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# First Detection of TEM-116 and SHV-75 Producing Enterobacteria Isolated from Two Ivorian Teaching Hospitals: Case of Abidjan and Bouaké

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**A B S T R A C T**

The aim of this study was to detect and identify $bla_{TEM}$, $bla_{SHV}$ and $bla_{OXA}$ genes in Abidjan and Bouaké. A total of 73 strains of Enterobacteriaceae from Abidjan and Bouaké and resistant to at least two third generation cephalosporins have been taken into account. Maldi-Tof and Vitek-2, double-disc synergy method were used for identification, determination of minimum inhibitory concentrations (MIC) and the ESBL detection. The $bla_{TEM}$, $bla_{SHV}$ and $bla_{OXA}$ genes were determined by PCR and the β-lactamases identified by sequencing. The overall prevalence of 56.2% was observed with rates of 65.8% for *K. pneumoniae*, 24.4% for *E. coli*, 7.3% for *E. cloacae*, 2.4% *M. morganii* and 0% in *P. mirabilis*. The $bla_{TEM}$, $bla_{SHV}$, $bla_{OXA}$ genes were detected at respective rates of 90.2%, 87.8% and 12.2%. The identification revealed the presence of TEM-1, TEM-116, SHV-11, SHV-12, SHV-75 and OXA-1. The strains were resistant to ceftazidime and cefotaxime (100%), to cefepime (95%) and susceptible to ertapenem and meropenem (100%). Our study showed an increase in the prevalence of TEM and OXA ESBLs and the first detection of TEM-116 and SHV-75 in Côte d'Ivoire.

## Introduction

The production of β-lactamase extended spectrum (ESBL) represents the mechanism of resistance to β-lactams most widespread among Enterobacteriaceae (Valverde *et al*., 2004; Wei-Hua and Zhi-Qing, 2013).

Several of these ESBL derive from TEM mutations (Temoneira) and SHV (Sulphydryl Variable) and confer resistance to penicillins, cephalosporins and aztreonam (Gangoué-Piéboji *et al*., 2005; Menezes *et*

*al*., 2012). However, they are inhibited by inhibitors such as clavulanic acid, tazobactam and sulbactam (Jacoby and Medeiros, 1991; Bradford, 2001). ESBLs have changed dramatically in recent years due to their location on genetic materials consist of mobilized self-transmissible plasmids that are responsible for their rapid and horizontal spread among the different species of enterobacteria (Bradford *et al*., 1994; Chaïbi *et al*., 1999). ESBL-producing organisms often carry plasmids encoding for other types of β-lactamases. OXA type β-lactamases constitute the fast growing group of oxacillinases (Poirel *et al*., 2010). These enzymes are characterized by their strong hydrolytic activity against certain penicillins such as methicillin, oxacillin and cloxacillin. They escape the activity of the β-lactamase inhibitors (Bradford, 2001; Poirel *et al*., 2010). Thus, β-lactamase production by enterobacteria is a global public health problem that deserves special attention as responsible for treatment failure in infections where these pathogens are involved. The objectives of this study were to detect and identify the presence of $bla_{TEM}$, $bla_{SHV}$ and $bla_{OXA}$ in some enterobacteria strains isolated in Côte d'Ivoire.

## Materials and Methods

### Bacterial Strains

A total of 73 infectious strains belonging to the Enterobacteriaceae family were considered. These strains are from the network monitoring antibiotic resistance in Côte d'Ivoire (ORMICI) and concern the two most important towns: Abidjan (South) and Bouaké (Center). The isolation of the bacterial strains was carried out by a 18h culture on Mac Conkey agar. A first identification was performed using biochemical characters and confirmed with Maldi-Tof and Vitek-2 at Teaching hospital of Liège (Belgium) according to the manufacturer's recommendations. Enterobacteria species considered were *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae, Proteus mirabilis* and *Morganella morganii*. The reference strain of *Escherichia coli* ATCC 25922 was also used as positive control for antibiotic susceptibility testing and as negative control for the detection of *bla* genes.

### Antimicrobial Susceptibility Testing and Detection of ESBL Producers

Antibiotic sensitivity was performed on isolated strains of 18h on MacConkey agar using Vitek-2. The Minimum Inhibitory Concentration (MIC) of antibiotics was determined using the AST-N236 card. The antibiotics tested were: temocillin, ampicillin, amoxicillin + clavulanic acid, piperacillin + clavulanic acid, cefuroxime, cefotaxime, ceftazidime, cefepime, ertapenem, meropenem, amikacin, gentamicin, ciprofloxacin, tigecycline, fosfomycin, nitrofurantoin, colistin and cotrimoxazole.

The double-disc synergy test (Jarlier *et al*., 1988) was used for detection strains producing ESBL. This test consisted to have around a disk of amoxicillin/clavulanic acid as the cross cefotaxime disks, ceftazidime and cefepime on Mueller Hinton agar (bioMérieux, France). Distortion of the peripheral inhibition zones of surrounding antibiotics toward the central disk with clavulanate was indicative for an ESBL.

### Molecular Analysis Techniques

DNA plasmid extractions were performed on the positive strains from the double-disc synergy test (41 strains) using Miniprep GeneJET plasmid kit according to manufacturer's recommendations. The

*bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{OXA}$ genes were detected by multiplex PCR using the primers summarized in table 1 (Dallenne *et al*., 2010).

The amplification reaction was performed in a final reaction volume of 25 µL composed of Master Mix (12.5 µL), of mixture of the primers (0.6 µL), water (10.9 µL) and of DNA (1 µL). The amplification program consists in an initial denaturation of 10 min at 94 °C. Cycle ramping for PCR consisted of 30 cycles of denaturing at 94 °C for 40 s, annealing at 60 °C for 40 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were analyzed by electrophoresis in a 2% agarose containing Midori Green (Nippon Genetics).

The purified PCR products were sequenced at platform GIGA Technology of the Teaching Hospital of Liège (Belgium) using the BigDye® Terminator v3.1 Cycle Sequencing Kit according to manufacturer's recommendations in a genetic analyzer ABI Prism.

## Results and Discussion

### Detection of ESBL-producer Isolates

Of 73 Enterobacteria strains isolated, 41 were ESBL-producers. A correspondence of 100% was observed between the Vitek-2 test and the double-disc synergy test. The overall prevalence of ESBL-producing Enterobacteriaceae (ESBLE), by considering all of the strains included in this study, was 56.2%. This rate was higher among *Klebsiella pneumoniae* (27 strains, 65.8%) followed by *Escherichia coli* (10 strains, 24.4%). The rate in other species, namely *Enterobacter cloacae* and *Morganella morganii* was 7.3% and 2.4% respectively. Contrary to the other enterobacteria studied, the ESBL phenotype was not detected in *Proteus mirabilis*.

All isolated strains were resistant to cefotaxime and ceftazidime with MICs between 2 µg/mL and 64 µg/mL. A resistance to cefepime of more than 95% was also observed. All strains were sensitive to meropenem (MIC less than 0.25 µg/mL) and to colistin (MIC less than 0.5 µg/mL) (Table 2). One strain of *E. cloacae* was intermediate to ertapenem (MIC equal to 1 µg/mL). Resistance to amikacin was observed in only two strains (*E. cloacae* and *M. morganii*). A high rate of resistance to gentamicin, ciprofloxacin and cotrimoxazole was also observed in the strains producing β-lactamase.

### β-lactamase Types

The multiplex PCR allowed the detection of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{OXA}$ genes. The amplicon size was 800 bp for TEM, 713 bp for SHV and 564 bp for OXA. A rate of 90.2% was observed for the gene *bla*$_{TEM}$. *bla*$_{OXA}$ gene was carried by 36 strains (87.8%) while *bla*$_{SHV}$ gene was found in only five strains (12.2%). *bla*$_{TEM}$ gene was found alone in three strains of *K. pneumoniae* when *bla*$_{OXA}$ gene was found alone in three strains of *E. coli*. Four strains (three *K. pneumoniae* strains and one *E. cloacae* strain) carried concomitantly three genes *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{OXA}$. The association from *bla*$_{TEM}$/*bla*$_{OXA}$ genes (30 strains) and *bla*$_{TEM}$/*bla*$_{SHV}$ (one strain) was also observed in one strain. One strain of *E. cloacae* (73L/14) did not carry any of the searched genes, however, it presents an ESBL phenotype.

PCR products sequencing showed the presence of TEM-116 (2 strains) and TEM-1 (35 strains). The sequence analysis of *bla*$_{SHV}$ identified SHV-11 (two strains), SHV-12 (one strain) and SHV-75 (one strain). In the case of *bla*$_{OXA}$ gene, all sequences obtained were identified as OXA-1.

The prevalence of β-lactamases type TEM, SHV and OXA increased considerably thoughout the world since they first appearance. In Côte d'Ivoire, they have been identified in Enterobacteriaceae strains of human origin (Guessennd *et al*., 2008b). In this study, we looked for $bla_{TEM}$, $bla_{SHV}$ and $bla_{OXA}$ genes among enterobacteria genera from two major Ivorian cities namely Abidjan and Bouaké. Our study involved 73 enterobacteria which were predominant *K. pneumoniae* (33 strains) followed by *E. coli* (17 strains). The overall incidence of ESBL-producing Enterobacteriaceae was 56.2%. This rate has evolved compared to that observed in Côte d'Ivoire (9%) in 2008. However, it is less than that observed in India (67.4%) in 2012 (Guessennd *et al*., 2008b; Menezes *et al*., 2012). The detection rates in different types were 81.8% in *K. pneumoniae*, 58.8% in *E. coli*, 50% in *E. cloacae* and 16.7% in *M. morganii*. These rates are higher than those observed in Korea (30% and 9.2%), Cameroon (18.8% and 17.6%), in Tunisia (32.38% and 12.34%) and Mali (36% and 56%) respectively in *K. pneumoniae* and *E. coli* (Jeong *et al*., 2004; Gangoué-Piéboji *et al*., 2005; Abbassi *et al*., 2008; Tandé *et al*., 2009). This could be explained by the fact that we included only resistant strains to at least two extended spectrum cephalosporins in the study. However, a higher prevalence of 77.9%, was observed in 2010 by Towne *et al.* in *E. cloacae* (Towne *et al*., 2010).

The search for β-lactamases genes concerned $bla_{TEM}$, $bla_{SHV}$ and $bla_{OXA}$. The rate of 90.2%, 9.7% and 87.8% were observed respectively for TEM, SHV and OXA. These rates are different from those observed by Jain and Mondal (2008) with 75% for TEM and 46.8% for TEM and SHV (Jain et Mondal, 2008). The identification of sequences of $bla_{TEM}$ showed the presence of TEM-1 (94.6%) and TEM -116 (5.4%).
TEM-1 is the original β-lactamase from which are derived all other TEM by mutation of one or more amino acids. Commonly encountered in Gram negative bacteria, it is responsible for more than 90% of ampicillin resistance observed in *E. coli*. This enzyme also hydrolyzes the first generation of cephalosporins (Bradford, 2001). TEM-1 was found in different enterobacteria worldwide (Perilli *et al*., 2002; Jeong *et al*., 2004; Szabo *et al*., 2005; Dallenne *et al*., 2010). A literature review conducted by Storberg and published in 2014 reported the predominance of TEM-1 to prevalence rates up to 100% all over Africa (Storberg, 2014).

**Table.1** Primers used for PCR and Sequencing

| Targets | Primers (5'⟶3') | Amplicon size (pb) |
|---|---|---|
| $bla_{TEM}$ | MultiTSO-T_for CATTTCCGTGTCGCCCTTATTC | 800 |
| | MultiTSO-T_rev CGTTCATCCATAGTTGCCTGAC | |
| $bla_{SHV}$ | MultiTSO-S_for AGCCGCTTGAGCAAATTAAAC | 713 |
| | MultiTSO-S-rev ATCCCGCAGATAAATCACCAC | |
| $bla_{OXA}$ | MultiTSO-O_for GGCACCAGATTCAACTTTCAAG | 564 |
| | MultiTSO-O_rev GACCCCAAGTTTCCTGTAAGTG | |

**Table.2** Susceptibility to β-lactam Antibiotics and *bla* Genes Detected in Enterobacteria Studied

| | | | | MIC (µg/mL) | | | | | | *bla* genes detected | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strains | Isolates | Rzgion | Source | AMC | CTX | CAZ | FEP | ETP | MEM | TEM | SHV | OXA |
| *Escherichia coli* | | | | | | | | | | | | |
| | 2L/14 | Abidjan | Urine | >= 32 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 15L/14 | Abidjan | Others | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-116 | | OXA-1 |
| | 34L/14 | Bouaké | Urine | >= 32 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | | | OXA-1 |
| | 35L/14 | Bouaké | Urine | >= 32 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | | | OXA-1 |
| | 36L/14 | Abidjan | Pus | 16 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | | | OXA-1 |
| | 42L/14 | Abidjan | Feces | >= 32 | >= 64 | 16 | >= 64 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 43L/14 | Abidjan | Feces | >= 32 | >= 64 | >= 64 | >= 64 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 50L/14 | Abidjan | Urine | 16 | >= 64 | 16 | >= 64 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 53L/14 | Abidjan | Urine | >= 32 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 58L/14 | Bouaké | Urine | >= 32 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| *Klebsiella pneumoniae* | | | | | | | | | | | | |
| | 3L/14 | Abidjan | Urine | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 5L/14 | Abidjan | Blood | 16 | >= 64 | 4 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 8L/14 | Abidjan | Urine | 16 | >= 64 | >= 64 | 8 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 13L/14 | Abidjan | Blood | 8 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | TEM-1 | | |
| | 14L/14 | Abidjan | Others | >= 32 | >= 64 | 4 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 18L/14 | Bouaké | Blood | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | |
| | 19L/14 | Bouaké | Blood | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 23L/14 | Abidjan | Urine | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-116 | | |
| | 24L/14 | Abidjan | Urine | >= 32 | >= 64 | >= 64 | 8 | <= 0,5 | <= 0,25 | TEM-1 | SHV-11 | OXA-1 |
| | 25L/14 | Abidjan | Urine | | | 4 | 2 | | <= 0,25 | TEM-1 | SHV-11 | |
| | 31L/14 | Abidjan | Others | >= 32 | >= 64 | 8 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 32L/14 | Abidjan | Blood | >= 32 | >= 64 | 8 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 37L/14 | Abidjan | Pus | 16 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 39L/14 | Bouaké | Blood | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 44L/14 | Bouaké | Blood | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 45L/14 | Bouaké | Blood | 16 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 46L/14 | Abidjan | Pus | >= 32 | >= 64 | 16 | 32 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 47L/14 | Abidjan | Pus | >= 32 | 8 | 4 | <= 1 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 51L/14 | Abidjan | Urine | 16 | >= 64 | 4 | 2 | <= 0,5 | <= 0,25 | TEM-1 | SHV-75 | OXA-1 |
| | 57L/14 | Bouaké | Blood | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 59L/14 | Bouaké | Blood | >= 32 | >= 64 | 16 | 16 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 60L/14 | Bouaké | Blood | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 61L/14 | Abidjan | Feces | >= 32 | >= 64 | 16 | 16 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 67L/14 | Abidjan | Blood | >= 32 | >= 64 | 16 | 16 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 69L/14 | Abidjan | Blood | >= 32 | >= 64 | 16 | 8 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 70L/14 | Abidjan | Urine | >= 32 | >= 64 | 16 | 2 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 71L/14 | Bouaké | Urine | >= 32 | >= 64 | 16 | 16 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 72L/14 | Bouaké | Blood | >= 32 | >= 64 | >= 64 | 8 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| *Enterobacter cloacae* | | | | | | | | | | | | |
| | 21L/14 | Bouaké | Blood | >= 32 | >= 64 | >= 64 | 16 | 1 | <= 0,25 | TEM-1 | SHV-12 | OXA-1 |
| | 29L/14 | Abidjan | Blood | >= 32 | >= 64 | >= 64 | >= 64 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |
| | 73L/14 | Abidjan | Blood | >= 32 | >= 64 | >= 64 | >= 64 | <= 0,5 | <= 0,25 | | | |
| *Morganella morganii* | | | | | | | | | | | | |
| | 6L/14 | Abidjan | Urine | >= 32 | >= 64 | 2 | 4 | <= 0,5 | <= 0,25 | TEM-1 | | OXA-1 |

AMC: amoxicillin/clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, ETP: ertapenem, MEM: meropenem

In Côte d'Ivoire, this gene was also identified at a high rate but lower than ours (63.4%) showing his involvement in the resistance to extended-spectrum antibiotics observed in that country (Guessennd *et al*., 2008a). Different from TEM-1 in two mutation points (Val84Ile and Ala184Val), TEM-116 was detected for the first time in Korea, in Spain and Uruguay in *K. pneumoniae* and *E. coli* (Hu *et al*., 2008). It was subsequently detected in China in *Shigella flexneri* isolated from chicken droppings. Two studies have given the presence of TEM-116 in South Africa in *Salmonella* strains and Tunisia in *Providencia stuartii* (Usha *et al*., 2008; Lahlaoui *et al*., 2011). This β-lactamase was identified for the first time in Côte d'Ivoire in this study. TEM-116 is an extended spectrum β-lactamase with preferential hydrolytic activity to ceftazidime and cefotaxime (Jeong *et al*., 2004).

The $bla_{SHV}$ gene was detected in three strains of *K. pneumoniae* and one strain of *E. cloacae*. The identification showed the presence of SHV-11 and SHV-75 which have not ESBL activity and SHV-12 which is an ESBL (Heritage *et al*., 1999). SHV-11 and SHV-12 were reported for the first time in Switzerland in 1997 before reaching the world (Nüesch-Inderbinen *et al*., 1997). A recent study of *K. pneumoniae* strains from seven developing countries including Côte d'Ivoire showed the presence of SHV-11 (Breurec *et al*., 2012). SHV-12 has already been identified in Côte d'Ivoire (Guessennd *et al*., 2008a) and in other countries in Africa and worldwide (Perilli *et al*., 2002; Valverde *et al*., 2004; Jeong *et al*., 2004; Gangoué-Piéboji *et al*., 2005; Dallenne *et al*., 2010; Towne *et al*., 2010). For some of these studies, SHV-12 was the most expressed gene. This β-lactamase has an important epidemiological character because it is able to hydrolyze ceftazidime, cefotaxime and aztreonam (Kim *et al*., 1998). SHV-75 comes from SHV-1 by the substitution of histidine at position 254 by asparagine (www.lahey.org/studies). This gene was detected in Senegal in *K. pneumoniae* (Breurec *et al*., 2012). To our knowledge, this study represents the first detection of SHV-75 in Côte d'Ivoire.

The $bla_{OXA-1}$ gene was the only class D β-lactamase gene present in enterobacteria studied. Some previous studies showed his presence in Enterobacteriaceae (Abbassi *et al*., 2008; Lim *et al*., 2009; Ruppé *et al*., 2009; Storberg, 2014). OXA-1 is an enzyme generally identified in resistant enterobacteria to ampicillin. As OXA-31, it has the ability to hydrolyze cefepime and cefpirome, two fourth generation cephalosporins. However, it remains sensitive to ceftazidime. (Poirel *et al*., 2010) This gene is often found in combination with genes encoding other β-lactamases on the same plasmid. In our study, OXA, TEM and SHV associations were found. This increases the resistance of studied enterobacteria to antibiotic with extended spectrum.

The resistance point to antibiotics show a high resistance to β-lactam with extended spectrum except for ertapenem and meropenem. However, one strain of *E. cloacae* was intermediate to ertapenem without carbapenemase production. This decreased sensitivity could be explained by a decrease or loss in the permeability of the outer membrane of the enterobacteria (Dallenne *et al*., 2010). Another strain of *E. cloacae* presented a high level of resistance to third generation cephalosporins but none searched genes was found. It is likely that this strain produces other β-lactamase with extended spectrum. In addition to resistance to β-lactam antibiotics, resistance to ciprofloxacin was also observed. Some

previous studies have shown that plasmids carrying *bla* gene could also carry resistance genes to other families of antibiotics in particular *qnr* gene (Guessennd *et al*., 2008a; Ruppé *et al*., 2009).

In conclusion, the present study took into account enterobacteria from two regions of Côte d'Ivoire, showed changes in the prevalence of ESBL especially TEM and OXA. In addition, it has shown a diversification of $bla_{TEM}$ and $bla_{SHV}$ genes by detecting for the first time of TEM-116 and SHV-75 β-lactamases in the country. In the future, some studies on the detection and identification of ESBL circulating throughout the country should be undertaken. The identification of these enzymes is important in the care of patients, optimizing treatment and the establishment of measures to counteract the spread of antibiotic resistance, including β-lactams.

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