | 205 (48.2) | 197 (46.3) | 210 (49.4) | 153 (36) | 271 (63.7) | 257 (60.4) | 168 (39.5) | 423 (99.5) | 2 (0.4) |

PEN = penicillin; OXA = oxacillin; CAZ = ceftazidine; CRO = ceftriaxone; AMX = amoxicillin; AMP = ampicillin; AMC; amoxicillin + clavulanic acid; IMP = imipenem; Nbre T = total number.
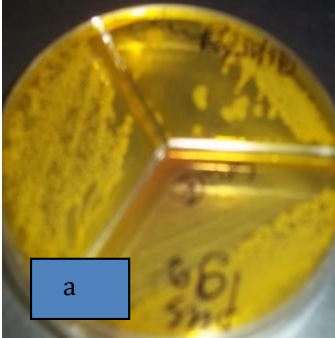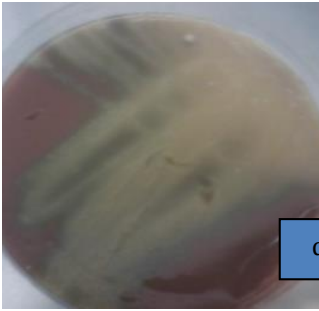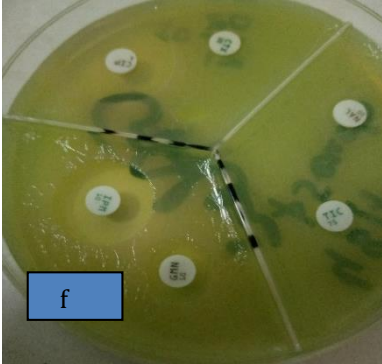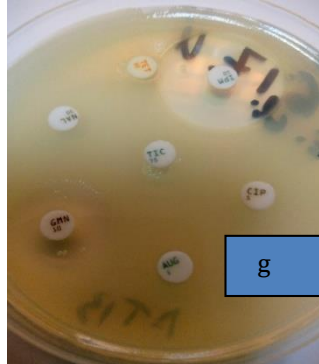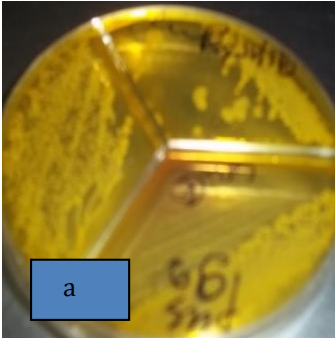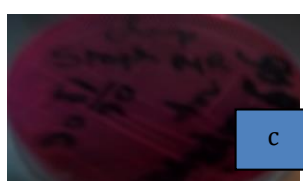
**Table 5** Biochemical profile of bacterial agents isolated from pus in surgical site patients
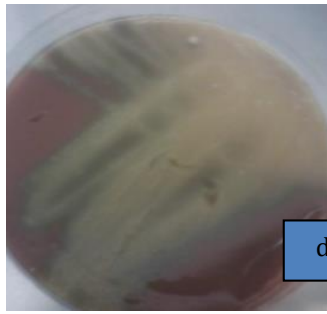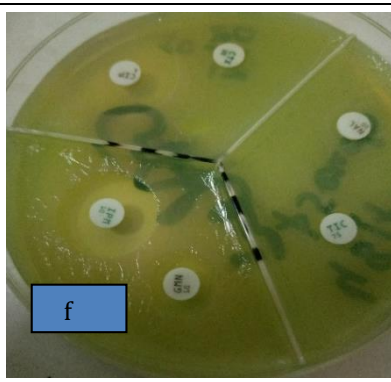
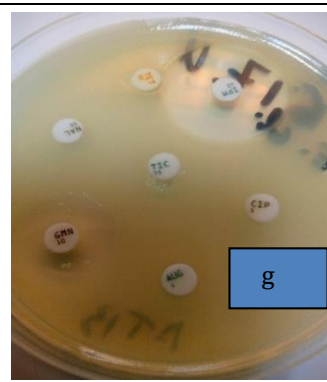| Bacterial agent | Carbohydrate | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ONPG | ADH | LDC | ODC | CIT | H₂S | URE | TDA | IND | VP | GEL | GLU | MAN | INO | SOR | RHA | SAC | MEL | AMY | ARA | COA | CAT | GAL | OX |
| *Staphylococcus aureus* | | + | | | | | +/- | | | + | | + | | | | | + | | | | + | + | | |
| *Staphyloccocus spp* | | +/- | | | | | +/- | | | +/- | | + | | | | | +/- | | | | - | + | | |
| *Streptoccocus agalactae* | | | | | | | | | | +/- | + | | | | | | | | | | - | - | | |
| *Streptoccocus faecalis* | | + | | | | | | | | +/- | + | | | | + | | | | | - | - | + | | |
| *Streptococcus spp* | | +/- | | | | | | | | | + | | | +/- | | | | | +/- | | - | +/- | | |
| *Enterobacter cloacae* | + | - | - | - | + | - | +/- | - | - | + | - | + | + | - | - | +/- | - | - | +/- | - | | + | | - |
| *Proteus mirabilis* | - | - | + | - | - | + | + | + | - | - | - | + | + | - | - | - | - | - | - | - | - | + | | - |
| *Proteus spp* | - | - | +/- | - | - | + | +/- | + | - | +/- | +/- | + | +/- | - | - | - | - | +/- | - | - | - | + | | - |
| *Pseudomonas spp* | - | +/- | - | - | _ | - | - | - | - | - | - | + | +/- | - | - | - | - | - | - | - | - | - | | + |
| *Pseudomonas aeruginosa* | - | + | - | - | - | - | - | - | - | - | - | + | +/- | - | - | - | - | - | - | - | - | - | | + |
| *Escherichia coli* | +/- | +/- | +/- | - | - | - | - | - | + | - | - | + | + | - | - | +/- | - | - | - | +/- | - | + | + | - |

+ = positive (use of carbohydrate by the microorganism); - = negative (non-use of carbohydrate by the microorganism); +/- = sometimes positive or negative.
ONPG = Ortho-Nitro-Phenyl-Galactosidase; ADH = Arginine Hydrolase; LDC = Lysine Decarboxylase, ODC = Ornithine Decarboxylase; CIT = Simmons Citrate; H2S = Dihydrogen sulphide; Urea; TDA = Tryptophan Deaminase; IND = Indole; VP; Vogues-Proskauer; GEL = Gelatine; GLU = Glucose; MAN = Mannitol; INO = Inositol; SOR = Sorbitol; RHA = Rhamnose; SAC = Sucrose; MEL = Melibiose; AMY = Amygdalin; ARA = Arabinose, COA = Coagulase, CAT = Catalase; GAL = Galactose; OX = Oxidase. NB: empty boxes: these are biochemical tests not carried out.

**Table 6** Morphological and biochemical characteristics of the bacteria isolated

| 1 | a: Yellow golden colonies giving the morphological appearance of *Staphylococcus aureus* on Chapman agar after 24 hours culture of pus at 37 o C<br><br>b: Images illustrating the formation of a champagne cork indicating the production of ESBL by a strain of *Staphylococcus* spp<br><br>c : Colonies de *Staphylococcus* spp sur gélose Chapman |  |  |
|---|---|---|---|
| 2 | d: Greyish colonies giving the morphological appearance of *Streptococcus* after 24 hours culture of pus at 37 o C.<br><br>e: Colonies of *Escherichia coli* (pink on Hektoen agar). |  |  |
| 3 | f: antibiogram of *Pseudomonas aeruginosa* resistant to ceftazidine and susceptible to imipenem and ciprofloxacin.<br><br>Greenish colonies indicating the production of pyoverdine and giving the morphological appearance of *Pseudomonas aeruginosa*<br><br>g: antibiogram of *Escherichia coli* multi-resistant and sensitive to imipenem. |  |  |
| 4 | Biochemical characteristics of *Pseudomonas aeruginosa* (API 20E Gallery) |  | |
| 5 | Biochemical characteristics of *Escherichia coli* (API 20E Gallery) |  | |
| 1 | a: Yellow golden colonies giving the morphological appearance of *Staphylococcus aureus* on Chapman agar after 24 hours culture of pus at 37 o C<br><br>b: Images illustrating the formation of a champagne cork indicating the production of ESBL by a strain of *Staphylococcus* spp<br><br>c : Colonies de *Staphylococcus* spp sur gélose Chapman |  |  |

| 2 | d: Greyish colonies giving the morphological appearance of *Streptococcus* after 24 hours culture of pus at 37 o C.<br><br>e: Colonies of *Escherichia coli* (pink on Hektoen agar). |  d |  e |
|---|---|---|---|
| 3 | f: antibiogram of *Pseudomonas aeruginosa* resistant to ceftazidine and susceptible to imipenem and ciprofloxacin.<br><br>Greenish colonies indicating the production of pyoverdine and giving the morphological appearance of *Pseudomonas aeruginosa*<br><br>g: antibiogram of *Escherichia coli* multi-resistant and sensitive to imipenem. |  f |  g |
| 4 | Biochemical characteristics of *Pseudomonas aeruginosa* (API 20E Gallery) |  h | |
| 5 | Biochemical characteristics of *Escherichia coli* (API 20E Gallery) |  i | |

## 5. Discussion

Our study took place at the Bacteriology Unit of the laboratory of the National Reference University Hospital of N'Djamena from August 1, 2018 to August 1, 2019. It was a prospective observational study during which we analyzed 527 pus samples from the sites. Operations from the General Surgery 145 (27.5%), Urology 211 (40%) and Traumatology 171 (32.5%) departments.

The study involved the remaining 527 patients. Of the 527 cultures performed 453 were positive (86%) and 74 cultures were negative (14%). This high frequency of positive cultures could be explained in part by the promiscuity of patients in the inpatient departments.

The low culture negative rate in this study could be justified by the fact that all operated patients had received antibiotic therapy.

The average age of the patients was 52 with the extremes of 15 to 90 years and 43% of them belonged to the age group of 20 to 30 years. Our results were comparable to those obtained elsewhere [4, 11, 15]. They were higher than those obtained by Madougou in 2010 in Benin, which had obtained an average age of 40 years out of a sample of 2218 [14].

Age is thought to play a role in the genesis of an infection at the surgical site [19, 18, 20].

Our results could be explained by the fact that the Chadian population is predominantly young. This age group is the most socio-economically active. She is often the victim of road accidents causing open fractures and wounds. According to the World Health Organization (WHO), road accidents are the leading cause of death among young people aged 15 to 29. [17]

Male sex was predominant with a sex ratio of 2.29. This result is consistent with the data in the literature. It is higher than that obtained by Bassolé I. in Ouagadougou in 2012. [13] who also found a male predominance with a sex ratio of 1.63 on a sample of 360. It is lower than that of Madougou in Cotonou in Benin in 2010 which obtained a male predominance with a sex ratio of 3.19 out of a sample of 88 [14]. This male predominance could be explained by the difficulty women have in accessing health care. In the literature, opinions are divided. For some authors, gender is a factor influencing the occurrence of ISO [5, 7, 8].

In contrast, others believe that ISO is more common in women [30].

Our results could also be explained by a high frequency of accidents in men due to their daily activities causing fractures and penetrating wounds. This predominance of SSI on fracture has also been reported by Madougou et al. [14] and by Pivot Diane [16] who found 88.7% and 75.2% respectively of fractures.

In our study, the most represented profession was that of pupils / students (27.7%) followed by truck drivers with 15.6%. This result is comparable to that found by Bouare in Mali in 2010 [9] which found 39% of pupils / students and higher than that of Mariam who found in 2012 in Mali 12.5% of pupils / student and 25% civil servants [10]. This difference could be explained by the high number of postoperative complications recorded in this vulnerable age group. This result could also explain the fact that, the pupils / students by going daily to their places of course were often victims of the accidents of the public highway requiring most often surgical interventions.

During our study, the search for the germs responsible for SSI resulted in 453 (86%) positive cultures and 74 (14%) sterile cultures: In total, we isolated 453 germs. These results are comparable to that obtained by Ouedraogo in 2011 with 84.4% positive culture in his study entitled bacteriological profile of surgical site infections at the Souro Sanou University Hospital Center in Bobo-Dioulasso in Burkina Faso [22]. It is superior to those obtained by Faye-kette et al. in Côte d'Ivoire with 70% in their study entitled Microbial Epidemiology of Surgical Site Infections (SSI) in a trauma service in Abidjan [8]. This result demonstrates that culture remains the key element in the etiological diagnosis of ISOs.

*Escherichia coli* was the most identified bacterial germ 139 (32.7%) followed by *Staphylococcus* spp 89 (20.9%). These results are comparable to those obtained elsewhere [15]. On the other hand, Katienga in 2012 Burkina Faso found E coli 61.5% and *Pseudomonas aeruginosa* 30.7% [19]. Abdoulaye O. et al reported during their study entitled Epidemiological and Bacteriological Aspect of Surgical Site Infections in the Surgery Services of the Niamey National Hospital that the S. aureus species was largely in the majority with 31% [18]. These differences could be explained by the fact that these germs are responsible for infectious hospitalism or nosocomial infection.

The isolates tested showed varying susceptibility to antibiotics. This sensitivity ranged from 33 to 100% to gentamicin. It ranged from 93.7 to 100% for imipenem, which is a last resort antibiotic. On the other hand, these isolates showed strong resistance to other antibiotics of the beta-lactam class. This resistance ranged from 0 to 85.9%. This result is comparable to those obtained by Bessimbaye et al. [1] who reported a prevalence of BMR at 66.6% and ESBL at 33.3% to beta-lactams during their study on the prevalence of bacterial multi-resistance in a hospital in N'Djamena [1].

Quinolones exhibited varying susceptibility to isolates. This sensitivity varies according to the germs: *Staphylococcus aureus* was sensitive 27 times out of 37 tests to nalidixic acid (72.9%), and sensitive 25 times out of 37 tests, or 78.3% to ciprofloxacin. *Escherichia coli* was 77% resistant to nalidixic acid and sensitive to norfloxacin 16 times out of 24 tests, or 66.6%. These results corroborate those of Bassolé in Burkina Faso in 2012 [13], who found a sensitivity of 64.9% of bacteria to ciproflaxacin.

During our study, *Escherichia coli* was 100% susceptible to imipenem. This result is superior to that of Bassolé in Burkina Faso who reported a 95.7% sensitivity of *Escherichia coli* to imipenem [13]. However, there was variable resistance in *Escherichia coli* to other beta-lactams: 73.9% resistance to penicillin and 71.9% to ampicillin. This result is comparable to that of Bessimbaye et al. [1] who reported a 64% resistance of *Escherichia coli* to beta-lactam. This result could be explained by the fact that these bacteria produce broad-spectrum beta-lactamases which allow them to resist antibiotics.

The antibiotic sensitivity study of strains of *Staphylococcus* spp showed a sensitivity of 98.8% to imipenem. Sensitivity of approximately 67.4% to amoxicillin-acid + clavulanic acid. However, they showed variable resistance to other beta-lactams: 68.5% to oxacillin and 66.3% to penicillin. Bassolé found a sensitivity of 83.3% of streptococci to penicillin [13]. These variations in resistance of the different bacterial strains isolated towards antibiotics could be explained by the absence of a strategy for prescribing antibiotics, self-medication, ignorance of the problems of resistance to antibiotics which are the direct causes of inactivity of beta-lactams to isolated bacterial strains.

During our study, 52 strains of *Staphylococcus aureus* were isolated which showed 100% susceptibility to imipenem. However, variable resistance of *Staphylococcus aureus* strains to other beta-lactams has been noted. It was 55.5% on penicillin and 83.3% on ampicillin. Bassolé in Burkina Faso [13] reported a sensitivity of 100% of *Staphylococcus aureus* strains to imipenem and a sensitivity of 81.8% to ciproflaxacin. This resistance to beta-lactams could be explained by the production of extended-spectrum beta-lactamases (ESBL) by bacteria.

The sensitivity of *Pseudomonas aeruginosa* to fluoroquinolones in particular with ciprofloxacin (68%) and ofloxacin (63%) was significant in this study, thus revealing their power against pathogens. However, widespread use can lead to resistance to fluoroquinolones [1].

In this study, *Pseudomonas aeruginosa* showed high resistance to nitrofurantoin (79%), which contrasts with a report that nitrofurantoin is a urinary antiseptic and has better sensitivity and is considered safe by some authors [21]. *Pseudomonas aeruginosa* is widely known for its high rate of resistance and has been reported for this unique ability of intrinsic and extrinsic resistance to drugs commonly used for antimicrobial chemotherapy [24, 25]. This situation is very worrying because it has been confirmed by several studies which have linked multidrug resistance and increased morbidity and mortality [27, 30]. According to the results of this study, all *Pseudomonas aeruginosa* isolated from operated patients were resistant to multiple antibiotics. This high prevalence of multiple antibiotic resistance (MAR) is alarming and in line with the conclusions of previous researchers [26,28]. Resistivity of isolates to various antibiotics could be due to overuse of antibiotics and self-medication resulting in drug resistance, in particular by *Pseudomonas aeruginosa* [29].

## 6. Conclusion

Surgical site infections are frequent complications in surgery and cause morbidity and death. During our study, we analyzed 527 samples and 453 cultures were positive (86%). ISOs affect young populations much more, with a predominance of men. The etiology of SSIs is dominated by germs, most often bacterial.

Probabilistic antibiotic therapy, self-medication, the use of antibiotics at inappropriate doses lead to the selection and maintenance of multi-resistant strains in hospital settings. The cytobacteriological examination of the pus allowed us to identify the responsible germs, the most frequent of which were *Escherichia coli* followed by *Staphylococcus* spp which is increasingly resistant to antibiotics.

The antibiotic most sensitive to the isolated organisms was imipenem with a sensitivity ranging from 93.7 to 100% to isolates. The isolates as a whole showed marked resistance to beta-lactams.

## Compliance with ethical standards

*Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

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