Survey of the Germ Antibiorésistance Implied In Infections of the Surgical Site Infection (SSI) To the General Hospital Of National Reference (HGNR) Of N’Djamena (CHAD)

Hassan Mahamat Ali,1 ; Cheikna ZONGO2 ; Ali Haroun Hissethin3 ; Yeri Esther HIEN4 ; Deniș Erbi5 ; Doumani Djonabaye6 ; Roumane Moukhtar7 ; Abdelsalam TIDJANI8 ; Aly Savadogo9

1, 2, 3 Sectoral Program to Fight AIDS and Sexually Transmitted Infections (PSLS / IST), PO Box: 440 in N’Djamena (Chad)
2, 3, 5, 7, 8. Food Science and Nutrition Research Laboratory (LaRSAN) / University of N’Djamena (Chad)
2, 3, 5, 9. Laboratory of Applied Biochemistry and Immunology (LaBIA) / Joseph Ki-Zerbo University of Ouagadougou (Burkina Faso)
2, 3, 4, 9. Training and Research Unit in Life and Earth Sciences, Joseph Ki-Zerbo University. 03 B.P 7021 Ouagadougou 03
6. Pharmacology and Toxicology Laboratory Center for Research in Infectious Diseases (CRID) Yaounde, Cameroon University of Yaoundé

Abstract:
The objective of this survey is to study the antibiorésistance of germs implied in infections of the operative site to the General Hospital of National Reference (HGRN) of Ndjama to Chad. A survey was conducted between July and November 2018, and a total of 51 cases of surgical site infections have been recorded. The infections were swabbed and streaked on Chapman, Mac Conkey, Cétrimide, Sabouraud and Chocolate agars to isolate the causal germs. API20ES gallery and Vitek2 were used for the identification of the different germ isolates. Antimicrobial resistance profiles of the isolates were determined following classical antibiogram on Muller Hinton agar and Vitek2 analysis. The infections were majorly caused by E. coli (29.4%), Klebsiella spp (22.5%), Staphylococcus aureus (16.6%), Pseudomonas aeroginosa (11.7%), Proteus mirabilis (8.8%), and Enterobacter cloacae (5.8%), and rarely by Serratia odorifera, Shigella sonnei, Corynebacterium spp (0.98% each) and Candida albicans (1.9%). All S. aureus isolates were resistant to penicillin and oxacillin. A few isolates of Klebsiella spp, Enterobacter cloacae, Serratia odorifera and Shigella spp. were multiresistant. There is a need of training in hygiene and antimicrobial stewardship at surgery unit of the national referral hospital in N’Djamena (CHAD).

Key words: National General Hospital in N’djamena, Chad, Surgical Site Infection, antimicrobial resistance.

Introduction:
The resistant or multi-resistant microorganisms present a major threat in the world (13). They are responsible for infections nosocomialeses and communal (5). Infections nosocomial are especially a preoccupying reality in services to high risk that receive some extremely vulnerable patients to the colonization of microorganisms and therefore to the infection (10). The Post - Operative Infections (POI) or Surgical Site Infections (SSI) constitute the most important infections with a representation of 15% to 25% (25). They are responsible for morbidity and a very important mortality. They increase the hospitable living length and the cost of cares (29). These infections are the most often caused sometimes by the resistant or multi-resistant bacteria circulating in the hospitable environment communal (23).
An analysis of the SSI in sub-Saharan Africa showed a prevalence spaced out between 6.8-26% (18) according to countries, of the nature of the survey and the encouraging factors the intervening of these infections. In the developed countries the setting up of systems of prevention and surveillances of infections nosocomialeses permitted to reduce the prevalence of these infections, who is SSIs are considered like being one of the first reasons of deaths and the post-operative complications considerably (4-9-30). A recent survey led by the 24 countries Africans showed that, the operated patients are two times more susceptible to die following the post-operative complications after a surgical intervention compared to patients operated in the other continents (3). The SSIs as well as the post-operative complications of it is the main reasons. Chad like the remained countries Africans confronted to these problems. However, studies led on the post-operative complications in a general manner, particularly infections of the operative site are rare or non-existent. Thus, such a survey proves to be primordial in order to give a general preview on the situation of the SSI. From where the objective of this work that aims to determine the frequency of these infections, to isolate the implied germs and to study the antibiorésistance of these germs to the HGRN.

Material and methods:
Setting and period of the survey
The survey has been achieved to the service of the general surgery of the General Hospital of National Reference (HGNR) of Ndjamena in collaboration with the Laboratory of Biochemistry and Immunology Applied (LaBIA) of the University Joseph KI-ZERBO. She took place of July to November 2018.

Type and population of survey:
It is about an analytic prospective survey to the service of the general surgery of the HGRN of Ndjamena. It concerned patients who had developed the SSI and that have an antibiothérapie in progress after having undergone a surgical intervention during the period of the survey. On the other hand, have been excluded in the survey the deceased patients, patients hospitalized in the service but not having undergone a surgical intervention and patients or patient's accompanying parents who refuse to sign the card of consent.

Size of the sample:
The size of the sample was 51 patients having developed some SSI after their surgical interventions in the service of general surgery and the service of emergencies during the period of the survey.

Withdrawal and transportation of samples:
Withdrawals concerned puses gotten by ecouvillonnage on the operated sites, sites of suture, sons or on drainses of patients having developed the SSI. They have been achieved days of bandaging or at the time of the daily visit of patients by surgeons.
Samples gotten by ecouvillonnage have been routed directly to the laboratory for the microbiological analyses without undergoing some previous specific treatments.

Isolation of germs:
The sowing of pus samples has been achieved by streak with the help of a dares or a pipette pastor in glass on the middle Mac Conkey for the research of the Entérobactéries, Chapman for Staphylococcus aureus, the gélose to the Cétrimide for Pseudomonas aeruginosa and the Sabouraud for the Candidases. The chocolate gélose has been used for the research of most the demanding bacteria. On the basis of the protocol of laboratory of the General Hospital of Reference of Ndjamena, the surroundings Mac Conkeys, Chapman, Cétrimide and Sabouraud have not been hatched in aérobiose to 37°C during 18 to 24h. The middle chocolate gélose is placed in aéro-anaérobiose with 5% of CO2 in a jarre of aerobiose, then hatched to 37°C during 18 to 24h. The characteristic colonies of every culture middle have been planted out on the middle Mac Conkey for the demanding germs and Gélose Chocolat (GC) for the demanding germs in view of tests of characterization.

Strains isolation and characterization
The isolated germ characterization has been achieved in several stages. First of all, A morphological characterization of colonies according to their shapes, sizes, aspects and colour has been achieved before the recording. Then, a coloration of Gram before and after recording of every colony. Then a characterization by the minimal gallery (test of oxydase, of the metabolisation of glucose, of the lactose, of the production of H2S, mannitol - mobility, the catalase and the coagulase). Entérobactéries have been characterized by the API20E gallery. All stumps, passed then to the Vitek2 for the
confirmation and also to the detection of the bêta-lactamase production at the isolated entérobactérieses.

**Antibiogramm:**
The antibiogramm has been achieved according to the method of diffusion in surroundings gelose described by Kirby Bauer. Nineteen (19) disks were tested, as indicated in Table 1.

**Table 1 Antibiotics used to study the antibiotic resistance of G- bacteria**

| Antibiotics used for Gram negative bacteria | Doses | Abbreviations |
|--------------------------------------------|-------|---------------|
| Ceftriaxone                                | 5µg   | CRO           |
| Cefotaxime                                 | 30µg  | FOX           |
| Gentamycin                                 | 10 µg | GM            |
| Tobramycin                                 | 10 µg | TOB           |
| Ampicillin                                 | 10 µg | AMP           |
| Amoxicillin                                | 25 µg | AMX           |
| Augmentin (Amox+A.Clav)                    | ½ µg  | AUG           |
| Imipenêm                                  | 10 µg | IMP           |
| Ciprofloxacin                              | 30 µg | CIP           |
| Norfloxacin                                | 15 µg | NOR           |
| Nalixidic Acid                             | 30 µg | NA            |
| Tetracycline                               | 30µg  | TET           |
| Chloramphénicol                            | 30 µg | C             |

**Table 2: Antibiotics used to study the antibiotic resistance of G + bacteria**

| Antibiotics used for Gram negative bacteria | Doses | Abbreviations |
|--------------------------------------------|-------|---------------|
| Cefotaxim                                  | 30µg  | FOX           |
| Gentamycin                                 | 10 µg | GM            |
| Penicillin                                 | 30µg  | P             |
| Oxacyclin                                  | 30µg  | OX            |
| Imipenêm                                   | 10 µg | IMP           |
| Ciprofloxacin                              | 30 µg | CIP           |

**Data analysis:**
The analyses were performed using Microsoft Excel version 2010.

**Isolated stump conservation**
Stumps have been preserved to -8°C in cryotubeses containing the Cœur - Brains Soup (CBS) with 10% of glycerol.

**Results:**
**Prevalence of germs**
Microbiological analysis results show that, on the set of samples (51) appropriated and sowed to the laboratory for the isolation of germs implied, 50 have been revealed positive cultures. Either a percentage of 98.04% of which forty two (42) cultures have been revealed polymicrobiennes (84%) and eight (8) monomicrobienneses (16%).

**Figure 1: Different germs isolated and characterized during the study period**
A total of 102 germs have been isolated and with an average of 2.04 germs per patient. Entérobactérieses constituted the cultivated bacterium majority very well 70 stumps on 102 (68.6%). E. Coli is the germ the more prevail (29.4%) on the set of the isolated stumps, follow-up of Klebsiella spp (22.5%), of Staphylococcus aureus (16.6%), of Pseudomonas aeroginosa (11.7%), of Proteus mirabilis (8.8%), of Enterobacter cloacae (5.8%), of Serratia odorifera, of Shigella sonnei, of Corynebacterium spp (0.98%) each, Candida albicans (1.9%).

**Antibiotic resistance:**
The survey of the isolated stump antibiorésistance frequently concerned antibiotics prescribed within HGRN, of which the accessibility and the availability for the operated patients were possible.

**Enterobacters:**
Curves presented on the figure 2, describe profiles of resistance of the different isolated entérobactéries stumps. The resistance to aminopenicillineses was most important (66/70), of which E. Coli presented a resistance of 90% and stumps of Klebsiella spp, Enterobacter spp, Proteus spp, Serratia spp and Shigella spp a resistance of 100%. This resistance lowered slightly with the association amoxicillin+acide clavulanique for certain stumps (E. Coli: 63.3% and Proteus spp: 55.50%), but remained elevated always for stumps of Klebsiella spp (91.3%), Enterobacter spp (83.30%), Serratia spp and Shigella spp (100%). The resistance to the C3G was 86.41%. Stumps of Enterobacter spp, Serratia spp and Shigella spp presented 100% of resistance to the ceftriaxone. Also, (the last two) these two last, presented 100% of resistance to the cefotaxime. The resistance to the imipenème was from afar weaker for the set of stumps of entérobactéries isolated 3.9% (4/102). From where the resistant stumps are E. coli, Klebsiella spp and Enterobacter spp. The resistances to aminoglycosideses for the set of the isolated entérobactérieses were 65% with a more elevated resistance opposite the gentamycine 80% (48/60). Stumps of Serratia spp and Shigella spp presented a resistance of 100% to the gentamicine. For what is quinoloneses, the rate of the resistance for the set of entérobactérieses was 62%. Serratia spp presented a resistance of 100% to the ciprofloxacine and the norfloxacine. The resistance to phénicoléses was 76.9% for the set of the isolated entérobactérieses. The rate of the resistance to the tétracycline was 91.3% for the set of stumps of entérobactéries tested. On the other hand, they presented a resistance of 100% to the tétracycline but E. coli. It is necessary noted also, a prévalence of 54.2% (38/70) stump producers of bétalactamases have been recorded at entérobactérieses. Of which 17 stumps of E. Coli, 11 stumps of Klebsiella spp, 4 stumps of Enterobacter cloacae, 4 stumps of Proteus mirabilis, a stump of Serratia odorifera and a stump of Shigella sonnei.

**Pseudomonas aeroginosa**
The curve on the present figure 3 the profile of resistance of stumps of P. aeroginosaes isolated. The rate of the resistance to aminopenicillineses and C3G was respectively 75% and 88.8%. This resistance lowered considerably with the association amoxicillin+acide clavulanique (58.3%). A resistance of 65.1% to aminoglycosideses (with 66.6% to the gentamycine and 62.5% to the tobramycine) has been determined. The resistance to quinoloneses was 54.3% (with 58.3% of resistance to the ciprofloxacine, 50% to the acidic nalixidique and 44.4% to the norfloxacine). The resistance to phénicoléses and the tétracycline was respectively 75% and 66.6%.

![Figure 2: Curves representative of the resistance profiles of the Enterobacteriaceae isolated against the antibiotics tested](image-url)
Staphylococcus aureus and Corynebacterium spp

The histogram below (figure 4) presented the profile of Staphylococcus aureus resistance opposite the different tested antibiotics. Levels of the most important resistance have been observed at the level of penicillines (with 100% of resistance to the penicillin and the oxacilline), 82.3% to tetracyclines, 71% to phénicoléses and 64.7% to macrolideses. The resistance opposite the C3GS was 46% (with 50% of resistance has the ceftriazone and 41.1% to the cefotaxime). The rate of resistance to aminoglycosides was 58.8%, to quinoloneses 52.9%, to rifampicines 41.1% and to glycopeptides 5%.

The stump of Corynebacterium was appreciable to all of antibiotics tested, except to the penicillin (100%).

Discussion:

Implied germ

Prevalence of germs polymicrobienses gotten are superior to most results of studies led to Niger: 71% (22), to Benin: 61.6% (8), to the Burkina Faso: 84.8% (24). But, they are near of a survey led to USA by (13) on the surgical infection microbiology that determined a percentage of 78.5%. In certain studies the SSIs seem on the other hand, to be a lot more monomicrobiales (2-23). These differences could explain themselves by differences in the used methods, to the hospitable
ecology, to the nature of studies led and to the fixed objective. It could be also due to types of surgery exercised, to the concerned anatomical sites and to the efficiency of the antibiotic prophylaxis setting up. For what is the negative culture, it is difficult to give the exact reasons, but several hypotheses are obvious. Germs implied in this infection are not arable on the plain environment or they are only other bacteria and mushrooms. Also, these implied germs could be some strict anaerobe of which conditions of withdrawal, of transportation, as well as the test of diagnosis is not required. Or, the antibiotic prophylaxis setting up answered well and that microorganismeses implied in this infection are in moribund state, of which the culture on surroundings geloses could not revive them. In term of the prevalence important of entérobactérieses in this survey it could explain himself by a likely fecal contamination (26) or to the circulation important of these germs to the breast of the aforesaid hospital (1). E. coli was the germ the more implied in this survey, these results are similar to those which gotten by (26) to the Burkina Faso. On the other hand, achieved other studies indicated Staphylococcus aureus as germ the more implied (28-19-8-22). This difference of results could explain himself by the microbial ecology of the hospitable environment and the source of contaminations. Contrary to É. colis and to Staphylococcus aureus classically recovered in the SSI Shigella spp was very little present. The presence of this germ has been signalled in infections of the operative site in a survey achieved in RDC (10). The presence of Shigella spp in this survey pushes fecal contamination hypotheses or contaminations of no one to no one (inter - contaminations), because these bacteria are only present in the fecal matters of patients reached of shigellooses or the healthy carriers. The presence of candida and Corynebacterium spp in the ISOS has been signalled by other studies (2-19-12), what corroborates with our results. The isolated Corynebacterium stump seems to be a wild stump. The presence of Staphylococcus aureus stumps could explain himself by the portage healthy of these stumps by patients, the accompanying of patients or the nursing staffs. The presence of Pseudomonas aeroginosas among the isolated germs could come from flora commensale of the sick of the nursing staffs, of water that it constitutes the normal flora, of humid soil of rooms of hospitalizations or the vegetation of the hospitable environment.

**Antibiorésistance:**
The resistance of entérobactérieses to betalactamineses (amoxicilline, ampicilline, ceftriazone, cefotaxime) was most frequent (92.2%). Our results are similar those gotten by (5) to Cameroon on the evolution of the resistance to antibiotics of entérobactérieses isolated of puses. This resistance important of the enterobacterieses leash to think about a mechanism of resistance enzymatique. Because, in presence of the inhibitor of the betalactamase (acidic clavulanique) a considerable decrease of resistance rate has been observed. Does the production of betalactamase by stumps producers permit the hydrolysis of the cycle lactame that succeeds to the destruction of the active site of the antibiotic molecule (17). In the same way, these antibiotics are the more managed in case of infections in hospitable environment all as in communal environment in the country in development (10-27). What could also explain the level of resistance important of these bacteria opposite antibiotics of the bétalactamine family? The rate raised of the enterobacteries resistance to betalactamineses could be also in relation with the antibiotic prophylaxis that is not adapted really well to seen it of our results. The use inadequate of these antibiotics for the antibiotic prophylaxis also targets bacteria commensaleses, of where by a phenomenon of transformation bacteria pathogeneses having colonized the intestine acquire plasmideses of flora lyse bacteria and integrate in their genetic heritage, what confers them the resistance (17). This observation has also been made by (23) to the Burkina Faso. The resistant stump presence to the imipenemme recorded among entérobactérieses could explain himself by the production of the carbapénmase. This shape of resistant must be taken with a lot of serious, because carbapénmeses are antibiotics of recourses in the treatment of germ shapes multiresistanteses. A proliferation important of the resistant stumps to carbapénmesses could be dangerous for the humanity (20-27).

The presence of the numerous stumps of enterobacterses (Klebsiella spp, Enterobacter spp, Serratia spp and Shigella spp) 100% resistant to phénicoloses, macrolidyses, tétracyclineses, aminoglycosidases and quinoloneses, could explain himself by conditions of hygiene precarious, or to the abusive and very little controlled use of antiseptics in this establishment. These last, puts microorganismeses under pressure,
pushing them to develop all shapes of resistance. She could also be owed to the phenomenon of genetic material exchanges between bacteria (conjugation), because the genetic material exchange between bacteria only requires a narrow contact to permit the directional transfer of DNA of a bacterium donor toward a receiving bacterium (6). What is practically obvious in most our samples (most samples were polymicrobises). The prevalence of bétalactamase enterobacterie producer gotten in this survey (54.2%) was superior to those gotten by (1) and (21) that was respectively 33.3% and 47.72%. This difference could explain himself by the studied stump origin or the rate of bétalactamases stumps producers is in increase.

For what is the resistance of \( P. aeroginosa \), a survey led in Iran by (11) on the emergence of the resistance to the cephalosporine of bacteria to negative Gram implied in infections nosocomialeses had indicated that 73.1% of \( Pseudomonas \) isolated spps were resistant to the cephalosporine, what is similar to our results. The works of (14), on the epidemiology and resistance to antibiotics of \( Pseudomonas aeruginosa \) isolates in a hospital pediatrie Moroccan, had shown a level of weak resistance of \( Pseudomonas aeruginosa \) to aminoglycosides and quinoloneses. This difference of results could explain himself by the hygenic conditions of the hospitable environment and the origin of the source of contamination. But also, by the capacity of \( Pseudomonas aeruginosa \) adaptation to the difficult conditions and the presence of numerous naturally resistant stumps to most antibiotics (15).

It is that could explain the level of resistance of this germ also opposite different antibiotics tested in this survey. The works of (16) in Mauritania on the survey of the sensitivity of stumps of \( Staphylococcus \) communal aureuses in the region of Nouakchott had shown a rate of resistance between 96 and 100% to the penicillin, what is similar to our results. On the other hand, they indicated a weaker resistance rate to the oxacilline (26.3%) that ours. This difference of results could explain himself by the origin likely of \( Staphylococcus aureus \) stumps isolated. The strong resistance (100%) stumps of \( Staphylococcus aureus \) opposite the penicillin and the oxacilline could explain himself by the production of penicillinase and the oxacillinase. These produced enzymes disrupt the activity of these antibiotics by a change of the link of molecules of the antibiotic to the partition of the bacterium returning stumps resistant producers thus (15). The level of production important of these enzymes, as well as the level of important resistance to tetracycline’s (82.3%), to phenicol (71%) and to macrolides (64.7%), could explain themselves by use anarchical, abusive and very little to control these antibiotics in the hospitable and communal environment in Chad. The presence of a resistant stump to the vancomycine (1/17) could explain himself by the exhibition prolonged of the stump opposite this antibiotic (7).

**Conclusion:**

The survey on the antibioresistance of germs implied in infections of the operative site (ISO) to the General Hospital of National Reference (HGNR) of N’djamena (CHAD) permitted to show that the stump \( E. coli \) (29.4%) was the germ the more implied. Also, permitted to note a strong resistance of bacteria isolated opposite the tested antibiotics. A resistance of enterobacteria stumps has the imipeneme has been recorded also. The antibioprophylaxie setting up must be change, because the antibiotic principals used for the antibioprophylaxie with the exception of the ciprofloxacin present a level of very reduced activity. A sensitization of nursing staff and the population for an efficient management of antibiotics is imperative.

**Conflicts of interests: Authors don't declare any conflict of interests.**

Contributions of the author: this survey was the fruit of the memory works, of which the conception, the collection, the analysis and the interpretation of data have been achieved by HMA. The coordination of the survey and corrections of the handwritten have been achieved by Cheikna ZONGO. The final version has been corrected by Pr Aly SAVADOGO, Dr Abdelsalam TIDJANI, Dr Yeri Esther HIEN.

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