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Full Length Research Paper

Prevalence of *bla$_{SHV}$* genes in clinical isolates of *Klebsiella pneumoniae* at Saint Camille medical Center in Ouagadougou. Isolation of *bla$_{SHV11}$*-like gene

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Five bacterial strains (4 *Klebsiella pneumoniae* and 1 *Escherichia coli*) representative of pathogenic species and resistant to β-lactam antibiotics are investigated to isolate the genes responsible of β-lactamase activity. The use of engineering techniques enables us to show the widespread of *bla$_{SHV}$* genes particularly in clinical isolates of *K. pneumoniae*. Our results highlighted an atypical *bla$_{SHV-11}$* gene.

Keys words: β-Lactamases, *Klebsiella pneuminiae*, *bla$_{SHV}$* gene, Saint Camille medical centre, Ouagadougou.

INTRODUCTION

The major mechanism of bacterial resistance to β-lactam antibiotics is the production of β-lactamases. These enzymes are able to inactivate the β-lactam antibiotic by hydrolysing the β-lactam ring (Abraham and Chain, 1940; Frère et al, 1991; Reid et al, 1987). It is reported that up 90% ampicillin resistance is due to the production of TEM-1 β-lactamase in *Escheridia coli* (Livermore, 1995) and SHV-1 β-lactamase in *Klebsiella pneumoniae* (Tzouvelekis and Bonomo, 1999). Many bacteria commonly isolated from human biological specimens like *E. coli, klebsiella pneumoniae Shigella spp., and Salmonella spp.* responsible for infection of gastroenteritis, urinary disease and the inflammation of the ear (children) were collected at the Saint Camille medical center of Ouagadougou and these strains exhibit an important β-lactamase activity against the usual β-lactam-antibiotics (Zeba et al., 2003). The present work is an attempt to establish what types of β-lactamase genes are occurring in our area may be related to these activities. Therefore, one *E. coli* and 4 *K. pneumoniae* strains were screened. This is the first epidemiological study at this level, carried in Ouagadougou and Burkina Faso.

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MATERIALS AND METHODS

Bacterial strains.

K. pneumoniae No. 291, 312, 392, 1201 and *E. coli* No.1004 were isolated from clinical specimens (faeces, urine, pus etc.) and were identified with API 20 system (Bio Merieux France).

Culture conditions

These clinical wild strains were grown on trypticase soy agar (Difco) plates (solid medium) or in Luria Bertani (LB Difco).

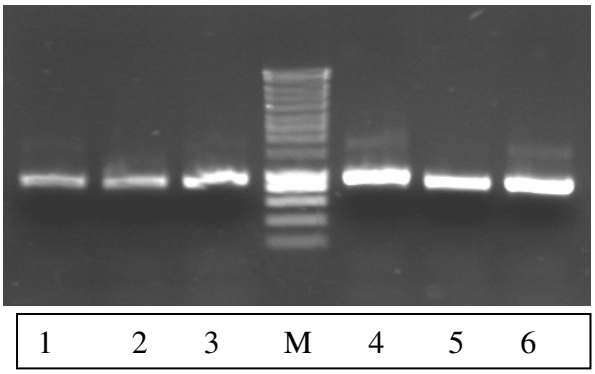

Figure 1. Purified PCR fragments obtained with SHV primers. Lane 1: Amplified fragment from shot gun clone BglII of *K. pneumoniae* N°291. Lane 2: Amplified fragment from genomic DNA of *K. pneumoniae* N° 312. Lane 3: Amplified fragment from shot gun clone BamHI of *K. pneumoniae* N° 312. M: DNA marker Smart Ladder. Lane 4: Amplified fragment from shot gun clone BamHI of *K. pneumoniae* N°312.Lane 5: Amplified fragment from shot gun clone BamHI of *K. pneumoniae* N° 392. Lane 6: Amplified fragment from shot gun clone Sau3AI of *K. pneumoniae* N° 1201. Cloning these fragments into pGEM-T Easy vector provided three recombinant plasmids: Fragment of lane 2 gave clone 2. Fragment of lane 5 gave clone named clone 5. Fragment of lane 6 gave clone 6. Recombinants 2, 5 and 6 are now reported on figure 2 under N° 1, 2 and 3 respectively.

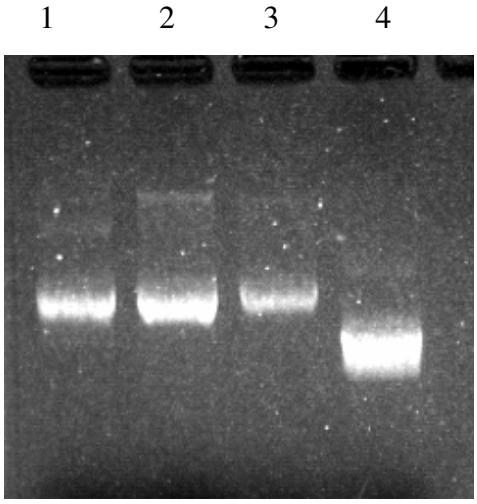

Figure 2. PCR fragments cloned into pGEM-T Easy vector. These fragments were obtained by amplification of genomic DNAs or shotgun clones with primers for SHV. Lane 1: Amplified fragment from genomic DNA of *K. pneumoniae* N° 312 cloned into pGEM-T Easy. Lane 2: Amplified fragment from shotgun clone BamHI fragment of *K. pneumoniae* N°392 cloned into pGEM-T Easy. Lane 3: Amplified fragment from shotgun clone Sau3aI of *K. pneumoniae* N° 1201 cloned into pGEM-T Easy. Lane 4: pGEM-T Easy without insert (for comparison). Sequencing of fragments from 1 and 2 gave *bla* $_{SHV-1}$ gene and *bla*$_{SHV-11}$ like gene respectively.

The *E. coli* competent DH5-α cells used in transformation experiments were grown in SOC medium which allow quick recuperation of cells and ensure maximum transformation efficiency.

Shotgun cloning

Genomic DNA**s** of the strains were extracted and purified by using Wizard kits of genomic DNA purification (Wizard genomic DNA purification kit, Promega corporation, Madison, WI, USA). Different types of Fragments from genomic DNAs were obtained by using specific different restriction enzymes (Sau3AI, BamHI, BglII, EcoRI etc.) These fragments were ligated into cloning vector PK18, carrying kanamycin resistance gene and transformed into *E. coli* competent DH5-α cells. The PK18 recombinant clones were selected on plates containing 50 µg of kanamycin per ml + 60 mM IPTG + 40 µg of Xgal per ml (KIX plates).

PCR

The PCR is both carried out to detect the β-lactamase genes or to amplify and isolate them (Mabilat and Goussard (1993), Brinas et al. (2002). PCR amplifications of the *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{ampC}$ genes were carried out using specific primers pairs (for example SHVF and SHVR, TEMF and TEMR, ampCF and ampCR) for amplification of correspondent genes (Sutcliffe, 1978; Mercier and Levesque, 1990) when the template is genomic DNA or shotgun clones. Theses primers were obtained from Eurogentec Bel. S.A. The PCR products were ligated into pGEM-T Easy Vector carrying ampicillin resistance gene and as previously, transformed into *E. coli* competent DH5-α cells. The correspondents clones were always selected on plates containing 50µg of ampicillin per ml + 60 mM IPTG and 40 µg per ml X-gal (AIX plates).

RESULTS AND DISCUSSIONS

Four recombinants plasmids carrying ampicillin-resistant genes were recovered from genomic DNAs of strains No. 291, 312, 392 and 1201. The attempt to obtain recombinant plasmids from strain 1004 DNA with this approach failed.

The PCR with primers specific for the *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{AMPC}$ genes were performed both directly on genomic DNAs and on recombinant plasmids. Positive PCR results were obtained from recombinant plasmids and from genomic DNA 291, 312, 392 and 1201 with primer of SHV (Figure 1). Positive PCR result was obtained with genomic DNA of strain N^{o}. 1004 with primer TEM (result not reported). PCR with primer AmpC was negative on all the strains investigated.

These results clearly point out that the *K. pneumoniae* strain N° 291, 312, 392 and 1201 harbour *bla*$_{SHV}$ genes and the *E. coli* N°1004 have *bla*$_{Tem}$ gene.

Among the 4 identified *bla*$_{SHV}$ genes, 2 were isolated by cloning PCR products into pGEM-T Easy Vector (Figure 2) but only two were successfully sequenced. One is a typical *bla* $_{SHV-1}$ gene [clone 1 from figure 2 (coming from fragment 2 of Figure 1)]. The second is the *bla* $_{SHV-11}$ -like gene [clone 2 from Figure 2

```
shv-11    1   atgcgttatattcgcctgtgtattatctccctgttagccaccctgccgctggcggtacac 60
                M  R  Y  I  R  L  C  I  I  S  L  L  A  T  L  P  L  A  V  H
clone 5   1   ATGCGTTATATTCGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGTACAC 60
                M  R  Y  I  R  L  C  I  I  S  L  L  A  T  L  P  L  A  V  H

shv-11    61  gccagcccgcagccgcttgagcaaattaaacaaagcgaaagccagctgtcgggccgcgta 120
                A  S  P  Q  P  L  E  Q  I  K  Q  S  E  S  Q  L  S  G  R  V
clone 5   61  GCCAGCCCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTCGGGCCGCGTA 120
                A  S  P  Q  P  L  E  Q  I  K  Q  S  E  S  Q  L  S  G  R  V

shv-11    121 ggcatgatagaaatggatctggccagcggccgcacgctgaccgcctggcgcgccgatgaa 180
                G  M  I  E  M  D  L  A  S  G  R  T  L  T  A  W  R  A  D  E
clone 5   121 GGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGCCTGGCGCGCCGATGAA 180
                G  M  I  E  M  D  L  A  S  G  R  T  L  T  A  W  R  A  D  E

shv-11    181 cgctttcccatgatgagcacctttaaagtagtgctctgcggcgcagtgctggcgcgggtg 240
                R  F  P  M  M  S  T  F  K  V  V  L  C  G  A  V  L  A  R  V
clone 5   181 CGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGCGGCGCAGTGCTGGCGCGGGTG 240
                R  F  P  M  M  S  T  F  K  V  V  L  C  G  A  V  L  A  R  V

shv-11    241 gatgccggtgacgaacagctggagcgaaagatccactatcgccagcaggatctggtggac 300
                D  A  G  D  E  Q  L  E  R  K  I  H  Y  R  Q  Q  D  L  V  D
clone 5   241 GATGCCGGTGACGAACAGCTGGAGCGAAAGATCCACTATCGCCAGCAGGATCTGGTGGAC 300
                D  A  G  D  E  Q  L  E  R  K  I  H  Y  R  Q  Q  D  L  V  D

shv-11    301 tactcgccggtcagcgaaaaacatcttgccgacggcatgacggtcggcgaactctgcgcc 360
                Y  S  P  V  S  E  K  H  L  A  D  G  M  T  V  G  E  L  C  A
clone 5   301 TACTCGCCGGTCAGCGAAAAACACCTTGCCGACGGCATGACGGTCGGCGAACTCTGTGCC 360
                Y  S  P  V  S  E  K  H  L  A  D  G  M  T  V  G  E  L  C  A

shv-11    361 gccgccattaccatgagcgataacagcgccgccaatctgctgctggccaccgtcggcggc 420
                A  A  I  T  M  S  D  N  S  A  A  N  L  L  L  A  T  V  G  G
clone 5   361 GCCGCCATTACCATGAGCGATAACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGC 420
                A  A  I  T  M  S  D  N  S  A  A  N  L  L  L  A  T  V  G  G

shv-11    421 cccgcaggattgactgccttttgcgccagatcggcgacaacgtcacccgccttgaccgc 480
                P  A  G  L  T  A  F  L  R  Q  I  G  D  N  V  T  R  L  D  R
clone 5   421 CCCGCAGGATTGACTGCCTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGC 480
                P  A  G  L  T  A  F  L  R  Q  I  G  D  N  V  T  R  L  D  R

shv-11    481 tgggaaacggaactgaatgaggcgcttcccggcgacgcccgcgacaccactaccccggcc 540
                W  E  T  E  L  N  E  A  L  P  G  D  A  R  D  T  T  T  P  A
clone 5   481 TGGGAAACGGAACTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCC 540
                W  E  T  E  L  N  E  A  L  P  G  D  A  R  D  T  T  T  P  A

shv-11    541 agcatggccgcgaccctgcgcaagctgctgaccagccagcgtctgagcgcccgttcgcaa 600
                S  M  A  A  T  L  R  K  L  L  T  S  Q  R  L  S  A  R  S  Q
clone 5   541 AGCATGGCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTCGCAA 600
                S  M  A  A  T  L  R  K  L  L  T  S  Q  R  L  S  A  R  S  Q

shv-11    601 cggcagctgctgcagtggatggtggacgatcgggtcgccggaccgttgatccgctccgtg 660
                R  Q  L  L  Q  W  M  V  D  D  R  V  A  G  P  L  I  R  S  V
clone 5   601 CGGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCCGTG 660
                R  Q  L  L  Q  W  M  V  D  D  R  V  A  G  P  L  I  R  S  V

shv-11    661 ctgccggcgggctggtttatcgccgataagaccggagctggcgaacggggtgcgcgcggg 720
                L  P  A  G  W  F  I  A  D  K  T  G  A  G  E  R  G  A  R  G
clone 5   661 CTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAACGGGGTGCGCGCGGG 720
                L  P  A  G  W  F  I  A  D  K  T  G  A  G  E  R  G  A  R  G

shv-11    721 attgtcgccctgcttggcccgaataacaaagcagagcgcattgtggtgatttatctgcgg 780
                I  V  A  L  L  G  P  N  N  K  A  E  R  I  V  V  I  Y  L  R
clone 5   721 ATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATCGTGGTGATTTATCTGCGG 780
                I  V  A  L  L  G  P  N  N  K  A  E  R  I  V  V  I  Y  L  R

shv-11    781 gatacgccggcgagtatggccgagcgaaatcagcaaatcgccgggatcggcgcggcgctg 840
                D  T  P  A  S  M  A  E  R  N  Q  Q  I  A  G  I  G  A  A  L
clone 5   781 GATACGCCGGCGAGCATGGCCGAGCGAAATCAGCAAATCGCCGGGATCGGCGCGGCGCTG 840
                D  T  P  A  S  M  A  E  R  N  Q  Q  I  A  G  I  G  A  A  L

shv-11    841 atcgagcactggcaacgctaa 861
                I  E  H  W  Q  R  *
clone 5   841 ATCGAGCACTGGCAACGCTAA 861
```

Figure 3. Alignment between typical *bla* $_{SHV-11}$ gene and the sequence of clone 5 obtained by PCR amplification of BamHI from *K. pneumoniae* No. 392.

(coming from fragment 5 of Figure 1)]. An attempt to sequence fragment N°3 of Figure 2 (coming from fragment 6 of Figure 1) failed.

Substrate profiles of β-lactamase from eleven *K. pneumoniae* genes *and* those currently studied (Zeba et al, 2003) indicate a close behavioural similarity in their spectral activity. These phenotype similarities can be related to those of the genotypes. Thus the bla_{SHV} gene may be common among *K. coli* species. Our results also indicate the existence of parental gene of bla_{SHV} (bla_{SHV-1}) on clinical wild strains of *K. pneumoniae* in Ouagadougou (Burkina Faso) and highlight an atypical bla_{SHV11} gene. This gene of 861 bp (clone 2, Figure 3) was isolated from *K. pneumoniae* N°392. It is not identical with bla_{SHV-11} genes available in gene Bank (NCBI-DB). The nucleotide, alignment of bla_{SHV-11} gene and that of clone 5 reveals substitutions at position 324 (cytosine replace thymine), 357 (thymine replace cytosine), 762 (cytosine replace thymine) and 795 (cytosine replace thymine). It appears that the change always occurs between cytosine and thymine. Therefore, on the basis of nucleotide sequences, the two genes (classical bla_{SHV-11} and clone 5) are slightly different, but the translation of the two genes yields the same variant SHV-11 β-lactamase. Our investigations are continuing to establish clearly the different types of bla_{SHV} genes encountered in Burkina Faso. The PCR analysis of the single *E. coli* detects bla_{TEM} type gene. We hope to further investigate these and similar genes in subsequent studies.

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