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# Detection of TEM and SHV Genes in Clinical *Escherichia coli* and *Klebsiella pneumoniae* Strains ESBL Isolated in Neonatology and Pediatric Units

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors VMG and SAA designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors VMG, SAA, AAT and TFBD managed the analyses of the study. Authors VMG, SAA, KNG, ASPNG and MD managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To detect *bla*$_{TEM}$ and *bla*$_{SHV}$ genes in *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae* (*K. pneumoniae)* strains resistant to β-lactams isolated from neonatal and pediatric infections.
**Place and Duration:** Bacteriology-Virology Department and Molecular Biology platform of Pasteur Institute of Côte d'Ivoire from January 2012 to November 2015.
**Methods:** A total of 38 strains of *E. coli* and *K. pneumoniae* ESBL isolated and identified according to classical bacteriology techniques from neonatal and pediatric infections, were subject of our study. Search for *bla*$_{TEM}$, and *bla*$_{SHV}$ genes were carried out by conventional PCR.

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**Results:** Molecular research showed that 52.6% of strains were carrying *bla*$_{TEM}$ gene and bla$_{SHV}$ gene was present in 36.9%. *bla*$_{TEM}$ and bla$_{SHV}$ genes were present simultaneously in 36.9% of strains.
**Conclusion:** This study revealed a predominance of *bla*$_{TEM}$ genes in *E. coli* and *K.* p*neumoniae* ESBL strains.

*Keywords: bla$_{TEM}$; bla$_{SHV}$ genes; E. coli; K. pneumoniae; neonatal and pediatric infections.*

## 1. INTRODUCTION

Selection pressure exerted by misuse of antibiotics results in the development of resistance mechanisms by bacteria, including a production of broad-spectrum beta-Lactamases (ESBL). ESBLs are a group of enzymes with the ability to hydrolyze penicillins, 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ generation of cephalosporins and aztreonam but these are inhibited by clavulanic acid [1,2]. *Enterobacteriaceae* including *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae* (*K. pneumonia*) are bacteria mostly involved in the production of ESBLs [3,4]. The existence of ESBL is global with various epidemics reported in several countries [5]. However, the prevalence of ESBL among strains differs by region [6]. African countries, particularly Côte d'Ivoire, are not immune to this phenomenon of bacterial resistance [7-9]. Recent studies demonstrated the presence of *bla*h genes in humans, animals and environment [7,10]. ESBLs characterized by a very large diversity, mainly derived plasmid type narrow penicillinases TEM (TEMoneira) and SHV (sulfhydryl Variable). These enzymes are frequently found in strains of *E. coli* and *K. pneumoniae* [1]. The hospital environment is conducive to spread of ESBL producing bacteria. ESBL producing strains are increasingly isolated in emergency Departments, paediatrics and maternity hospitals [11]. Transmission between patients, from mother to child, contaminated medical equipment or colonized health care workers would be risk factors in the acquisition of ESBL in these services [12,13]. The aim of this study is to detect the presence of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes in *E. coli* and *K. pneumoniae* producing ESBL isolated from neonatal and pediatric infections.

## 2. MATERIALS AND METHODS

### 2.1 Samples

Our study included a total of 38 non-redundant strains of *Enterobacteriaceae* including five strains of *E. coli* and 33 strains of *K. pneumoniae* identified according to classical bacteriology techniques (VITEK 2 system). Production of beta-lactamases has been demonstrated by double synergy method. These different strains isolated from neonatal or pediatric infections were collected during the period from January 2012 to November 2015 and are part of bio collection of Pasteur Institute in Côte d'Ivoire. These strains were stored at -80°C in brain heart broth supplemented with 10% glycerol.

### 2.2 Methods

#### 2.2.1 Identification of broad spectrum beta-lactamase-producing bacteria by the double synergy method

This method consisted of placing around a disk containing a β-lactamase inhibitor (amoxicillin / clavulanic acid (AMC)) and at a distance of 3 cm (centre-to-centre) Cefotaxime (CTX), Ceftazidime (CAZ) and Aztreonam (ATM) disks. Production of β-lactamases will be materialized by the appearance of a champagne-capped image reflecting the potentiating effect of clavulanic acid [14].

### 2.3 Extraction of Plasmid DNA

Plasmid DNA was extracted from 18 to 24 hours colony cells of *E. coli* and *K. pneumoniae* by alkaline lysis method with phenolization. After series of centrifugations, plasmid DNA obtained was stored at -20°C.

### 2.4 Detection and Amplification of Genes

A mix was prepared for each strain by adding to 5 µl of DNA, 5 µL of 5x colored Buffer, 5 µL of 5x non-colored buffer, 3 µL of $Mgcl_2$ (25 mM), 0.5 µL of dNTPs (10 mM), 1 µL of each primer and 0.3 µL of Taq polymerase in a final volume of 45 µl adjusted with water for injection preparation. Primers used for amplification of *bla*$_{TEM}$ genes were 5' ATAAAATTCTTGAAGACGAAA 3 'and 5'-GACAGTTACCAATGCTTAATCA-3'of number of accession AB 282997 and for genes *bla*$_{SHV}$ 5'

TTATCTCCCTGTTAGCCACC 3' and 5' GATTTGCTGATTTCGCTCGG3' of number of accession X 98098. Amplification of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes took place in 30 consecutive cycles of three main steps on the Gene Amp® AB applied biosystem 9700 thermocyclers. Each cycle included an initial denaturation at 94° C for 5 min followed by cyclic denaturation at 94° C for 1 min, hybridization at 50° C for *bla*$_{TEM}$ gene and 60°C for *bla*$_{SHV}$ gene for 1 min, cyclic elongation at 72° C for 1 min and final elongation at 72° C for 7 min. Migration of PCR products was carried out on a 2% agarose gel at 120 V for 30 minutes. Visualization of DNA strips was performed using an imaging system (Gel Doc$^{TM}$ EZ Imager).

### 2.5 Data Analysis

Exact Fischer test was used for comparison of two qualitative variables. Interpretation of significance between the variables consisted in comparing the P-value found with a previously defined threshold (generally 5%).

## 3. RESULTS

### 3.1 Genes Prevalence

*Bla*$_{TEM}$ and bla$_{SHV}$ genes have been identified respectively at 52.3% (20/38), 36.8% (14 / 38) and association of these two genes in 36.8% (14/38) of cases. Fig. 1 shows bands specific to PCR products for detecting *bla*$_{SHV}$ gene.

### 3.2 Genes Distribution According to Species

Distribution of genes detected according to species presented in Table 1. *bla*$_{TEM}$ gene is the most prevalent in both species with 100% for *E. coli* and 36.5% for *K. pneumoniae*. In both species, the presence of *the bla*$_{SHV}$ gene is almost always associated with that of the *bla*$_{TEM}$ gene, in 75% of cases with *E. coli* (Table 1).

### 3.3 Genes Distribution According to Year

Detection of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes according to a year of isolation of strains shows a predominance of *bla*$_{TEM}$ genes during four years from 2012 to 2015. *Bla*$_{SHV}$ gene was only found in 9.1% in 2012. *Bla*$_{TEM}$ and bla$_{SHV}$ genes were more frequently detected in strains of both species isolated in 2015 respectively at 100% and 92.3%. Rates are stable between 2012 and 2014 with p-Values significant (Table 2).

### 3.4 Genes Distribution According to Clinical Services and Biological Products

Distribution of biological products according to clinical services and detected *bla* genes showed that ESBL strains studied mainly came from blood cultures (7/19 in pediatric infections and 15/19 in neonatal infections). Differences observed between biological products, and two ESBL genes are not statistically significant (p> 0.05) (Table 3)

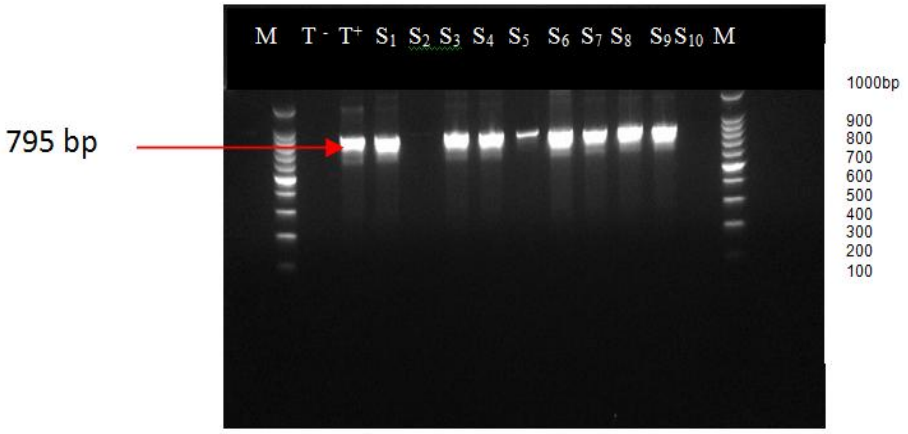


**Fig. 1. Electrophoretic profile of PCR amplification products for the detection of *the bla*$_{SHV}$ gene**

*M: Size marker 100 bp, T$^{+}$: Positive control, T$^{-}$: Negative control and S1 to S10: Samples analyzed. bp: bases pairs*

**Table 1. Distribution of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes according to *E. coli* and K. *pneumoniae* strains ESBL**

| Species (Nb) % (Nb ) | *bla*$_{TEM}$ % ( Nb ) | *bla*$_{SHV}$% (Nb) | *bla*$_{TEM}$ +*bla*$_{SHV}$ |
|---|---|---|---|
| *E. coli* (5) | 100 (5) | 80 (4) | 80 (4) |
| *K.pneumoniae*(33) | 36.4 (15) | 30.3 (10) | 30.3 (10) |
| Total (38) | 52.6 (20) | 36.8 (14) | 36.8(14) |

**Table 2. Distribution of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes according to years of isolation and clinical services**

| Years of isolation (Nb) | Types of genes *bla (*% ) (Nb) | | | p-Value |
|---|---|---|---|---|
| | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{TEM}$ +*bla*$_{SHV}$ | |
| 2012 (11) | 27.3 (3) | 9.1 (1) | 9.1 (1) | 0.0068 |
| 2013 (6) | 16.7 (1) | 0 | 0 | |
| 2014 (8) | 37.5 (3) | 12.5 (1) | 12.5 (1) | 0.001 |
| 2015 (13) | 100 (13) | 92.3 (12) | 92.3 (12) | |
| Clinical services(Nb) | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{TEM}$ +*bla*$_{SHV}$ | p-Value |
| Neonatology (19) | 73.7 (14) | 68.4 (13) | 68.4 (13) | |
| Pediatrics (19) | 31.6 (6) | 5.3 (1) | 5.3 (1) | > 0.05 |

**Table 3. Distribution of types of *bla* genes according to biological products and clinical services**

| Clinical services/ biological products (Nb) | Types of *bla* genes (Nb) | | | p-Value |
|---|---|---|---|---|
| **Neonatology** | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{TEM}$ +*bla*$_{SHV}$ | |
| Blood (15) | 15 (100%) | 15 (100%) | 15 (100%) | |
| Urine (1) | 1(100%) | 1 (100%) | 1 (100%) | > 0.05 |
| Cerebrospinal fluid (2) | 0 | 0 | 0 | |
| Bronchial aspiration (0) | 0 | 0 | 0 | |
| Suppuration (1) | 1 (100%) | 0 | 0 | |
| Probe tip (0) | 0 | 0 | 0 | |
| Pediatrics | | | | |
| Blood (7) | 2 (28.6%) | 0 | 0 | |
| Urine (6) | 3 (50%) | 0 | 0 | |
| Cerebrospinal fluid (2) | 0 | 0 | 0 | > 0.05 |
| Bronchial aspiration (1) | 0 | 0 | 0 | |
| Suppuration (2) | 0 | 0 | 0 | |
| Probe tip (1) | 0 | 0 | 0 | |

## 4. DISCUSSION

*Bla*$_{TEM}$ genes were more frequently detected (52.6%) compared to *bla*SHV genes (36.8%). These results are similar to previous work in Trinidad and Tobago which showed that 100% of *E. coli* ESBL strains carried the *bla*$_{TEM}$ genes against 4.1% of *bla*$_{SHV}$ and 84.3% of *K. pneumoniae* ESBL strains carried *bla*$_{TEM}$ genes against 34.5% of *bla*$_{SHV}$ [15]. In a study conducted in Iran, TEM and SHV genes were produced by *E. coli* strains and *K. pneumoniae* in respective proportions of 20.6% and 14.4% [16]. This gene distribution varies according to geographical region. Indeed, no ESBL was detected among 267 strains of *E. coli* and 53 strains of *K. pneumoniae* in a study in China [17].

An association of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes in proportions of 80% and 30.3% were observed respectively in *E. coli* and *K. pneumoniae*. One study in 2011 and another in 2010 in India revealed that this combination of genes was observed only in strains of *K. pneumonia* in the respective proportions of 42.6% and 20.11% while in *E. coli*, TEM genes were associated with the CTX-M genes [18,19]. A study in Côte

d'Ivoire also reported the association of $bla_{TEM}$ and $bla_{SHV}$ genes in the *Enterobacteriaceae* studied [20]. According to many authors, an association of genes in same bacteria can pose a diagnostic and therapeutic problem [21].

Evolution of gene prevalence according to years has shown a decrease from 2012 to 2013 (p=0.0068) but a considerable increase from 2013 to 2015 (p=0.001). Differences between the years of isolation and proportion of genes are statistically significant. This alarming situation calls for an urgent need to rationalize increasing use of antibiotics and to implement preventive hygiene strategies in our hospitals [22].

Study of distribution of *bla* genes according to the type of infections underlined a higher proportion of ESBL TEM and SHV in neonatology. This could be explained by a clonal distribution of strains harbouring these genes within this service. Indeed, the presence of the same gene profiles coding for β-lactamases observed in many isolates would be favoured by the proliferation of clonal strains or by transposable genetic elements bearing genes for antibiotic resistance [23]. Neonatology department can be subject of nosocomial epidemics which required closure and repair of this service [24,25].

Prevalence of ESBL genes showed a predominance of TEM and SHV gens isolated from blood cultures especially in neonatology. Studies elsewhere have shown a global emergence of ESBL-producing *Enterobacteriaceae* involved in the occurrence of pediatric blood infections [26]. These infections are becoming more frequent in neonatology with a high mortality rate [27].

## 5. CONCLUSION

This study revealed a high prevalence of $bla_{TEM}$ genes among strains of *E. coli* and K. *pneumoniae ESBL* isolated infections including neonatology. The spread of ESBL strains carrying TEM gene underscores need to implement restrictive measures in uncontrolled use of antibiotics, the real problem in Africa.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Bradford AP. Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology and detection of this important resistance threat. Clin Microbiol Rev. 2001;14(4):933-951.
2. Wiegand I, Geiss HK, Mack D, Sturenburg E, Seifert H. Detection of extended-spectrum beta-lactamases among *Enterobacteriaceae* by use of semi-automated microbiology systems and manual detection procedures. J Clin Microbiol. 2007;45:1167-1174.
3. Villegas M, Blanco M. Increasing prevalence of extended spectrum β-lactamase among Gram-negative bacilli in Latin America—2008 update from the Study for Monitoring Antimicrobial Resistance Trends (SMART). Braz J Infect Dis. 2011;15:34–39.
4. Jones RN, Guzman-Blanco M, Gales AC, Gallegos B, Castro AL, Martino MD, Vega S, Zurita J, Cepparulo M, Castanheira M. Susceptibility rates in Latin American nations: A report from a regional resistance surveillance program (2011). Braz J. Infect Dis. 2013;17:672–681.
5. Paterson DL, Bonomo DR. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev. 2005;18(4):657-686.
6. Sangaré SA, Maiga AI, Guido I, Maiga A, Camara N, Savadogo S, Diallo SF. Bougoudogo F, Armand-Lefevre L, Andremont A, Maiga II. Prevalence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* isolated from blood cultures in Africa. Médecine et Maladies Infectieuses. 2015;45:374–382.
7. Guessed N, Kacou-N'Douba A, Gbonon V, Yapi D, Ekaza E, Dosso M, Courvalin P. Prévalence et profile de résistance des entérobactéries product prices de β-lactamases à spectre élargi (BLSE) à Abidjan de 2005 à 2006. J Sci Biol. 2008; 9(1):63-70.
8. Magoué CL, Melin P, Gangoué-Piéboji J, Okomo Assoumou MC, Boreux R, De Mol P. Prevalence and spread of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in grounders, Cameroon. Clin. Microbiol. Infect. 2013; 19:E416-420.
9. Schaumburg F, Alabi A, Kokou C, Grobusch MP, Köck R, Kaba H, Becker K, Adegnika AA, Kremsner PG, Peters G,

Mellmann A. High burden of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in Gabon. J. Antimicrob. Chemother. 2013;68:2140–2143.

10. Toty AA, Guessennd N, Akoua-Koffi C, Otokoré DA, Meex C, Mbengue GV, Djaman AJ, Dosso M, Galleni M. First detection of TEM-116 and SHV-75 Producing *Enterobacteria* isolated from two Ivorian teaching hospitals: Case of Abidjan and Bouaké. Int Curr Microbiol App Sci. 2016;5(5):1-9.
11. Mariani-Kurkdja P, Doit C, Bingen E. Entérobactéries product prices de β-lactamases à spectre étendu en pédiatrie. Archives de pédiatrie. 2012;19:93-96.
12. Nelson E, Kayega J, Seni J, Mushi MF, Kidenya BR, Hokororo A, Zuechner A, Kihunrwa A, Mshana SE. Evaluation of existence and transmission of extended spectrum β- lactamase producing bacteria from post-deliverywomen to neonates at Bugando Medical Center Mwanza-Tanzania. BMC Res. Note. 2014;7:279.
13. Mshana SE, Hain T, Domann E, Lyamuya EF, Chakraborty T, Imirzalioglu C. Predominance of *Klebsiella pneumonia* ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. BMC Infect Dis. 2013; 13:466.
14. Jarlier V, Nicolas M, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer β- lactam agents in *Enterobacteriaceae*; hospital prevalence and susceptibility patterns. Rev Infect Dis.1988;10(4):867–78.
15. Akpaka PE, Legall B, Padman. Molecular detection and epidemiology of extended-spectrum β-lactamase genes prevalent in clinical isolates of *Klebsiella pneumonia* and *Escherichia coli* from Trinidad and Tobago. West Indian Med J. 2010;56(6): 591-596.
16. Zaniani RF, Meshkat Z, Nasab NM, Karamadini-Khaje M, Ghazini K, Rezaee A, Esmaily H, Nabavinia SM, Hoseini DM. The prevalence of TEM and SHV Genes among Extended- Spectrum β-lactamases producing *Escherichia coli* and *Klebsiella pneumonia.* Iran J Basic Med Sci. 2012;15(1):654-660.
17. Liao K, Chen Y, Wang M, Guo P, Yang Q, Ni Y, Yu Y, Hu B, Sun Z, Huang W,Wang Y, Wu A, Feng X, Luo Y, Hu Z., Chu Y., Chen S, Cao B, Su J, Gui B, Duan Q, Zhang S, Shao H, Kong H, Xu Y. Molecular characteristics of extended-spectrum *β*-lactamase producing *Escherichia coli* and *Klebsiella pneumonia* causing intra-abdominal infections from nine tertiary hospitals in China. Diagn Microbiol Infect Dis. 2017;87(1):45-48.
18. Manoharan A, Premalatha K, Chatterjee S, Mathai D, SARI Study Group. Correlation of TEM, SHV and CTX-M extended-spectrum β-lactamases among *Enterobacteriaceae* with their *in vitro* antimicrobial susceptibility. Indian J Med Microbiol. 2011;29:161-164.
19. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Prevalence of $bla_{TEM}$ ,$bla_{SHV}$ and $bla_{CTX-M}$ genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. Indian J Pathol Microbiol. 2014;357:249-54.
20. Guessennd N, Bremont S, Gbonon V, Kacou-N Douba A, Ekaza E, Lambert T, Dosso M, Courvalin P. Résistance aux quinolones de type qnr chez les entérobactéries product prices de β-lactamases à spectre élargi à Abidjan en Côte d'Ivoire. Pathologie Biologie. 2008;56(7-8):439-446.
21. Roh KH, Uh Y, Kim JS, Kim HS, Shin DH, Song W. First outbreak of multi drug resistant*Klebsiella pneumonia* producing both SHV-12-type extended-spectrum β-lactamase and DHA-1-Type AmpCβ-lactamase at a Korean Hospital Yonsei. Med J. 2008;49(1):53-57.
22. Naas T, Cuzon G, Robinson AL, Andrianirina Z, Imbert P, Ratsima E, Ranosiarisoa ZN, Nordmann P, Raymond J. Neonatal infections with multidrug resistant ESBL producing *E. cloacae* and *K. pneumonia* in neonatal units of two different Hospitals in Antananarivo, Madagascar. BMC Infect Dis. 2016;16:275.
23. Yu WL, Chuang YC, Walther-Rasmussen J. Extended spectrum β- lactamases in Taiwan: epidemiology, detection, treatment and infection control J Microbiol Immunol Infect. 2006;39:264–277.
24. Leroyer C, Lehours P, Tristan A, Boyer F, Marie V, Elleau C, Nolent P, Venier AG, Brissaud O, de Barbeyrac B, Megraud F, Rogues AM. Outbreak in newborns of methicillin-resistant *Staphylococcus aureus* related to the sequence type 5 Geraldine clone. Am J Infect Control. 2016;44:e9-e11).

25. Stapleton PJ, Murphy M, McCallion N, Brennan M, Cunney R, Drew RJ. Outbreaks of extended spectrum β-lactamase producing *Enterobacteriaceae* in neonatal intensive care units: A systematic review. Arch Dis Child Fetal Neonatal ED. 2016;101(1);F72-78.
26. Dramowski A, Cotton MF, Rabie H, Whitelaw A. Trends in paediatric bloodstream infections at a South African referral hospital. BMC Pediatrics. 2015;15(1):33.
27. Flokas ME, Karanika S, Alevizakos M, Mylonakis E. Prevalence of ESBL Producing *Enterobacteriaceae* in Pediatric Blood stream Infections: A Systematic Review and Meta Analysis. PLoS ONE. 2017;12(1):e0171216.



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