First Description of Extended-Spectrum β-Lactamases and OXA-48 Carbapenemase in Enterobacteriaceae Isolates in Brazzaville, Congo

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ABSTRACT

Objective: To characterize genotypically Extended-Spetrum-Beta-Lactamase (ESBL) and OXA-48 carbapenemases producing Enterobacteriaceae especially Klebsiella, Enterobacter, Serratia (KES) and Citrobacter species in portage and infection processes at the Brazzaville hospital center.

Material and Methods: The study was carried out for 7 months. Clinical samples (urine, pus, and blood cultures) were collected from inpatients and outpatients at the Brazzaville University Hospital. Strains were identified by API20E and confirmed by MALDI-TOF. Antibiotics susceptibility testing was performed on isolated strains by diffusion method on MH agar plates. ESBL and OXA-48 phenotypes were identified according to the CA-SFM synergy technique and by a decrease in inhibition diameter around the Ertapenem disk and confirmed by PCR and sequencing. MLST K. pneumoniae genotyping of OXA-48 strains was performed.

Results: Thirty-four no duplicate Enterobacteria strains were isolated from thirty-four patients, of which 12/34 (35.29%) were from outpatients and 22/34 (64.70%) from inpatient patients. Except for imipenem, colistin; the amikacin and fosfomycin, tested antibiotics show high resistance to the majority of the beta-lactam, as well as a resistance very frequent aminoglycosides, to sulfamides, tetracyclines and Fluoroquinolones.

PCR revealed that 30/34 (88.24%) produced ESBLs, of which 2 strains harbor both ESBL and OXA-48 enzymes. blaSHV gene was the most common ESBL gene detected with 20/30 (66.67%), blaCTX-M was detected in 14 isolates (60.87%), blaTEM 15/30 (50%), blaOXA-48 2/30 (6.67%), blaCTX-M-9 1/30 (3.33%). 70% of the isolates (n=24) were isolated from urine samples. Sequencing of the amplification products revealed that the blaCTX-M1 strains were all CTX-M15; 13 variant enzymes were detected for blaSHV. Four types for TEM. Both strains OXA-48 were OXA-181 non-plasmid-borne and CTX-M-9 was CTXM-27. These strains were resistant to gentamycin and fluoroquinolone. MLST K. pneumoniae OXA-48 showed two different standard sequences known in the ST464 and ST15 literature.

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INTRODUCTION

The introduction of semi-synthetic penicillins in the 1960s was soon followed by the appearance of Ambler narrow-spectrum Ambler class A beta-lactamase strains with the active serine site (SXXX) [1], named TEM-1, TEM-2, and SHV-1 [2]. These enzymes, encoded by plasmid genes, have spread to different species of enterobacteria [2]. The resistance to amoxicillin and the first-generation cephalosporins they cause has favored the prescription of aminoglycosides, quinolones and especially third-generation cephalosporins (C3G).

However, this exposure to C3G led to the appearance in the 1980s of Extended-Spectrum Beta-Lactamases (ESBL), class A enzymes, destroying most beta-lactams with the exception of cephapinem and carbapenens [2]. At the same time, resistance genes to fluoroquinolones and aminoglycosides (qnr or aac (6')-Ib-cr) have spread within these same bacterial populations following widespread use of these antibiotics.

Thus, prescribers have used beta-lactam of the latest generation: carbapenens. Their use was soon followed by the appearance of the carbapenemase-producing strains, especially in K. pneumoniae, then in other species of enterobacteria. These carbapenemas are of different types: metallo-β-lactamases (IMF, VIM, NDM), beta-lactamases class A (KPC, GHG, SME) and class D (OXA-48) [3]. Class D essentially corresponds to the oxacillinase type enzymes comprising OXA-48, which have 5 variants (OXA-162, OXA-163, OXA-181, OXA-204, OXA-232 and the recently described enzymes OXA-244 and OXA-245 [4,5].

The OXA-48 gene first described in K. pneumoniae is one of the most recently described carbapenemas, structurally different from the previous ones and essentially identified in Mediterranean countries [6,7].

These enzymes strongly hydrolyze carbapenens but little or no third-gener of cephalosporins (with the exception of OXA-163) [8]. They are resistant to suicide inhibitors of β-lactamases. However, their presence is often coupled with the presence of ESBL, which leads to a multi-resistance of these secretory strains [7].

Work on the production of ESBLs and carbapenemas in enterobacteria in Africa has been limited in North Africa. Data on West and Central Africa are scarce and scattered. Some studies have reported the presence of ESBL and carbapenemas in Nigeria [9], Senegal [10] and Burkina Faso [8].

Data on Central Africa are scarce and scattered. Some studies have been reported in Cameroon [11] and in Gabon [12].

In Congo Brazzaville, no studies on the characterization of ESBL genes and OXA-48 carbapenemas have been reported. This justifies the framework of the present study which aimed to genotypically characterize ESBL and OXA-48 carbapenemas gene variants harbored by strains of enterobacteria isolated in portage and in infectious processes at the CHU of Brazzaville.

MATERIALS AND METHODS

Patients and samples

It is a prospective descriptive study, carried out over a seven-month period from January to July 2017. It focused on enterobacterial strains isolated from inpatients and outpatients from diagnostic specimens at Brazzaville University Hospital. The samples corresponded to the various sites of colonization (urine, pus, and blood cultures), essentially isolated to the various services of general surgery, pediatric intensive care, cardiology, rheumatology and urology.

Some epidemiological data such as age, sex, and origin of service were first identified for each patient. Duplicates (same strain in the same patient for the duration of the study) were eliminated. Samples for pus were taken by a swab, this technique consists of having a sterile swab moistened with sterile distilled water at the site of pathological colonization.

The strains were isolated according to conventional techniques and identified by the API20E system (Biomerieux, Marcy-l’Etoile, France), then an identification confirmation was carried out by MALDI-ToF using the Biotyper, a database and a Microflex spectrometer (Bruker Daltonics, Bremen, Germany). The correct identification at the level of the species was defined when the MALDI-TOF score >1.9 on all the spots [13].

The collection of strains was made at the laboratory of bacteriolog -virology of the Hospital and University Center of Brazzaville and the genotypic study was conducted at the Research Unit on Infectious Diseases, Emerging Tropical Diseases (URMITTE) at the IHU Marseille La France.

Study of the sensitivity

Antibiotics susceptibility testing was carried out for each strain by the method of diffusion in agar medium on Mueller-Hinton medium (Becton Dickinson, The Bridge of Clai, France) according to the recommendations of the Committee of the Antibiogram of the French Society of Microbiology (CA-SFM) [14]. The agar plates were inoculated with a swab from a culture inoculum calibrated at 0.5 McFarland before the deposit of the disks impregnated with an antibiotic (Bio-Rad, Marnes-La-Coquette, France). After incubation of agar plates at 37°C for

Conclusion: We report here for the first time in Congo Brazzaville, the presence of β-lactamase genes including blaTEM, blaSHV, blaCTX-M and blaOXA-48 genes at disturbing frequencies within Enterobacteriaceae strains at the Brazzaville University Hospital. It proves the need to promote an infection prevention program with antibiotic regulation in hospitals in Congo Brazzaville.

Keywords: Enterobacteriaceae; Antibiotic resistance ESBL; OXA-48; MLST; CHU; Brazzaville
18h to 24h inhibition diameters, due to antibiotic concentration gradients established from the disc, were compared to the critical values given by CA-SFM, thus allowing define the susceptibility or resistance of a strain to an antibiotic.

ESBL production was confirmed phenotypically according to CA-SFM recommendations, including the MH agar synergy test between the clavulanic acid disc and cephalosporin discs of the 3rd ceftriaxone: CRO, cefepime: FEP). The carbapenemase OXA-48 phenotype was determined when there was a decrease in inhibition diameter around the Ertapenem disk on MH agar in an imipenem-sensitive strain [15]. 16 antibiotics were tested: amoxicillin AMX (25 μg), amoxicillin/clavulanic acid AMC (20/10 μg), cefepime FEP (30 μg), Piperacillin-Tazobactam TZP (75/10 μg), cefotaxin CF (30 μg), ceftriaxone CRO (30 μg) ERT ertapenem (10 μg), imipenem IMP (10 μg), fosfomycin FF (50 μg), nitrofurantoin FNitro (300 μg), trimethoprim+Sulphamethazole SXT (1.25/23.75 μg), amikacin (30 μg), ciprofloxacin CIP (5 μg), doxycycline DO (30 UI), CT colistin (50 μg) and gentamicin GEN (15 μg). The different strains tested were classified into sensitive (S), Intermediate (I) and Resistant (R) categories. The strain of E. Coli ATCC 25922 was used as a sensitive reference for antibiogram.

Molecular detection of antibiotic resistance genes

DNA extraction: The bacterial strains were cultured overnight on MacConkey agar at 37°C. Then, the colonies were removed using a sterile plastic loop of 1 μl and transferred into 200 μl of sterile water. Total DNA was extracted with the EZ1 Advanced XL (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The DNA was eluted in a final volume of 200 μL. The extracted DNA was stored at -70°C for further analysis.

Detection by PCR of Oxa-48 and ESBL genes: Initial molecular gene detection was performed on all strains using the real-time PCR (RT-PCR) technique using a C1000 Touch CFX96 Real-Time System (Bio-Rad) (Figure 1) thermocycler using primers and probes specific to the blaTEM group, blaCTXMA, blaSHV [16] and blaOXA-48 (Table 1) and the Quantitect Probe PCR assay kit (QIAGEN, Hilden, Germany). Each qPCR reaction was carried out in a 20 μL reaction mixture containing 10 μL of Quantitect Probe PCR, 0.5 μL of the probe (2.5 μM), 0.5 μL of each primer (20 μM), 2 μL of sterile distilled water and 5 μL of DNA diluted to 10th was added.

Table 1: Primers and probes used for RT-PCR reactions, classical PCR and sequencing then MLST.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer/Probe-name</th>
<th>Sequence (5'-3')</th>
<th>Size</th>
</tr>
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<td>TEM_F</td>
<td>TTCTGCTATGTGGTGCGGTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TEM_R</td>
<td>GTCCTCCGATCGTTGTCAGA</td>
<td>213</td>
</tr>
<tr>
<td></td>
<td>TEM_probe</td>
<td>AACTCGGTCGCGCAGACATGATTCAGA</td>
<td></td>
</tr>
<tr>
<td>SHV_F</td>
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<td>TCCTGCTGGCGGATAGTGCAT</td>
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<tr>
<td>SHV_R</td>
<td></td>
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<td>105</td>
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<td></td>
<td>CTXMA_probe</td>
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<tr>
<td>OXA48_RT_Probe</td>
<td></td>
<td>6-FAM-AGCTTGGATCGCCCTGATTGTG-TAMRA</td>
<td></td>
</tr>
</tbody>
</table>

Conventional PCR and sequencing
The primer sequences and probes used are shown in the Tables, respectively. The thermal cycle for PCR amplification consisted of a denaturation step at 50°C for 2 minutes, followed by 35 cycles each comprising a hybridization step at 95°C for 15 seconds; an elongation step at 95°C for one second then a final extension 60°C for 35 seconds. The q-PCR was carried out in 96-well microplates with a positive control (E. coli CMUL64 for OXA-48 and K. pneumoniae KPNASEY for TEM, CTX-M and SHV) and a negative control contained ultra-pure water instead of the template DNA to ensure the absence of contamination during the preparation of PCR reactions.
To test the real-time PCR results received, conventional PCR formats were made. Using the primer pairs specific to the blaTEM group [17], the blaCTX-M-1 group, blaCTX-M-9, blaSHV [16] and the blaOXA-48 group [18] (Table 1).

Standard PCRs were performed in a final volume of 25 μL. The standard PCR conditions consisted of an initial denaturation step at 95°C for 15 min; followed by 35 cycles of amplification each comprising a hybridization step at 55°C for 50 seconds, an elongation step at 72°C for 1 minute, and a final extension step at 72°C for 7 minutes.

The amplification products were detected by electrophoresis using 1.5% SYBR-safe agarose gels (Invitrogen, Leek, The Netherlands), as well as a DNA molecular weight marker (bench top pGEM, ®DNAMarker, promega, madison, wisconsin, USA). Visualization of the gels was performed using the leaning ADNPPEM marker (promega, madison, wisconsin, USA) under ultraviolet illumination.

DNA sequencing

Classical PCR products positive for ESBL and OXA-48 were purified using the NucleoFast 96 PCR plate (Machery-Nagel EURL, France) and sequenced using BigDye terminator chemistry on a ABI3730 Automatic Sequencer (Applied Biosystems, Foster City, California, USA). The sequences obtained were assembled and corrected with the Codon Code Aligner software and then analyzed from the Antibiotic Resistance Gene-ANNOTation (ARG-ANNOTation) database using a local BLAST program in the Bio-Edit software [19].

Phylogenetic and molecular evolutionary analyzes were conducted using MEGA version 7 [20].

Molecular epidemiology

To elucidate the spread and epidemiology of OXA-48 carbapenem-resistant K. pneumoniae strains, the molecular genotyping technique: MLST K. pneumoniae was undertaken.

To do this, 7 household genes were studied: gapA (Glyceraldehyde-3-phosphate dehydrogenase), infB (translation initiation factor 2), mdh (Malate dehydrogenase), pgI (phosphoglucose isomerase), phoE (Phosphoporine E), rpoB (beta-subunit of RNA polymerase B) and tonB (Periplasmic energy transducer).

The classical PCR amplification and sequencing were performed using primers specific for each gene (Table 1) [21].

For each locus any different sequence has been designated by a distinct allele number; for each isolate the combination of 7 integers corresponds to the alleles of 7 loci defines the allelic profile or the Sequence Type (ST). The numbers of the STs were allocated by referring to the MLST database of K. pneumoniae.

Conjugation and transformation

To test the transferability of the plasmid carrying the OXA-48 gene, conjugation experiments were performed using the sodium azide resistant E. coli J53 strain as the recipient strain. The transconjugants were selected on the medium Luria Bertani (LB) Agar containing 200 mg/L of sodium azide and 0.5 mg/L of ertapenem the tests were redone three times [22]. When no transconjugants were obtained, transformation tests were carried out. Plasmids were extracted using the high purity miniprep kit (Neobiotec, Los Angeles, CA) according to the manufacturer's protocol.

Statistical analysis

The data were captured using the GraphPad Prism 7 software. The Chi² (X²) test was used to compare the production frequencies of ESBL genes between enterobacterial strains isolated from inpatients and outpatients. The confidence interval was 95% with a degree of freedom of 1. The difference between the frequencies was considered significant when the p-value was less than 0.05.

RESULTS

A total of 34 enterobacterial strains were selected on MacConkey agar with reduced β-lactams susceptibility. Bacterial identification by MALDI-TOF reveals 23 K. pneumoniae, 4 Serratia marcescens, and 4 Enterobacter cloacae, 3 Citrobacter freundii isolates.

The antibiotic susceptibility testing on 16 different antibiotics shows that all strains were resistant to amoxicillin (Table 2), 97.05% (n=33) of the isolates were resistant to amoxicillin/acid clavulanic, 73.53% (n=25), resistant to cefalotin, and 50% (n=17) resistant to ceftriaxone. A low level of resistance to Piperacillin-Tazobactam was observed with 8.82% of the isolates (n=3); 14.70% (n=5) to cefepime, 11.76% (n=4) to Ertapenem (K. pneumonia only) and 11.76% (n=4) to Colistin mainly in Serratia marcescens (naturally resistant). In contrast, all strains (100%) were sensitive to imipenem and fosfomycin sensitivity was observed for 97.06% of isolates Table 2. Regarding aminoglycosides, there is a fairly strong resistance was observed against gentamicin and amikacin with 38.23% (n=13) and 2.94% (n=1) respectively. In addition, against fluoroquinolones, a resistance level of 29.41% (n=10) was detected for ciprofloxacin; tetracyclines and furans remain 58.82% (n=20) resistant; as for the sulfonamides, the latter remain resistant to 94.12% (n=32) of the strains studied (Table 2).
Table 2: Resistance profile of enterobacterial strains (n=34).

Enterobacteriaceae strains ESBL (n=34)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Klebsiella pneumoniae (n=23)</th>
<th>Enterobacter cloacae (n=4)</th>
<th>Serratia marcescens (n=4)</th>
<th>Citrobacter freundii (n=3)</th>
<th>Total (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% R</td>
<td>% R</td>
<td>% R</td>
<td>% R</td>
<td>% R</td>
</tr>
<tr>
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<td>11,76</td>
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<td>100</td>
<td>94,12</td>
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</table>

AMX: Amoxicillin; AMC: Amoxicillin/Acid clavulanic; TZP: Piperacillin-Tazobactam; CF: Cefalotin; CRO: Ceftriaxone; FEP: Cefipime; ERT: Ertapenem; IMP: Imipenem; CS: Colestin; AK: Amykacin; GEN: Gentamycin; CIP: Ciprofloxacine; FF: Fosfomycine; F: Nitrofurantoin; DO: Doxycillin; SXT: Trimethoprim+Sulfaphametoxazol.

The ESBL-encoding enterobacterial strains had a frequency of 40% (12/30) in community strains and 60% (18/30) in hospital strains. The difference in ESBL production in this study between the community and hospital settings was not statistically significant with p-value >0.05 (p=0.161). They were most often isolated from urine, 70% (21/30) (Table 3).

Table 3: Molecular characterization of ESBL genes and OXA-48 carbapenemase Klebsiella pneumonia producing strains (n=30).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Code</th>
<th>Date of isolation</th>
<th>Type of sampling</th>
<th>Ward</th>
<th>Other phenotypes</th>
<th>bla genes detected</th>
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<tbody>
<tr>
<td>K. pneumoniae (n=23)</td>
<td>K01</td>
<td>February 21, 2017</td>
<td>Pus</td>
<td>Chirurgie</td>
<td>F DO SXT</td>
<td>SHV-106</td>
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<tr>
<td></td>
<td>K04</td>
<td>March 28, 2017</td>
<td>Urine</td>
<td>MI</td>
<td>F DO SXT</td>
<td>CTX-M-15</td>
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<td>K05</td>
<td>March 27, 2017</td>
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<td>Externe</td>
<td>CIP F DO SXT</td>
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<td>K06</td>
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<td>SXT</td>
<td>SHV-89</td>
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<td></td>
<td>K08</td>
<td>February 28, 2017</td>
<td>Urine</td>
<td>Cardiology</td>
<td>GEN SXT</td>
<td>TEM-135, SHV-70, CTX-M-15</td>
</tr>
</tbody>
</table>
The PCR amplification products revealed that 20/30 (66.67%) Figure 2 isolates carried the blaSHV gene; 17/30 (56.67%) isolates were blaTXM-1 and 17/30 (56.67%) isolates were blaTEM, then 2/30 (6.67%) isolates expressed the blaOXA-48 gene. In addition, 1/30 (3.33%) expressed for blaCTXM-9 (E. cloacae). The ensemble distributed as follow: a quadruple blaTEM-SHV-CTXM-1-OXA-48 expression in two isolates (6.67%), a triple blaTEM-SHV-CTXM-1 expression was
identified mainly in 7 isolates of *K. pneumoniae* (23.33%), followed by two double expressions of blaTEM-CTX-M-1 and blaTEM-SHV respectively in 6 isolates (20%) and in one isolate (3.33%).

Four different types of TEM enzymes were detected: TEM-163 with accession number: EU813939 (n=7), TEM-198 AB700703 (n=4), TEM-135 JQ060998 (n=3) and TEM-141 AY956335 (n=1). For the blaSHV gene, 13 types of enzymes were detected: SHV-65 DQ174305 (n=1), SHV-89 DQ193536 (n=2), SHV-106 AM941847 (n=4), SHV-70 DQ013287 (n=2), SHV-99 AM922305 (n=1), SHV-161 JX121128 (n=3), SHV-38 GQ407124 (n=1), SHV-11 HQ877614 (n=1), SHV-27 FJ685656 (n=1), SHV-63 EU342351 (n=1), SHV-1 AF148850 (n=1), SHV-85 DQ322460 (n=1) and SHV-61 AJ866284 (n=1). One enzyme was detected after sequence comparison based on ARG-ANNOT data for blaOXA-48: OXA-181 with accession number HM992946 (n=2). The CTX-M- gene of group 9 was CTX-M-27 (Table 3).

MLST molecular typing of *K. pneumoniae* OXA-48 strains showed that both strains belonged to two different sequences types ST464 and ST15. Despite the clinical and epidemiological data, including hospitalization in the same service and the lack of familial links between patients, the typing results of the isolates exclude the infection of these two patients by the same multidrug-resistant strain.

The ST464-associated strain *K. pneumoniae* (KP20) expressed three types of ESBL enzymes: TEM-163, SHV-38, CTX-M-15 was sensitive to imipenem, colistin, amikacin, fosfomycin and doxycycline. *K. pneumoniae* strain (KP30) having for ST15, expressed the ESBL genes TEM-135, SHV-161, CTX-M-15; was sensitive only to imipenem, colistin, and amikacin (Table 4).

Alignment of ClustalW protein sequences of OXA-48 genes with their counterpart OXA-181 whose accession number is HM992946 has shown the existence of a mutation at position 218 (Figure 3), the substitution of the amino acid valine by glycine. Since both amino acids are hydrophobic, this is a conservative substitution.
Very much higher than the frequency found in the studies conducted in 2016 at the Omar Bongo Ondimba military hospital in Libreville, Gabon (8.8%) [12].

The results of our study show that 88.23% (30/34) of the strains studied were producing ESBL, of which 5.88% (2/34) strains of K. pneumoniae co-produced OXA-48 carbapenemases. This rate (88.23%) is quite close to that recorded by Zineb et al. (80%) in Algeria [25].

The frequency of ESBL-producing enterobacteria observed in our study is undoubtedly the consequence of selection pressure due to the inappropriate prescription and misuse of broad-spectrum antibiotics in both hospital and community settings (pharmacy dispensing). No prescription (self-medication); not to mention the impact of poorly controlled food and where more and more antibiotics are used in agriculture and livestock. Added to this is the variety and quality of antibiotics sold, linked to the lack of rigorous rules for their acquisition in developing countries [4].

The difference in ESBL production in this study between the community and hospital settings was statistically significant with p-value >0.05 (p=0.161).

According to the results of our study, 70% of ESBL strains were isolated most often from the urine sample (21/30). This is consistent with published data that ESBLs are usually isolated from urine and respiratory secretion samples [24,25].

The strains of ESBL are multi-resistant and have a growing infectivity risk for our hospitals, their intra and extra-hospital spread requires all health centers to monitor their diffusion and resistance to other families of antibiotics. Indeed, the genes responsible for these resistance phenotypes often carry the determinants conferring resistance to other families of antibiotics [2,26]. The antimicrobial resistance of enterobacterial strains revealed high co-resistance rates for gentamicin (38.23%), ciprofloxacin (29.41%), Furantoin (58.82%) and doxycycline (58.82%) and sulfamethoxazole-trimethoprim (94.12%), which is consistent with the work of Ahoyo et al. [27].

From a therapeutic point of view, ESBL strains maintain a good susceptibility to ertapenem (88.24% sensitivity), amikacin (97.07% sensitivity) and fosfomycin (97.07% sensitivity), colistin (88.24%) and imipenem (100% sensitivity).

Therapeutic options for such infections are often limited to the use of carbapenem. The problem is the cost and availability of these antibiotics on the Congolese market, sometimes causing other mechanisms of resistance in carbapenems respectively, related to non-compliance with the prescribed antibiotic dose. Resistance to colistin was only observed in Serratia marcescens, which is inconsistently sensitive to this antibiotic as confirmed by the literature data.

A Ugandan study found that poverty was responsible for the premature withdrawal of antibiotic treatment in some patients or sharing a single dose of treatment by an entire family [28].

There is a frequent association between genetic determinants of OXA-48 type and those of ESBLs, which highlights the possibility of co-selection of these two resistance mechanisms [6].

Most ESBL strains belonged to the SHV family (66.67%); significantly higher than the 31.1% reported to the Annaba CHU in Algeria [25]. These results disagree with international data that report a predominance of the CTX-M1 gene [29]. The production rates of CTX-M1, CTX-M-9 and TEM ESBL then of OXA-48 genes at Brazzaville university hospital were 56.67%, 3.33%, 50%, and 6.67% respectively.

This study highlights, on the one hand, the increase in the frequency of ESBL production by enterobacterial strains isolated at Brazzaville University Hospital, and on the other hand confirms the worldwide spread of the CTX-M-15 gene.

Since its first description in 2001, the CTX-M-15 gene has been identified in several locations in Asia, Europe and Africa [30,31]. The first time the emergence of CTX-M-15 producing enterobacterial strains in Congo Brazzaville, CTX-M-15 differs from CTX-M-3 by an Asp-2403Gly substitution which increases...
activity against ceftazidime. The improved substrate spectrum of CTX-M-15 is probably a contributing factor to its spread [32].

In this study, the genetic diversity of ESBL genes and carbapenemases can be attributed to genes already detected and the emergence of new clusters in our geographical area (TEM-141, TEM-135, TEM-198, SHV-11, SHV-1, SHV-27, SHV-38, SHV-61, SHV-63, SHV-65, SHV-70, SHV-85, SHV-89, SHV-99, SHV-106, SHV-161, CTX-M-15, and OXA-181). SHV-11, SHV-1, SHV-28, SHV-98, SHV-99, CTX-M-15, CTX-M-27, OXA-181, have been reported in Algeria, Cameroon, Burkina Faso and Tunisia studies with CTX-M-15 being the most prevalent [4,32,33].

MLST K. pneumoniae OXA-48 revealed that these strains belonged to 2 different clones or Sequence Types (ST) already known in the ST464 and ST15 literature (Figure 4). For the ST15, our results are in agreement with Stolle et al., Which report strains of K. pneumoniae-OXA-48 ST15 isolated from pigs in Germany [34], a recent study conducted in Tunisia by Tanfous FB also reports the presence of epidemic strains of -OXA-48 ST15 [33].

It should be noted that the ST464 clone was never reported in Africa in K. pneumoniae except for this study. Nevertheless, the presence of this ST464 epidemic clone was reported in Colombia in Acinetobacter nosocomialis [35].

CONCLUSION
Congo Brazzaville, like the rest of the world, is facing a serious problem of the emergence of antibiotic resistance. Many factors including the health status and level of education of the population, the level of training of the prescribers, the infrastructures and the quality of the antibiotics provided favor their emergence and their propagation.

Prevalence and genetic studies provide fundamental information for BMR epidemiology, which is essential for understanding the control and control of these resistant pathogens. This monitoring must be generalized and must be an integral part of the fight against multidrug-resistant bacteria in all the most important health centers in the Republic of Congo.

REFERENCES


