Prevalence Multi-Resistant Bacteria in Hospital N'djamena, Chad

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Abstract

Antibiotic resistance is a phenomenon as old as the advent of antibiotics. Today, often of synthetic origin and produced by humans, antibiotics are from natural substances produced by fungi but also bacteria to defend against other bacteria. The first who learned to synthesize antibiotics developed at the same time the means to protect themselves. In assessing the prospects for the emergence of multi-resistant bacteria (BMR) in hospitals in Chad, samples of biological products for patients from different departments of the General Referral Hospital (HGRN) were examined for Search multi-resistant bacteria. 1,285 patients whose average age was 35.5 ± 14 years were included in this study. Isolation of bacteria was made after seeding of organic products on the following agar media: Hektoen, Mac Conkey, OLED, Chapman, Mueller-Hinton (MH) agar and polyvitex. The biochemical identification and antimicrobial susceptibility of bacteria were performed using the compact PLC VITEK© 2TM 15 to HGRN laboratory. Of the 1285 cultures analyzed, 328 (25.25%) were positive for bacterial infection. Of the 328 bacteria isolated and identified, 66 have submitted multiresistant phenotypes to different families of antibiotics for a prevalence rate of 20.12%. Of the 66 multiresistant bacteria, 44 (66.67%) strains of bacteria presented the multidrug-resistant phenotype to several families of antibiotics and 22 strains (33.33%) were only resistant to beta-lactams by producing beta-lactamase extended spectrum (ESBLs) (significant difference p ≥ 0.001). This study suggests a disturbing trend in the emergence of multi-resistant bacteria in Chad and therefore should prompt appropriate corrective reactions.

Keywords: Prevalence; Antibiotic; Bacteria; Multidrug resistance; Beta-lactamase extended spectrum; Chad

Introduction

The discovery of antibiotics in the early twentieth century was a true revolution in the treatment of bacterial infectious diseases [1]. However, the massive and sometimes misuse of antibiotics in medical circles as in self-medication has dramatically altered the microbial ecology and has helped increase the rate of resistant bacteria [2]. The bacteria adapt to is manifested by their ability to appropriate new properties or by modification of their genome (mutations) by acquisition of genetic information via mobile genetic elements such as plasmids and transposable elements [3,4]. Most bacterial species are capable of integrating into their genome, different resistance determinants. And dissemination of resistance genes between bacteria has led to the emergence of bacteria resistant to several antibiotics or multi-resistant bacteria (BMR) [5,6]. Production and dissemination of large amounts of antibiotics, for all uses (human and veterinary medicine, agriculture), formed last half-century a new stress to which the bacterial world has faced little difficulty [7]. This is notably what is going to beta-lactam antibiotics which are the major pillar of antibiotic treatment of infections with enterobacteria. Some potentially pathogenic enterobacteria such as Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae and Enterobacter aerogenes, are resistant to all molecules of this class, including the carbapenem [8]. These strains are often co-resistant to many other antibiotics which can make it very problematic treatment. Infections caused by BMR are associated with high morbidity and mortality, an extension of the length of hospital stay and increased cost of hospitalization [9]. Indeed, in hospital transmission between patients BMR can be done through the nursing staff or through a contaminated environment [10]. Therefore, the aim of this study was to evaluate the level of movement of BMR in hospital and outpatient settings in Chad.

Materials and Methods

Period the study site

It is a study for etiologic diagnostic purposes of multi-resistant bacteria (BMR), which was performed for six months from January to June 2013. The microbiological diagnostic testing biological products (urine, pus diverse, feces, blood, cerebrospinal fluid (CSF) has been made to the bacteriology laboratory unit of the National General Reference Hospital (HGRN) N'Djamena. This Hospital as its name suggests is a central hospital to which all patients middle-level hospitals can be referred if necessary. Every year, 15,000 patients, on average, there are hospitalized.
Population

The surveys were conducted with 10 patients in HGRN services (general medicine, infectious diseases (MI), surgery (visceral and general), gastroenterology, diabetology, pulmonology, urology, Ear, Nose and Throat (ENT) cardiology and diabetology). In addition, it also covered patients received outpatient at the Pavillon des Urgences (PU).

Data processing

The collected data were entered and analyzed using the software Excel 2010. They were treated in the light of the bacterial etiology, the misuse of antibiotics, the origin of the patients, age and sex, and according to the resistance to one or several families of antibiotics. The chi-square test (chi-square) was used to compare qualitative variables with a significance level set at 5%.

Microbiological analyzes and sensitivity

Isolation of bacteria was made after seeding of organic products (feces, urine, pus and various blood) on the following agars: Hektoen, Mac Conkey, CLED, Chapman and poly vitex (BIOMÉRIEUX). After 18 to 24 hours of incubation bacteriological incubator at 37°C, green and bluish colonies with or without black center Hektoen were considered suspicious Salmononella and Shigella. The yellow colonies on the agar Hektoen and yellowish in CLED agar were suspicious of Escherichia coli. On MacConkey agar, E. coli produces pink to red colonies and those of Salmonella and Shigella are small, translucent and colorless.

The colonies of staphylococcus aureus are of yellow color on the edge Chapman agar. Proteus invade all environments in general and have colonies in deep black center. Finally the agar poly vitex, colonies of bacteria are either white or translucent and is the only Gram stain allows the distinguished. The colonies on all agar were subcultured on Mueller-Hinton (MH) for the oxidase test and antigenic studies.

The biochemical identification and antimicrobial susceptibility of bacteria were performed using the compact PLC VITEK® 2TM 15. Using a Dispensette, 3 ml saline 0.45% were divided in tubes of 5 ml classified in a cassette. Then, using a Pasteur pipette, a bacterial colony was suspended in 3 ml of saline.

After homogenization, the turbidity was read with DensiChek McFarland each suspension. For each identification of Gram (-) bacteria or Gram (+), 145 .mu.l or 280 .mu.l of identification suspension were divided into 3 ml of saline for susceptibility testing. The biochemical identification cards and susceptibility testing were included in the suspensions disposed in the cassette and the whole was introduced into the Vitek2 for reading barcodes. The identification cassette was removed and the Vitek2 proceeds to analyze.

The agglutination test was performed following the Kaufmann instructions and White [11] using sera: Shigella versatile type 1, anti-flexneri, boydii and anti-anti-Sonnei (Bio-Rad) Research Shigella; anti-Salmonella (OMA, OMB, WTO, WCO, and the different antisera Vi flagellar H) (Bio-Rad) for the detection of Salmonella. The Vitek2 has determined the minimum inhibitory concentration of antibiotics according to the Committee of European Antimicrobial (CAEU).

To measure the extent of bacterial resistance phenotypes, it was used three cards antibiotic susceptibility of Gram-negative bacteria (AST N103, N222 AST, AST N233) and a susceptibility map of gram +bacteria (AST GP67) and include 13 families with 42 antibiotics tested. These are beta-lactam antibiotics (ampicillin, amoxicillin clavulanic acid+, cephalothin, cefoxitin, cefotaxime, ceftazidime tircaricillin pipercillin/tazobactam, ocxillin); carbapenem (imipenem, ertapenem); quinolone (nalidixic acid, pipemidic acid), fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin nitrofurantoin); aminoglycosides (nétilimicine amikacin, gentamicin, tobramycin); macrolides (erythromycin, azithromycin, spironomycin); tetracyclines (doxycycline, tetracycline, tigecycline, minocycline); glycopeptides (vancomycin); the polypeptides (colistin); trimethoprim sulphonamides (trimethoprim-sulfamethoxazole); lincosamides (lincomycin, clindamycin); the streptogamines (pristamycin, quinapristine-dalfopristin); oxazolidinone (linezolid) and various antibiotics (rifampicin, choramphénicoles ...).

The strains of Escherichia coli ATCC® 25922, Escherichia coli and Pseudomonas ATCC® 35218 aeruginosa ATCC® 27853 (CLS1) served as controls strains for quality control of Viteck2 PLC. The strains were stored at -86°C in brain heart broth (BCC) (Bio-Rad) at 15% glycerol for future following the guidelines of the National Committee on Clinical Laboratory Standards characterizations and Centers for Disease Control [12].

Results and Discussion

Bacterial etiologies

During the six months of the study period, 1,285 samples have been received and cultured in the laboratory of urine HGRN which 855 (66.54%), 171 stools (13.31%), 167 pus (13%), 62 LCR (5%) and 3 blood (0.23%). Among skin cultures, 328 (25.52%) were positive for bacterial infection. Of 328 strains of bacteria isolated and identified, 66 (20.12%) showed multi-antibiotic-resistant phenotypes. Among the multi-resistant bacteria identified, 44 have submitted multi-resistant phenotypes to different families of antibiotics and 22 were resistant only to beta-lactam phenotypes by producing beta-lactamases with extended spectrum (ESBLs) or rate respective prevalence of 66.67% and 33.33% (significant difference: $x^2 = 7.706$, df=1, $p \geq 0.001$). High levels of resistance to beta-lactams in ESBL production has also been reported in Benin [13].

Distribution of patients according misuse of antibiotics

As for the misuse of antibiotics, 865 (67%) patients reported purchasing their drugs at the edge of the streets for their treatment, 420 (33%) testified obtain medical prescription. In addition the abuse of drugs was reported by Bessimbaye et al. [14].

Distribution of multi-resistant bacteria according to the origin of the samples

Table 1 shows the distribution of bacterial strains according to the origin of the various services of the General Hospital of N'Djamena National Reference. The frequency of multi-resistant strains is higher in urology services, infectious diseases (MI) and the Emergency pavilion (PU) with respective prevalence rates of 23%, 15.15% and 26%. It is useful to note that the PU patients are sick from N'Djamena or referred by other hospitals (both in N'Djamena as other regions of Chad) to specialized services HGRN.

Infections caused by multi-resistant bacteria (20.12%) identified in the various departments of the hospital could be caused by nosocomial...
infections due to non-compliance with good basic hygiene practices in hospitals, as already reported by the other authors [15].

### Table 1: Distribution of bacterial strains by origin of patients.

<table>
<thead>
<tr>
<th>bacterial strains</th>
<th>urology</th>
<th>MI</th>
<th>ORL</th>
<th>surgeries</th>
<th>Gastroenterology</th>
<th>cardiology</th>
<th>diabetology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>4</td>
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<td>1</td>
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<tr>
<td>Enterococcus</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td>Shigella spp</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Proteus mirabilis</td>
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<td>0</td>
<td>1</td>
<td>0</td>
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<td>0</td>
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<td>Pseudomonas aeruginosa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 1:** Distribution of bacterial strains by origin of patients.

### Distribution of patients by age and sex

The study included 1,285 patients, aged 8-76 years. The average age of patients was 35.5 ± 14 years, 56% of them belonging to the age group of 20 to 35 years. The sample consisted of 550 women (43%) and 735 men (57.2%).

Patients who had bacterial infection (N=328) consisted of 185 (56.40%) women and 143 (44.6%) of men. Compared to sex, positivity to multi-resistant bacteria was 41 (62.12%) women and 25 (38%) among men (difference not significant: \( x^2=1.076, df=1, p=0.30 \)).

### Distribution of bacterial strains as biologics

Table 2 shows the distribution of bacterial strains as biological products analyzed. A high frequency of bacterial infections caused by *Escherichia coli* and *Staphylococcus* is observed, with prevalences of 38% and 33.33%. These results confirm that UTIs are cosmopolitan and typically relate to *Escherichia coli* [16]. Among the identified *Salmonella*, a strain of *Salmonella paratyphi A* and one strain of *Salmonella paratyphi B* multidrug-resistant were isolated respectively in the pus from a wound of a patient operated at lower leg level in General Surgery Service and the cerebrospinal fluid (CSF) of a patient from the emergency ward of the service. The isolation of these pathogens in different hospital departments testament circulation hospital BMR. The multi-resistant bacteria isolated in pathological biological products in hospitals were also reported elsewhere [17]. *Salmonella typhi* resistant to cotrimoxazole was isolated from the stool of a patient infected with human immunodeficiency virus (HIV) in the Department of Infectious Diseases (SMI). This could be due to the abusive use of cotrimoxazole commonly recommended for primary prophylaxis for opportunistic infections for all patients infected with HIV in Chad according to national guidelines for management of HIV/AIDS.

Table 2: Distribution of the bacterial strains according to the pathological biological products bacterial strains pathological organic products.

<table>
<thead>
<tr>
<th>bacterial strains</th>
<th>urine</th>
<th>selles</th>
<th>Pus divers</th>
<th>sang</th>
<th>LCR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>20</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Shigella spp</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>13</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>3</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>66</td>
</tr>
</tbody>
</table>

Twenty and two strains of *Staphylococcus*, including *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Staphylococcus hominis* were isolated and identified. *Staphylococcus aureus* were isolated from urine samples 6 and 4 in the pus of various
origins (ears and wounds). Five *Staphylococcus hominis* strains were isolated in the urine. September *Staphylococcus haemolyticus* strains were isolated in two samples of urine, blood sampling 4 others in various pus. Such diversity of hospital infections has also been reported [18].

*Salmonella typhi* was isolated from the stool of a sick infected with HIV (HIV) in the service of infectious diseases (SMI). It was resistant to cotrimoxazole. This could be due to the abusive use of cotrimoxazole commonly recommended for primary prophylaxis for opportunistic infections for all patients infected with HIV in Chad according to WHO and UNAIDS guidelines. 22 *Staphylococcus* isolated and identified, there are 10 including 6 *Staphylococcus aureus* in urine and pus in various 4 (ears and wounds). 5 *Staphylococcus hominis* in urine. 7 including 2 *Staphylococcus haemolyticus* in urine, 1 in the blood of a patient hospitalized in the cardiology department and the other 4 were isolated in various pus pus including 3 in 1 ear and into the wound of a sick from the emergency ward of the service (PU). The variety of infections (nosocomial, urinary, endocarditis, septicemia and food poisoning) was also reported [18].

**Distribution of strains of bacteria resistant to several families of antibiotics (BMR) and only those resistant to beta-lactams by producing beta-lactamases with extended spectrum (ESBLs).**

Table 3 shows the distribution of strains based on their resistance or to several families of antibiotics and multi-resistant bacteria (BMR) or to beta-lactams by producing beta-lactamases with extended spectrum (ESBLs). Therefore, the prevalence of strains BMR was 66.67% and that of ESBL was 33.33%. Of the 22 strains of bacteria producing beta-lactam extended spectrum (ESBLs) isolated and identified, three (13.63%) were producing natural penicillinase. They were resistant to penicillin G (MIC ≥ 32), ticarcillin (MIC ≥ 128) and oxacillin (MIC ≥ 4). A strain (4.54%) of penicillinase producing *Proteus mirabilis* was resistant to inhibitors (clavulanic acid, tazobactam or oxacillinases (OXA for "oxacillinhydrolysing abilites") and cephalosporinase extended spectrum (CTX-M "cefoxtimase"). A strain of *Staphylococcus aureus* (4.54%) penicillinase-producing acquired PLP Modified (penicillin-binding protein) induced by the mecA gene was detected. Two strains of *Staphylococcus aureus* (9.1%) were identified producers of cephalosporinases of the 2nd and 3rd generation. Acquired resistance to penicillin G was crossed with all beta-lactam antibiotics but at varying levels depending on the use of antibiotics for the most active molecules. Another example of cross-resistance was observed that resistance to methicillin two (2/10) strains (20%), *Staphylococcus* aureus producing a PLP which also provides resistance to other molecules of the family of beta-lactams. This PLP is encoded by the mecA gene carried by a mobile genetic element integrated into the chromosome of *Staphylococcus aureus*. The other five strains of *Staphylococcus aureus* 5/10 (50%) were resistant to several families of antibiotics.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Phenotypic characteristics</th>
<th>BMR</th>
<th>BLSE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td>16</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td></td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td></td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 3:** Distribution of resistant bacteria strains to multiple antibiotics from several families (BMR) and those resistant to beta-lactam antibiotics by producing beta-lactamases with extended spectrum (ESBLs).

The resistance due to the modification of membrane proteins and by acquisition of genes has also been reported by Kariuki et al. [19]. The acquisition of high-level resistance (MST) was demonstrated in (8/25) of *E.coli* strains (32%) induced by production of cephalosporinases AmpC types (adenosine cyclic monophosphate) is a resistance mechanism increasing the MIC of antibiotics of the family of beta-lactam antibiotics: amoxicillin+clavulanic acid (MIC ≥ 32), cephalothin (MIC ≥ 64), cefotaxin (MIC ≥ 64) and ceftazidime (MIC ≥ 6). But according to the literature, chromosomal cephalosporinases inducible AmpC are usually expressed at very low levels in *Escherichia coli* and *Shigella* and do not contribute significantly to beta-lactam resistance among his species. However the resistance to top level observed in *Escherichia coli* could be explained by the presence of strong promoters mutants (cAMP) that may exist and cause an overproduction of cephalosporinase [20]. An *Escherichia coli* producer of OXA-30 β-lactamase likeness "to oxacillinases" or OXA was also identified in this study and resistant to clavulanic acid. Indeed oxacillinases are characterized by their excellent hydrolysis rate cloxacillin and oxacillin but they are not inhibited by clavulanic acid. The nine (9/22) other strains of bacteria were identified in all producing cephalosporinases and extended spectrum carbapenemases. Furthermore, carbapenemases producing strains have been reported in the case of enterobacteria OXA-48, first described in *Klebsiella pneumoniae* and Turkey in *Escherichia coli* [21]. Such strains were identified as being responsible for multiple outbreaks of nosocomial infections in hospitals in Istanbul and Ankara. This hydrolysis enzyme carbapenem moderately and, more weakly, other β-lactams. The detection of these strains expressing OXA-48 (in the absence of ESBL) is therefore particularly difficult, based on a discrete reduction in sensitivity to carbapenems [22]. The most common is carbapenemase OXA-23 [23]. These enzymes hydrolyze only slightly cephalosporinases of 3rd generation [24].

Among the BMR 44, 21 have developed multi-drug resistance associated families (beta-lactams, glycopeptides, macrolides/lincomasides/ST/rapogranimes) by PLP modification which would be induced by the mecA gene, or inducible by MLSb. Fourteen are resistant to families (beta-lactams, aminoglycosides) by producing ESBL or by acquisition of a plasmid which would be resistance induced asparagine protein due to the presence of nucleotide AAC (6') with high MICs for *Escherichia coli* nètilimicine (MIC ≥ 16), amikacin (MIC ≥ 16), gentamicin (MIC ≥ 16), tobramycin (MIC ≥ 16). A strain...
of Klebsiella pneumoniae MDR-families (beta-lactams, carbapenems) with the higher MIC: ampicillin, amoxicillin-clavulanic acid (MIC ≥ 32), cephalothin, cefoxitin, cefotaxime, cefazidime (MIC ≥ 64) ticarcilline, piperacillin/tazobactam (MIC ≥ 128) ertapenem (MIC ≥ 8). Yet ertapenem is generally active against ESBL-producing Enterobacteriaceae. The observed resistance could be explained by the combination of the production of β-lactamases at a reduced permeability of the outer membrane by the bacterial strain in question.

In France today, the prevalence of resistance to glycopeptides remains low (<2%) and lower in than other European countries like Italy, Greece and Romania. [25]

Salmonella A paratyphi identified, was resistant to beta-lactams by producing ESBL and nalidixic acid (quinolones). A Staphylococcus aureus resistant to beta-lactams by producing penicillinase and families oxazolidones, sufamides by acquiring plasmids induced gene ACC (6') has been identified. It was resistant to antibiotics of these families with the following CMI: ampicillin/sulbactam (MIC ≥ 8), linezolid (MIC ≥ 8), trimethoprim-sulfamethoxazole (MIC ≥ 32) respectively. A Streptococcus pneumoniae was identified in CSF multi-pest resistant families (beta-lactams, glycopeptides, sufamides, streptogamines, tetracyclines, chloramphénicoles, carbapenems fluoroquinolone) and rifampicin with CMI: amoxicillin (MIC ≥ 8) cefotaxime and ceftriaxone (MIC ≥ 4); vancomycin (MIC ≥ 2); trimethoprim/sulfamethoxazole (MIC ≥ 32); chloramphenicol (MIC ≥ 32); levofloxacin, ofloxacin (MIC ≥ 8) and moxifloxacin (MIC ≥ 1); imipenem (MIC ≥ 2) and rifampicin (MIC ≥ 4).

And the 5 other bacteria remaining BMR, there Enterococcus 3 and Enterococcus faecium, having acquired a resistance to amoxicillin and glycopeptides, the number of antibiotics likely to be active is very small and associations with aminoglucosides may be little or no Synergistic [30].

Conclusion

In view of the upsurge of BMR in Chad, adequate measures are necessary. In particular, flawless hand hygiene is the basis of precaution to be applied at all times and by all health personnel to prevent the transmission of germs and protects against contamination in hospitals. Similarly, it is recommended that patients strict implementation of hygiene measures and domesticate plant to minimize contamination by BMR.

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