Original Research Article

Antibiotic resistance and production of extended spectrum β-lactamases by clinical Gram-negative bacteria in Benin.

ABSTRACT

Aims: The aim of this work was to determine the resistance profile and to investigate the production of extended spectrum β-lactamases (ESBL) of clinically relevant Gram-negative Bacillus (GNB) strains.

Methodology: About 191 strains were isolated from 1823 samples collected at the HKM National Hospital and University Center of Cotonou (Benin). Species identification was done with the Api 20th gallery. Two methods were used to search for β-lactamase production: the liquid acidimetric test for penicillinases and double halo method for ESBL. The susceptibility to conventional antibiotic molecules was investigated by the disk diffusion method. PCR was used for the research of $_{bla}$TEM and $_{bla}$SHV genes coding for the production of β-lactamases.

Results: A prevalence of 10.48% of GNB was recorded. Among the isolated strains, 51.31% came from samples collected from inside hospital-patient and 48.69% from out-of-hospital samples. The most contaminated samples were urinating (43.98%), pus (34.58%) and blood (9.42%). Five of the isolated species were in the majority: *Klebsiella pneumoniae* (28.27%), *Acinetobacter spp*. (18.32%), *Pseudomonas aeruginosa* (15.72%), *Escherichia coli* (14.15%) and *Enterobacter cloacae* (12.04%). More than the half (57.07%) of the strains were found to produce penicillinases whereas 16.76% of the isolated strains were ESBL-producing. Only four species, namely *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli* and *Enterobacter agglomerans* were ESBL-producers. The ESBL-producing strains are cross-resistant to beta-lactams. Imipenem is the most effective antibiotic on all isolated strains. ESBL-producing GNB strains possessed both the $_{bla}$*TEM* gene and the $_{bla}$*SHV* gene in a proportion of 25%; 37.5% of the strains had only the $_{bla}$TEM gene and 12.5% of the strains had only the $_{bla}$SHV gene.

Conclusion: GNB are composed of specific pathogenic species and normally commensal species but very quickly becoming opportunistic pathogens. Given this situation, it is necessary to continue research to identify resistance genes.

Key words: Gram-negative bacilli, ß-lactamases, antibiotics, clinic, Benin.

1. INTRODUCTION

The bacteria-related diseases are the second leading cause of death and lost years of productive life worldwide [1]. These bacteria are being involved in 70% of mortality due to humans infectious diseases [2]. Gram-negative bacilli occupy a very important place among the responsible microorganisms of infections [3]. These pathogens are part of different bacterial families such as *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*,

Enterobacter cloacae, *Proteus mirabilis*, *Serratia marcescens* etc.), *Pseudomonas*, *Stenotrophomonas* and *Acinetobacter*. Thought displaying various virulence level, these bacteria have natural (natural resistance), or develop (acquired resistance) resistance to antibiotics [4].

The discovery of antibiotics has been one of the most important therapeutic advances of the twentieth century; their use has reduced significantly the rate of morbidity and mortality associated with infectious diseases [5]. However, it has been at the origin of a strong increasing antibiotic resistance affecting more species with the use of a number of more and more antibiotics [6]. Since the beginning of the 1960s, there is an increase in the number of bacteria resistant to antibiotics, and the emergence of new resistance [7]. Thus, the emergence and dissemination of resistance to antibiotics represent a real threat to global public health [8-9]. The antibiotic resistance continues to grow in both the industrialized and the developing country [10]. The situation is alarming in countries with limited resources where infectious disease, poverty and malnutrition are endemic [11].

During the last ten years, bacterial resistance to antibiotics, particularly by β-lactamases with extended spectrum (ESBL) production, has become a major problem of public health [12]; because of the multidrug resistance and the choice of limited antibiotics molecules [13]. ESBL-producing microorganisms were associated with the expansion of hospital-acquired infections leading to prolonged hospitalization, increased morbidity and mortality [14-15]. Despite the importance of the problem and economic consequences, West Africa is having national program of surveillance to fight against resistance as recommended by the World Health Organization [10]. Furthermore, there is a lack of data assessing the impact of antimicrobial resistance in development country such as West Africa [16]. The emergence and dissemination of the ESBL in West Africa is therefore reported to be related to the expansion of CTX-M-15 allelic type [17]. Other ESBL allelic type of CTX-M type were reported by Duval et al. [18] in Mali (CTX-M-14), in Nigeria by Ogbolu et al. [19] and in Senegal by Breurec et al. [20] the identified form was CTX-M-3. Apart of the CTX-M enzymes, SHV enzymes (3 and 12) were recently characterized from various isolates in Mali [21] and Nigeria [22]. Plasmids carrying ESBL gene can also host other resistance genes conferring resistance to other families of antibiotics, including cotrimoxazole, fluoqinolone and to aminoglycosides [23].

In Benin, there is a lake of information on the ESBL. Thus, this study is a contribution on the epidemiology of clinical isolated Gram Negative Bacilli. The aim of our study was to isolate GNB from various clinical samples, to determine their prevalence, to make their biochemical characterization, to evaluate their antimicrobial resistance and to check the presence of some resistance genes.

2. MATERIAL AND METHODS

2.1. Study design and sampling

The study focused on the biological samples carried to the National Hospital and University center of Cotonou (Benin) from July–September 2012. Thus during 3 months, 1823 specimen were collected from both hospitalized patient and community. The specimen coming from hospitalize patient are considered as inside hospital-patient specimen. If not, they are out-of-hospital specimen. The entire specimen randomly carried to the reference center during the period were taken as our sample size. These specimens collected following the conventional protocols [24-25] include urethral, vaginal, urinary, semen, blood, catheters, Intra Uterine Devices (IUD) and cerebrospinal fluid specimen. For the study, only specimen with majority or pure culture (10^5 cfu/ml) of GNB were taken into account.

2.2. Isolation and identification of Gram negative bacilli

Specimen were seeded on Eosin Methylene Blue Agar (Oxoid, England) and incubated for 24 h at 37°C. From the obtained colonies, the Gram stain and the identification by the Gallery Api 20E was carried out.

2.3. Susceptibility of Bacilli Gram negative bacilli isolates to antibiotics

The susceptibility of the identified bacteria to 21 conventional antibiotics, provide by Oxoid (England) was performed using EUCAST recommended methods and interpretation [26]. The 21 antibiotics tested (Bio Mérieux, France) were: Cefotaxime (CTX 5 µg), Amoxicillin

(AMX 30 µg), Amoxicillin + clavulanic acid (AMC 20/10 µg), Ceftriaxone (CRO 30 µg), Cefuroxime (CXM), Cefoxitin (FOX 30 µg), Imipenem (IPM 10 µg), Nalidixic acid (NA 30 µg), Ciprofloxacin (CIP 5 µg), Ofloxacin (OFX 5 µg), Norfloxacin (NOR 10 µg), Gentamicin (GM 10 µg), Netilmicin (NET 10 µg), Chloramphenicol (C 30 µg), Trimethroprime sulfonamide (SXT 1.25/23.75 µg), Nitofurantoin (FT 300 µg), Ticarcillin (ICT 75 µg), Cefixim (CFM 30 µg), Aztreonam (ATM 30 µg), Doxycycline (DO 30 µg) and Tetracycline (TE 30 µg).

2.4. Penicillinase production assay by test acidimetrique in tubes

The production of the penicillinase was performed on all to GNB isolates by the broth medium method as previously describe [27]. The reagent used is a solution prepared extemporaneously with 600 mg of benzylpenicillin powder dissolved in 400 µl of sterile distilled water and 300 µl of 1N NaOH has been added to the mixture to obtain a pH of 8. Finally, to get a purple red color, 300 µl of 1% phenol red was added in distilled water. Two young (24 h) colonies have been emulsified in 500 µl of physiological water. Then, 150 µl of reagent to the benzylpenicillin previously prepared have been added. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 35657 were used as control. After 1 h of incubation at 37°C, a shift in the color of the original red purple to orange or yellow in the tubes indicates that the tested strain is penicillinase producing.

2.5. Analysis of the spectrum β-lactamase expanded (ESBL) by the method of double halo

Research of the ESBL on all the GNB isolates was performed using the method of disk synergy between third-generation cephalosporin and clavulanic acid [28]. On the Mueller Hinton agar previously seeded with the test strain, a disc of 3rd generation cephalosporin: (Cefotaxime (CTX) and Ceftriaxone (CRO) with clavulanic acid (amoxicillin + clavulanic acid), was applied and incubated for 18 h at 37°C [26]. The disc of amoxicillin + acid clavulanic (AMC) has been placed in the center of the two other cephalosporins at a distance of 30 mm. The positive result is a potentiation of the inhibition zone around the disks of CTX and CRO, in the presence of the antibiotic AMC, because of the synergy of betalactams with clavulanic acid [28].

2.6. Detection of resistance genes

Polymerase chain reactions was performed on total DNA of all confirmed ESBL producer GNB to detect genes encoding multidrug resistance (TEM and SHV) The DNA template was extracted by suspending a loop of GNB colony in 500-µL sterile, pure water and boiling for 10 min at 95°C. The suspension was then centrifuged for 5 min at 12000 rpm, and 10 µL of the supernatant was used as target DNA. DNA extracts were stored at -20°C until used.

The primers for $_{bla}$*TEM*, and $_{bla}$*SHV* were used for multidrug resistance gene investigation by PCR amplification in 30 µL containing for each: 5 µL of DNA, 0.5 µM of each primer (F and R), 1.5 mM $MgCl_2$, 250 µM dNTPs, 1X PCR buffer (Invitrogen) and 1U *Taq* DNA polymerase (Invitrogen). The PCR program used for amplification consisted, for $_{bla}$*TEM* (initial denaturation 94°C for 5 min followed by 30 cycles 94°C at 30 s, 52°C for 30 s, 72°C for 1 min and a final elongation step 10 min at 72°C). For $_{bla}$*SHV* (initial denaturation was performed at 96°C for 5 min, 30 cycles at 96°C for 15 s, 50°C for 15 s, 72°C for 1 min and a final elongation step 10 min at 72°C). The primers sequences and the expected fragments are presented in the Table 1.

Table 1. Primers used to search genes in this study.

Taguets genes	Primers	Primers sequences (5' ⟶ 3')	Amplicon size (bp)	Reference

bla_{TEM}	OT-F	5'-TTGGGTGCACGAGTGGGTTA-3'	465	[29]
	OT-R	5'-TAATTGTTGCCGGGAAGCTA-3'		
bla_{SHV}	SHV-F	5'-CGCCGGGTTATTCTTATTTGTCGC-3'	620	[30]
	SHV-R	5'-TCTTTCCGATGCCGCCGCCAGTCA-3'		

PCR products (10 µL) were visualized after electrophoresis at 150 V for 30 min on a 1.5 % agarose gel containing ethidium bromide and visualized with an UV trans-illumination. A 100 bp ladder standard was used as molecular weight ladder.

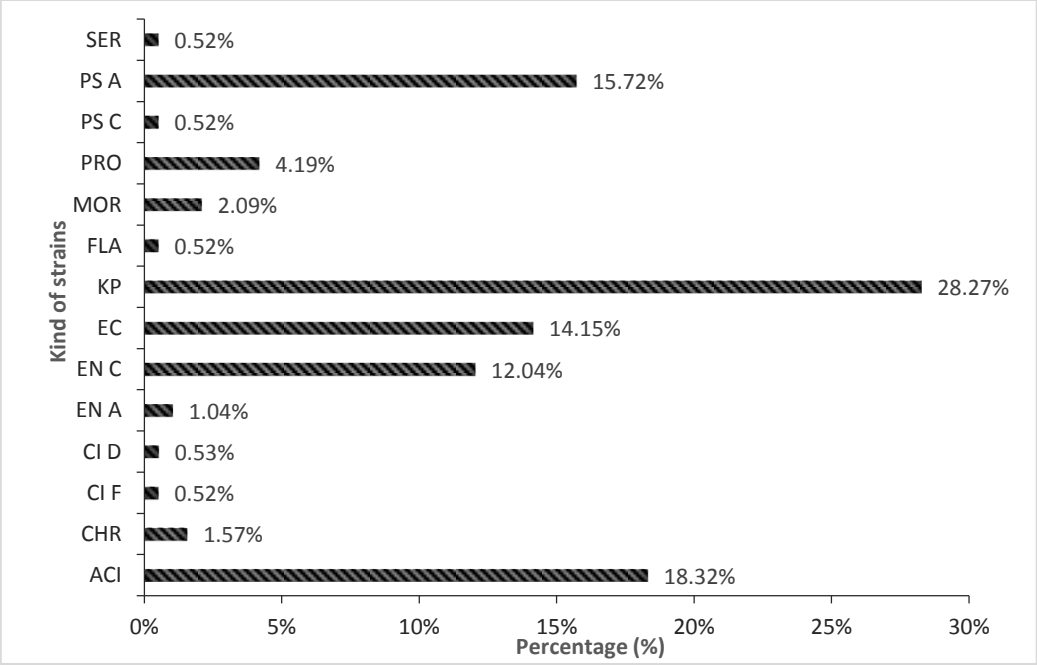
2.7. Data analysis

The Microsoft office Excel 2010 spreadsheet was used for processing data. GraphPad Prism 5 was used to perform the Chi-square test and make graphs. The test was considered statistically significant at $p < 0.05$.

3. RESULTS

3.1. Prevalence of GNB isolates

From the 1823 collected specimen, 191 strains (10.47%) of GNB were identified. The 191 strains were identified from both males (23.56%) and females (76.44%) patients. Thus, the females samples displays significant contamination rate compare to the males (p= 0.0001). Fifteen different species of GNB were isolated. The five most represented species are *Klebsiella pneumonia* (28.27%), *Acinetobacter* spp. (18.32%), *Pseudomonas aeruginosa* (15.72%), *Escherichia coli* (14.15%) and *Enterobacter cloacae* (12.04%) (Figure 1).



ACI : *Acinetobacter* spp, PS A: *Pseudomonas aeruginosa,* PS C : *Pseudomonas cepacia,* EC : *Escherichia coli,* KP : *Klebsiella pneumoniae*, EN A : *Enterobacter agglomerans,* EN C : *Enterobacter cloaceae,* PRO : *Proteus mirabilis,* MOR : *Morganella morgnii,* CHR : *Chryseomonas luteola,* CI D : *Citrobacter diversus*, CI F : *Citrobacter freundii*, FLAV : *Flavobacterium indologenes* et SER : *Serrati ficania.*

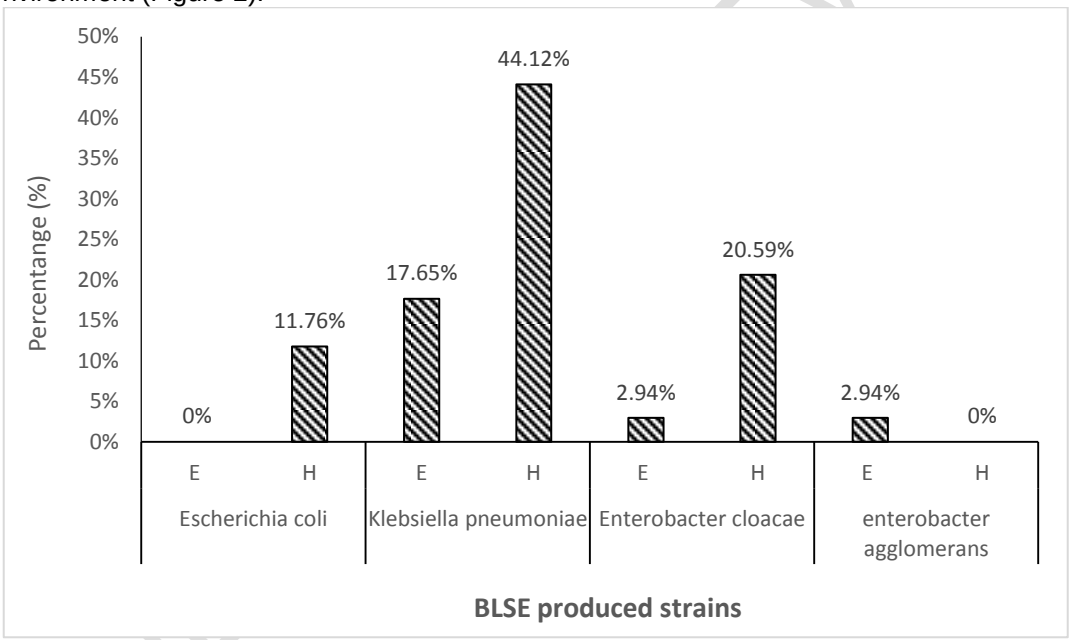
Figure 1. Distribution of different species of Gram-negative bacilli isolated from specimen.

Varieties of proportions of GNB were observed according to the type of specimen. The urine and pus are the specimen that contain more GNB with respectively, the proportions of 43.98% and 34.58% respectively. Blood specimen (9.45%), semen (2.61%), cerebrospinal

fluid (2.61%), vaginal specimen (2.09), pleural fluid (2.09), catheters (1.05%), IUDs (1.05%) and specimen of unknown origin (0.52%) respectively followed them.
The GNB isolates were grouped into hospital isolates (i.e., isolates from the specimen taken from patients hospitalized in different services of the CNHU) and outside isolates (i.e. the isolates obtained from the hospital environment's specimen). The hospital GNB's isolates (51.31%) were higher than those from outside the hospital (48.69%), and the difference seems not to be statistically significant (p> 0.05).

3.2. Production of penicillinase and ESBL by the GNB isolates

A proportion of 57.07% of the isolates were producing penicillinases. The isolates producing penicillinases were well found among hospital and extra-hospital isolates. Among the strains producing penicillinases, 61.47% are from the community hospital and 38.53% from the hospital environment. Thus, we find that hospital isolates produce more penicillinase than the isolates from outside the hospital; and the proportion difference was significant (p< 0.05).
Among the GNB isolates, 32 (16.76%) were expanded spectrum β-lactamases producers (ESBL). The ESBL producing isolates were recorded both in hospital and extra-hospital environment. However, hospital ESBL-producing isolates were substantially higher (75%) than the extra-hospital environment producers (25%) (p < 0.005). Among ESBL-producing isolates, only *E. coli*, *K. pneumoniae, Enterobacter clocae* and *En. agglomerans* strains' produced ESBL. The ESBL producers are *Klebsiella pneumoniae* (44.12%), *Enterobacter cloacae* (20.58%) and, *Escherichia coli* (11.76%) strains isolated from the hospital environment (Figure 2).



E: Extra-Hospital isolates and H: Hospital isolates

Figure 2. Gram Negative Bacillus strains producing ESBLs depending on the source.

3.3. Susceptibility to antibiotics of isolated strains of GNB

The investigation on the antibiotic resistance of the isolates revealed that Imipenem has experienced the lowest resistance (9.8%) and the Ticarcilline has experienced the highest resistance (100%) rate (Figure 3). All the intermediate resistance observed by the GNB were too strong, each being more than 50%. Thus, resistance level located between 50 and 80% was recorded for 12 antibiotics (Gentamicin, Netilmicin, Ciprofloxacin, Aztreonam, Trimethroprime sulfonamide, Cefoxitin, Ofloxacin, Ceftriaxone, Cefotaxim, Norfloxacin, Nitofurantoin and Cefixim). In addition, seven antibiotics (Amoxicillin + clavulanic acid, Nalidixic acid, Cefuroxime, Chloramphenicol, Amoxicillin, Tetracycline and Doxycycline) displayed an inhibition activity on between 80% and less than 100% of the tested GNB.

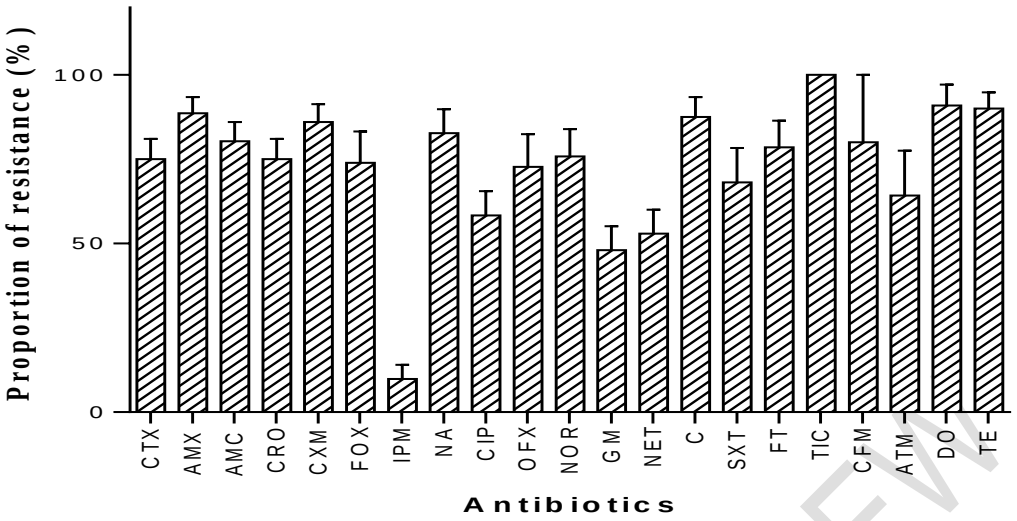


Figure 3. Antibiotic resistance of isolated Gram-negative Bacillus strains. CTX: Cefotaxime, AMX: Amoxicillin, AMC: Amoxicillin + clavulanic acid, CRO: Ceftriaxone, CXM: Cefuroxime, FOX: Cefoxitin, IPM: Imipenem, NA: Nalidixic acid, CIP: Ciprofloxacin, OFX: Ofloxacin, NOR: Norfloxacin, GM: Gentamicin, NET: Netilmicin, C: Chloramphenicol, SXT: Trimethroprime sulfonamide, FT: Nitofurantoin, TIC: Ticarcillin, CFM: Cefixime, ATM: Aztreonam, DO: Doxycycline and TE: Tetracycline.

3.4. Antibiotic resistance of GNB according to the production of ESBL

Globally, for the majority of the tested antibiotics (18), GNB isolates producing ESBL were more resistant than those that do not produce. However, Figure 4 shows a variation of resistance proportion according to the type of antibiotic. Eight antibiotics (Aztreonam, Nalidixic acid, Ticarcillin, Cefixim, Tetracycline, Doxycycline, Amoxicillin and Chloramphenicol) showed a resistance level between 2% and 15%. On the other hand, resistance of isolated strains varying between 20% and 38% was observed for eight antibiotics (Cefotaxim, Amoxicillin + clavulanic acid, Norfloxacin, Ceftriaxone, Ofloxacin, Ciprofloxacin, Trimethroprime sulfamide and Imipenem). To end, a highest resistance was obtained for Gentamicin (52.46%) and Netilmicin (56.67%).

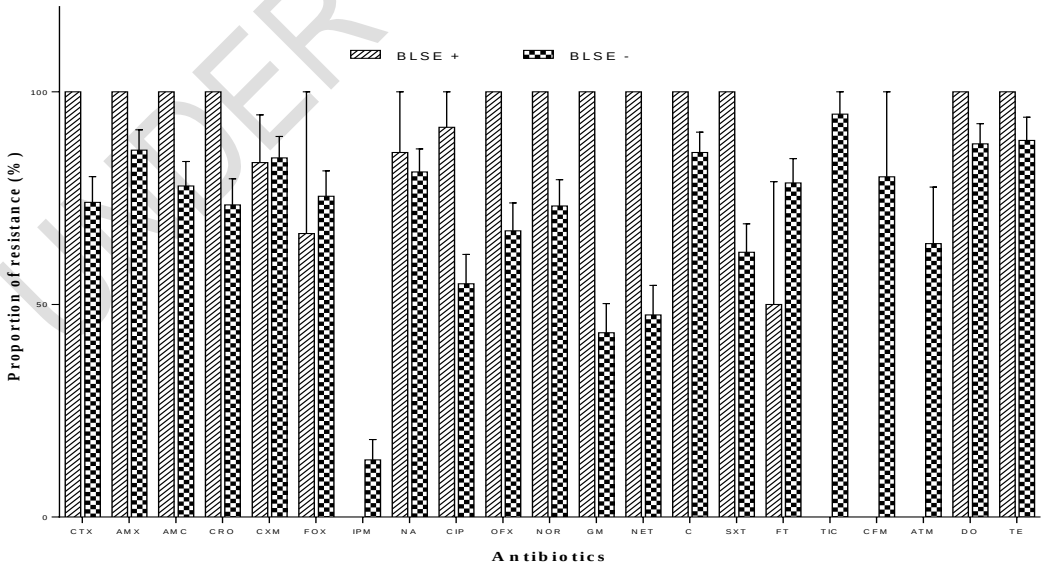


Figure 4. Antibiotic resistance of Gram Negative Bacilli strains depending on whether or not they produce ESBL. CTX: Cefotaxime, AMX: Amoxicillin, AMC: Amoxicillin + clavulanic acid, CRO: Ceftriaxone, CXM: Cefuroxime, FOX: Cefoxitin, IPM: Imipenem, NA: Nalidixic acid, CIP: Ciprofloxacin, OFX: Ofloxacin, NOR: Norfloxacin, GM: Gentamicin, NET: Netilmicin, C: Chloramphenicol, SXT:

Trimethroprime sulfonamide, FT: Nitofurantoin, TIC: Ticarcillin, CFM: Cefixime, ATM: Aztreonam, DO: Doxycycline and TE: Tetracycline.

3.5. Genotypic detection of gene resisting to $_{bla}$*TEM* and $_{bla}$*SHV*

The Table 1 shows that among the GNB isolates producing ESBL, 25% did not have $_{bla}$*TEM* and $_{bla}$*SHV* genes, 25% had at same time the two genes. Among the GNB producing ESBL, 3.5% of the strains had only $_{bla}$*TEM* gene and 12.5% of the strains had only $_{bla}$*SHV* gene. *K. pneumoniae* isolated from hospital specimen were the most carriers of the genes $_{bla}$*TEM* and $_{bla}$*SHV* then come the hospital isolated *Enterobacter cloacae* strains. *Enterobacter agglomerans* and *E. coli* strains isolated from hospital and ESBL producers did not carry the $_{bla}$*TEM* and $_{bla}$*SHV* resistance genes.

4. DISCUSSION

The five most important isolated species in this study were *Klebsiella pneumonia*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *E. coli* and *Enterobacter cloaceae*. The 4.40% was recorded for *E. coli* appear lower than the 11% previously obtained by [31] in Benin. This observation suggest an improvement in the management of *E. coli* infections in the countries. This observation can also be due to the emergence of other species. There are some studies in Benin (Abomey-Calavi and Cotonou) revealing the presence of *K. pneumoniae*, *Acinetobacter spp*., *P. aeruginosa* and *E. coli* [32] like in our study. We can deduce that *K. pneumoniae*, *Acinetobacter spp*., *P. aeruginosa* and *E. coli* are among the most founded GNB in hospitals environment in Benin.

The proportion of nosocomial isolates is high because hospitals are clusters of patients with infections, increasing the probability to infected “safe” people in the environment. The occurrence of hospital-associated infections is also favored by many factors including patient-related factors, exposures to invasive medical devices and deficiencies quality of care [33]. The same is true for cleaning, disinfection and sterilization defects, which are also potential factors for infections associated with care [34-35].

The specimen containing the majority of these bacteria in our work are urine, pus and blood . These results corroborate those obtained in a study conducted in Saudi Arabia where urine specimen were more contaminated than those of pus [36]. Toudji et al. [37] in Togo noted in there that the pus specimen (47.90%) were the most contaminated than the urine (40.78%) and blood specimen (7.77%). Despite this result, the percentage of contaminated urine obtained in our study is higher than that obtained by Toudji et al. [37]. These results show that colonization of urine is becoming a major public health problem [38].

The proportion of GNB producing penicillinase is 57.07%, while non-producing *Enterobacteriaceae* is 43.93%. Studying *Acitobacter baumannii* for example, the second major strain in our study, Endimiani et al. [39] found in Italy that the proportion of penicillinase-producing strains was 42%, a result not far from ours.

In Cameron, Gangoué-Piéboji et al. [29] reported 31% of GNB producing extended spectrum β-lactamases. This difference could be explained by the greater use of antibiotics, which increase the acquisition of resistance in this country. However, lowest (7.5%) rate of GNB producing extended spectrum β-lactamases were recorded in Barcelona [40]. A proportion of 75% of GNB producing extended spectrum β-lactamases strains was from hospital environment. During their investigations in Italy, Endimiani et al. [39] revealed that most of the strains isolated from extra-hospital patients had previously been hospitalized at least once during the last 12 months before the study. Thus, there is probable likelihood between strains isolated from extra-hospital specimen strains and hospitals’. About 4.5% of the GNB producing extended spectrum β-lactamases were from newborns; indicating that healthy individuals could be infected as non-carriers [41]. The acquisition of resistance can be a result of horizontal transfer of resistance genes [42].

Imipenem shows the lowest resistance (9.8%) and corroborates that obtained by Ahoyo et al [31] and Toudji et al. [37]. The resistance was recorded for 20 antibiotics (Doxycyclin, Aztreonam, Cefixim, Tetracycline, Ticarcillin, Amoxicillin, Amoxicillin + Clavulanic acid, Cefoxitin, Cefuroxime, Cefotaxim, Ceftriaxon, Gentamicin, Netilmicin, Chloramphenicol, Nalidixic acid, Norfloxacin, Ofloxacin, Ciprofloxacin, Trimethroprime sulfonamide, Nitofurantoin). Imipenem exerts a total inhibition on GNB that are resistant to many antibiotics such as Cefotaxim, Ceftriaxone, Aztreonam and Cefoxitin [43]. The loss of sensitivity to cephamycins (eg. cefoxitin) and carbapenems (eg Imipenem) that is normally maintained even in ESBL-producing strains [44], is believed to be due to resistance acquired

by these bacteria due to abuse of certain antibiotics; which calls for greater medical supervision. This antimicrobial resistance occurs naturally over time, usually because of genetic modification but the excessive or excessive use of antibiotics speeds up the process [45]. The proportion of bacteria resistant to antimicrobials in Benin could be explained by self-medication in patients and the use of antibiotics of dubious quality. Dougnon et al. [46] perceived that the quality of the antibiotic discs used for the performance of antibiograms could be a factor favoring the bacteria resistance.

GNB producing extended spectrum β-lactamases resisted more than those that do not produce. Aibinu et al. [47], in Nigeria, founded that most of producing ESBL *Enterobacter spp* resisted better than non-producing ones. Indeed, the ability to synthesize such enzymes allows bacteria to face a greater number of antibiotics as shown by our results and those of Aibinu et al. [47]. Our results revealed the existence of three antibiotics (Cefuroxime, Cefoxitin and Nitofurantoin) to which non-ESBL-producing GNB isolates resisted better than those ESBL-producing did. This could be explained by the absence of these antibiotics in the spectrum of action of these enzymes whose ability of synthesis would result from a mutation of the β-lactamase gene, which acted against these antibiotics.

Certainly, extended spectrum β-lactam resistance genes strains have the *blaCTX*-M that is the third gene that codes for the production of ESBLs. No *Enterobacter agglomerans* strains carried $_{bla}$*SHV* gene and 12.50% of *Enterobacter cloacae* strains had both the gene $_{bla}$*TEM* and $_{bla}$*SHV* gene. This result can be explained by the fact that ESBL-type are very rarely found in certain genera of *Enterobacteria* such as *Enterobacter*, *Serratia*, *Citrobacter*, in which the chromosomal cephalosporinase is predominant [48]. It was observed in our study, a simultaneous presence of $_{bla}$*TEM* and $_{bla}$*SHV* (12.50% for *Enterobacter cloacae* and 12.50% for *Klebsiella pneumoniae*). These associations are frequently reported in several studies. Thus, it was reported in Algeria the association of TEM -CTX-M1 and SHV-TEM genes in a group of strains [49]. The associations TEM-SHV, SHV-CTX-M-1 and TEM-CTX-M-1 were also reported [50]. The coexistence of the different β-lactamases in the same series may pose a diagnostic and therapeutic problem [51].

The ESBL-producing strains of *K. pneumonia* in the hospital environment were the major carriers of $_{bla}$*TEM* (15.62%) and $_{bla}$*SHV* (12.50%). A previous study proves that the enzymes initially derive plasmid-like narrow-spectrum penicillinases-temoneira (TEM 1/2) and variable sulphhydryl (SHV-1) by the modification of their active site were observed mainly in hospital strains of *Klebsiella pneumonia* [52]. It should also be noted that the different types of ESBL (TEM and SHV) found in our study are part of so-called transferable ESBLs, which explains the ease of transfer between *Enterobacteriaceae* [53]. The presence of ESBL-producing hospital strains of different types can act as a tank of resistance genes, which can be transmitted horizontally to extra-hospital or environmental strains. In addition, it is a risk factor for nosocomial epidemics [54]. Therefore, the inanimate environment of patients (surfaces and objects), contaminated by multi-resistant bacteria can serve as a secondary reservoir for cross-transmission [55]. We thus recommend to the patient to avoid the auto medication and to the practitioners to be sure of the strains before using antibiotics.

5. CONCLUSION

The study of GNB is an important scientific subject because of the strategic position of the gut, natural surroundings of life in the human body. The originality of our investigation was to explore the whole family by studying together its entire species encountered as pathogens in our environment. In this condition, bacteria living together often share their different characters over time. Indeed, almost all previous studies often addressed a species taken separately and even sometimes identified from a single type of sampling. *K. pneumoniae*, *Acinetobacter spp*., *P aeruginosa*, *E. coli* and *Enterobacter cloacae* were founded in majority with the capacity to produce penicilinase. However, this study would be interesting if the patient was followed for a long time. Especially with regard to patients coming from the outside world. It would also be interesting to expand this study to other hospitals across the country. For the depth of this study to explain the observations of our study, it will be interesting to study further the other resistance genes. This study enabled the National University Hospital Center of Benin to improve its diagnostic methods.

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