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.

Article Information

DOI: 10.9734/MRJI/2018/39354

Editor(s):
(1) Essam Hussein Abdel-Shakour, Professor, Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Reviewers:
(1) Abdullahi Sani Ahmad, Public Health and Diagnostic Institute, Northwest University, Nigeria.
(2) O. A. Thonda, Obafemi Awolowo University, Nigeria.

Complete Peer review History: http://www.sciencedomain.org/review-history/23880

Original Research Article

ABSTRACT

Aims: To detect blaTEM and blaSHV 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 blaTEM and blaSHV genes were carried out by conventional PCR.

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Results: Molecular research showed that 52.6% of strains were carrying blaTEM gene and blaSHV gene was present in 36.9%. blaTEM and blaSHV genes were present simultaneously in 36.9% of strains.

Conclusion: This study revealed a predominance of blaTEM genes in E. coli and K. pneumoniae ESBL strains.

Keywords: blaTEM, blaSHV 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, 1st, 2nd and 3rd generation of cephalosporins and aztreonam but these are inhibited by clavulanic acid [1,2]. Enterobacteriaceae including Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae) 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 blal 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 (sulhydryl 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 blaTEM and blaSHV 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 Mgcl2 (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 blaTEM genes were 5’ ATAAAAATTCTTGAAGACGAAA 3’ and 5’-GACAGTTACCAAATGCTTATCA-3’of number of accession AB 282997 and for genes blaSHV 5’

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TTATCTCCCTGTAGCCACC 3’ and 5’ GATTGTGGATGGCTGCTGG3’ of number of accession X 98098. Amplification of \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{SHV}} \) 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 \( \text{bla}_{\text{TEM}} \) gene and 60°C for \( \text{bla}_{\text{SHV}} \) 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™ 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

\( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{SHV}} \) 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 \( \text{bla}_{\text{SHV}} \) gene.

3.2 Genes Distribution According to Species

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

3.3 Genes Distribution According to Year

Detection of \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{SHV}} \) genes according to a year of isolation of strains shows a predominance of \( \text{bla}_{\text{TEM}} \) genes during four years from 2012 to 2015. \( \text{bla}_{\text{SHV}} \) gene was only found in 9.1% in 2012. \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{SHV}} \) 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 \( \text{bla} \) 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).
Table 1. Distribution of $\text{bla}_{\text{TEM}}$ and $\text{bla}_{\text{SHV}}$ genes according to $E. \text{coli}$ and $K. \text{pneumoniae}$ strains ESBL

<table>
<thead>
<tr>
<th>Species (Nb)</th>
<th>$\text{bla}_{\text{TEM}}$ % (Nb)</th>
<th>$\text{bla}_{\text{SHV}}$ % (Nb)</th>
<th>$\text{bla}<em>{\text{TEM}} + \text{bla}</em>{\text{SHV}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E. \text{coli}$ (5)</td>
<td>100 (5)</td>
<td>80 (4)</td>
<td>80 (4)</td>
</tr>
<tr>
<td>$K. \text{pneumoniae}$ (33)</td>
<td>36.4 (15)</td>
<td>30.3 (10)</td>
<td>30.3 (10)</td>
</tr>
<tr>
<td>Total (38)</td>
<td>52.6 (20)</td>
<td>36.8 (14)</td>
<td>36.8 (14)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Years of isolation (Nb)</th>
<th>Types of genes $\text{bla}$ (%) (Nb)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{bla}_{\text{TEM}}$</td>
<td>$\text{bla}_{\text{SHV}}$</td>
</tr>
<tr>
<td>2012 (11)</td>
<td>27.3 (3)</td>
<td>9.1 (1)</td>
</tr>
<tr>
<td>2013 (6)</td>
<td>16.7 (1)</td>
<td>0</td>
</tr>
<tr>
<td>2014 (8)</td>
<td>37.5 (3)</td>
<td>12.5 (1)</td>
</tr>
<tr>
<td>2015 (13)</td>
<td>100 (13)</td>
<td>92.3 (12)</td>
</tr>
<tr>
<td>Clinical services (Nb)</td>
<td>$\text{bla}_{\text{TEM}}$</td>
<td>$\text{bla}_{\text{SHV}}$</td>
</tr>
<tr>
<td>Neonatology (19)</td>
<td>73.7 (14)</td>
<td>68.4 (13)</td>
</tr>
<tr>
<td>Pediatrics (19)</td>
<td>31.6 (6)</td>
<td>5.3 (1)</td>
</tr>
</tbody>
</table>

Table 3. Distribution of types of $\text{bla}$ genes according to biological products and clinical services

<table>
<thead>
<tr>
<th>Clinical services/ biological products (Nb)</th>
<th>Types of $\text{bla}$ genes (Nb)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (15)</td>
<td>$\text{bla}_{\text{TEM}}$</td>
<td>$\text{bla}_{\text{SHV}}$</td>
</tr>
<tr>
<td>Urine (1)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Cerebrospinal fluid (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bronchial aspiration (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Suppuration (1)</td>
<td>1 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Probe tip (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pediatrics</td>
<td>2 (28.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Blood (7)</td>
<td>3 (50%)</td>
<td>0</td>
</tr>
<tr>
<td>Urine (6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrospinal fluid (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bronchial aspiration (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Suppuration (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Probe tip (1)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

4. DISCUSSION

$\text{bla}_{\text{TEM}}$ genes were more frequently detected (52.6%) compared to $\text{bla}_{\text{SHV}}$ genes (36.8%). These results are similar to previous work in Trinidad and Tobago which showed that 100% of $E. \text{coli}$ ESBL strains carried the $\text{bla}_{\text{TEM}}$ genes against 4.1% of $\text{bla}_{\text{SHV}}$ and 84.3% of $K. \text{pneumoniae}$ ESBL strains carried $\text{bla}_{\text{TEM}}$ genes against 34.5% of $\text{bla}_{\text{SHV}}$ [15]. In a study conducted in Iran, TEM and SHV genes were produced by $E. \text{coli}$ strains and $K. \text{pneumoniae}$ 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 $E. \text{coli}$ and 53 strains of $K. \text{pneumoniae}$ in a study in China [17].

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

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.

REFERENCES


