

## Review

# Overview of $\beta$ -lactamase incidence on bacterial drug resistance

Boukaré ZEBÀ

UFR-SVT/ Université de Ouagadougou/ Burkina Faso, 03 Bp 7021 Ouagadougou 03. E-mail: [aboubakrzeba@yahoo.fr](mailto:aboubakrzeba@yahoo.fr).

Accepted 15 September, 2005

**The bacterial drug resistance is its ability to grow in presence of high concentration of an antibiotic. The massive and often unsuitable use of antibiotic tends to select the resistant mutants that are then disseminated. The  $\beta$ -lactam antibiotics represent an antibiotic family widely used because of their remarkable tolerance by animal organism. This practice generated the resistance from many bacteria in clinical and environmental spaces. One of the main drug resistance mechanism used by bacteria is the production of inactivation enzymes named  $\beta$ -lactamases. This mini review attempts to pinpoint the  $\beta$ -lactamase incidence on the failure of clinical treatment using  $\beta$ -lactam antibiotic anywhere.**

**Key words:** Bacteria,  $\beta$ -lactamase, drug resistance.

## INTRODUCTION

Antibiotic resistance of pathogenic bacteria is more and more of high clinical relevance. Penicillin is the first antibiotic discovered by Fleming (1929). Penicillin belongs to  $\beta$ -lactam antibiotics which have a lethal effect on bacteria via an interaction with the target proteins known as penicillin-binding proteins (PBPs) located on the cell membrane. The PBPs are enzymes (transpeptidases) which are involved in bacterial wall peptidoglycan cross-linking. The damage caused by  $\beta$ -lactams to bacteria particularly in growing state come from their ability to bind transpeptidases and to inactivate them. This process of inactivation of the cross-linking enzymes disrupts the normal physiological constitution of cell wall peptidoglycan and induces cell lysis and death (Thomas et al., 1977). Unfortunately for chemotherapy and clinical treatment,  $\beta$ -lactamases which could be considered as bacterial absolute molecular weapons appeared. These enzymes probably existed before the discovery of  $\beta$ -lactam antibiotics, and may play other roles in bacteria. Until today  $\beta$ -lactamases have been exclusively found in bacteria.  $\beta$ -lactamase activity has not been detected in  $\beta$ -lactam-producing fungi such as *Penicillium* and *Cephalosporium* species (Ogawara et al., 1999). About the origin of these enzymes, the accepted idea today is that  $\beta$ -lactamases are the result of conversion of PBPs by genetic transformation. This means that both types of proteins have a common ancestor.  $\beta$ -Lactamase were first described as an enzyme from bacteria able to destroy penicillin by

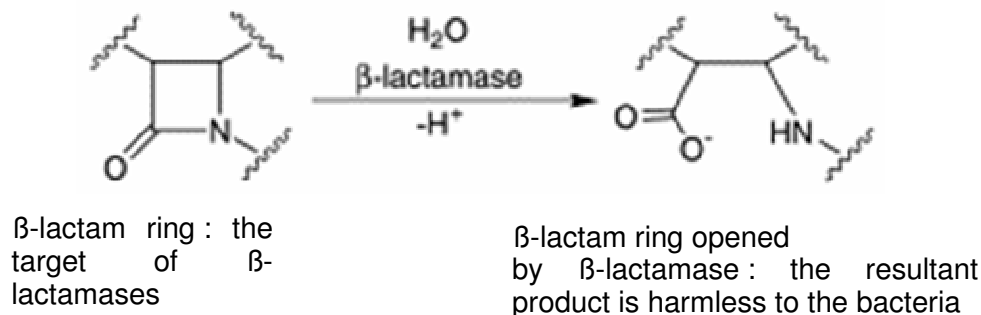
Abraham et al. (1940). The first factor responsible for bacterial resistance is the constitutive or inducible production of these hydrolytic enzymes. These enzymes were characterized by the Special Commission of the International Union of Biochemistry as enzymes acting on amide and cyclic amide bond (Webb, 1984; Figure 1). Therefore, they have the classification number (E.C 3.5.2.6) and received the systematic name of  $\beta$ -lactam hydrolases.

The aim of this short review on the great and complex subject of  $\beta$ -lactamases is to analyse  $\beta$ -lactamase mediated bacterial resistance throughout the world with a particular focus on the African area. We hope that this modest contribution will attract attention to the phenomenon and help devising a modified approach to antibiotic consumption in the African area.

## CLASSIFICATION OF $\beta$ -LACTAMASES

$\beta$ -Lactamases are grouped into 4 classes (Ambler, 1980) named A, C, D and B which derive from two main groups (serine and metallo- $\beta$ -lactamases) (Table 1, Figure 2). The following are the features of the different classes of  $\beta$ -lactamases:

1. The class A enzymes include many members of which TEM and SHV are the main representatives. Historically



**Figure 1.** Hydrolytic reaction of  $\beta$ -lactamase on the amide bond of the  $\beta$ -lactam ring.

**Table 1.** Two classification schemes for  $\beta$ -lactamases.

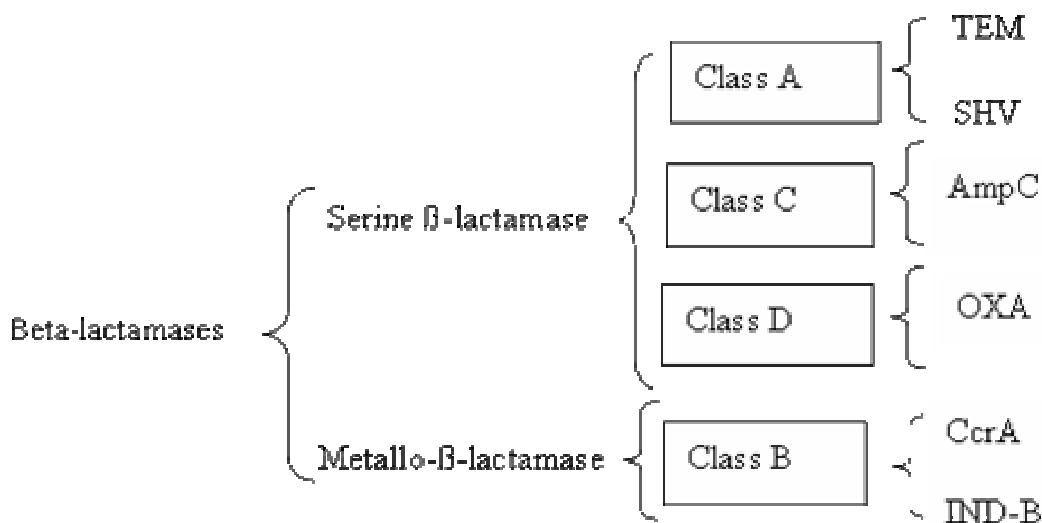
| Functional classification <sup>a</sup> | Molecular classification <sup>b</sup> | Characteristics                                                                                                                                                                                                              |
|----------------------------------------|---------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Group 2a                               | molecular Class A                     | 25-32 kDa Narrow spectrum penicillinases carried by Gram-negative bacteria and inhibited by active site inactivators (such as clavulanic acid). Example: <i>Klebsiella pneumoniae</i> chromosomal $\beta$ -lactamase, LEN-1. |
| Group 2b                               | molecular class A                     | 22-36 kDa Broad spectrum penicillinases, inhibited by clavulanic acid. Example: <i>Enterobacter cloacae</i> plasmid pDSO76 $\beta$ -lactamase, OHIO-1.                                                                       |
| Group 1                                | molecular Class C                     | 40 kDa Cephalosporinases not inhibited by active site inactivators. Example: <i>Pseudomonas aeruginosa</i> strain PA01 chromosomal AmpC $\beta$ -lactamase.                                                                  |
| Group 2be                              | molecular Class A                     | 28-29 kDa, extended spectrum $\beta$ -lactamases (ESBL) type TEM and SHV, inhibited by active site inactivators. Example: <i>Pseudomonas aeruginosa</i> chromosomal $\beta$ -lactamase, PER-1.                               |
| Group 2br                              | molecular Class A                     | 24 kDa Broad spectrum $\beta$ -lactamases not well inhibited by active site inactivators. Example: <i>Escherichia coli</i> strain GUER plasmid $\beta$ -lactamase, TEM-30.                                                   |
| Group 2c                               | molecular Class A                     | 22-34 kDa Carbenicillinase type PSE inhibited by active site inactivators. Example: <i>Acinetobacter calcoaceticus</i> strain A85-145 $\beta$ -lactamase, CARB-5.                                                            |
| Group 2d                               | molecular Class D                     | 29-53 kDa Oxacillinases, not well inhibited by active site inactivators. Example: <i>Salmonella typhimurium</i> strain type 1a $\beta$ -lactamase, OXA-2.                                                                    |
| Group 2e                               | molecular Class A                     | 27-48 kDa Cephalosporinases inhibited by active site inactivators. Example: <i>Yersinia enterocolitica</i> strain y56 chromosomal $\beta$ -lactamase.                                                                        |
| Group 2f                               | molecular Class A                     | 29-30 kDa Serine Carbapenemases. Example: <i>Serratia marcescens</i> strain S6 chromosomal $\beta$ -lactamase, Sme-1.                                                                                                        |
| Group 3                                | molecular Class B                     | 25-120 kDa, Metallo-carbapenemases) métrallo- $\beta$ -lactamases. Example: <i>Chryseobacterium (Flavobacterium) indologenes</i> chromosomal $\beta$ -lactamase found in Burkina Faso (Africa) named IND-B.                  |

<sup>a</sup>Bush et al., 1995.

<sup>b</sup>Joris et al., 1991.

the term "TEM" comes from the fact that the parental enzyme was found in the blood of a patient in Greece named Temoniera, hence this  $\beta$ -lactamase was designed TEM-1. The appellation SHV comes from Sulphydryl

Variable. Owing to the massive and intensive use of  $\beta$ -lactam antibiotics, the parental forms TEM-1 and SHV-1 underwent mutations yielding more than one hundred variants of TEM and more than twenty variants of SHV.



**Figure 2.** Main classes of  $\beta$ -lactamases. The representatives in each class are simply selected examples and do not correspond to all members of the class. For example class A and class B contain more than two types of enzymes. Only classes C and D comprise a single type, AmpC and OXA, respectively.

Most of these variants have high catalytic efficiencies and extended spectral activities. They are named ESBLs for Extended Spectral  $\beta$ -lactamases (Bradford, 2001). The TEM Variants are ubiquitous in *Escherichia coli* strains and SHV variants are predominant in *KLebsiella pneumoniae*.

2. The class C enzymes include non or few variants and the main representative remains AmpC which is chromosomally encoded in *Citrobacter freundii*, *Enterobacter cloacae* and *Morganella morganii* (Hanson et al., 1999). These  $\beta$ -lactamases are mainly inducible and highly active on first and second generation cephalosporins such as cefalotin, cephaloridin and cefalexin.

3. The class D enzymes belong as A and C to serine hydrolases and are essentially OXA (oxacillin-hydrolyzing) type enzymes which are frequently described in *Pseudomonas aeruginosa*. Like TEM and SHV, the OXA type includes at least thirty variants and some of them have an extended spectrum profile (Philipon et al., 1997; Poirel et al., 2001).

4. Class B enzymes differ from the 3 other classes in many respects. These enzymes are structurally and functionally distinct because of a vital need for one or two Zn(II) ion(s) in their active centre for activity. They are metallo- $\beta$ -lactamases and may confer multi-resistance to bacteria because of their ability to destroy most of the potent  $\beta$ -lactams including carbapenems. These enzymes are of the great clinical concern.

## WORLDWIDE DISTRIBUTION OF $\beta$ -LACTAMASES

During the last fifty years  $\beta$ -lactamases have received much attention because of their clinical relevance. Indeed, they have been responsible for a large number of therapeutic failures. In the early 1960s, the first plasmid-mediated  $\beta$ -lactamase in an *Escherichia coli* strain, TEM-1, was described in Europe (Datta et al., 1965). Thereafter the worldwide spread of the TEM-1 genetic element to other bacterial species (*P. aeruginosa*, *Haemophilus influenzae*, *Neisseria gonorrhoeae* etc) was observed (Bradford, 2001). At the same time another enzyme, SHV-1, appeared. This  $\beta$ -lactamase is chromosomally encoded in *K. pneumoniae* but plasmid encoded in *E. coli*. Owing to the introduction of the potent  $\beta$ -lactam antibiotics (oxymino-cephalosporins) into the market to suppress the resistance due to hydrolase activity, the wild types TEM-1 and SHV-1 spontaneously mutated, yielding extended spectrum enzymes. In this way, SHV-2 the pioneer of these enzymes appeared in *K. pneumoniae* in Germany (Kliebe et al., 1985). The number of these new types of enzymes able to destroy potent third generation  $\beta$ -lactams (designed ESBLs) is continuously increasing particularly in Europe and Asia.

## DRUG RESISTANCE INVOLVING $\beta$ -LACTAMASES IN AFRICA

The evolution of bacterial drug resistance in general and specifically via  $\beta$ -lactamases is not well investigated on the African continent. An important lack of scientific information about the subject is observed. The rare

reports about the role of  $\beta$ -lactamases often come from South Africa. Hence *Proteus mirabilis*, *E. coli* were found to produce TEM-26, SHV-2, and SHV-5 extended spectrum  $\beta$ -lactamases, while an AmpC related enzyme was found in *K. pneumoniae* (Pitout et al., 1998) in South Africa. Earlier, studies on bacterial strains isolated in Senegal/West African area have detected a high frequency of SHV-1  $\beta$ -lactamase in *E. coli* (Shaokat et al., 1987). These strains appeared to be particularly resistant to amoxicillin and ticarcillin. From 2000 to 2005 in a screening of *E. coli* and *K. pneumoniae* strains in Burkina Faso, we found a high frequency of SHV types  $\beta$ -lactamase in *K. pneumoniae* (Zeba et al., 2004) and TEM types  $\beta$ -lactamase in *E. coli* (results not published). Our results also indicate that the parental forms of these enzymes (SHV-1 and TEM-1) are most frequent. But two SHV-11 mutants were also identified in Burkina Faso in *K. pneumoniae* clinical strains. Our results are in agreement with previous studies (Shaokat et al., 1987). SHV-1 and TEM-1 are probably the most predominant  $\beta$ -lactam hydrolases in species such as *E. coli* and *K. pneumoniae*. But it is necessary to pay attention to the emergence of high efficiency variants of SHV-1 and TEM-1. The emergence of ESBLs such as SHV-5 in South Africa is a serious warning for the other countries. We also detected AmpC-type  $\beta$ -lactamases in strains of *Enterobacter cloacae* and *Citrobacter freundii*.

Another chapter to be opened for bacterial resistance mediated by  $\beta$ -lactamase in Africa is the emergence of enzymes conferring multidrug resistance to the producing bacteria. The identification of a metallo- $\beta$ -lactamase (MBL) in Burkina Faso during the last year (Zeba et al., 2005) is probably of grave concern. The gene encoding this Zn (II)  $\beta$ -lactamase was isolated from a clinical *Chryseobacterium (Flavobacterium) indologenes* strain and sequenced. It is a new variant of IND- $\beta$ -lactamases (results not yet reported). The particular characteristics and kinetic efficiencies of this enzyme underline the fact that, as in Japan particularly, Europe (Italy, France) and America (Canada, Bresil), the African continent must prepare for the challenge of metallo- $\beta$ -lactamases. A critical evolution of bacterial resistance mediated by  $\beta$ -lactamases is rapidly developing in many of the countries where resources are limited. The fragile situation of health will be dramatically complicated.

## ACKNOWLEDGMENT

I am very grateful to Professor Jean-Marie Frère of the Centre for Protein Engineering, University of Liège, Belgium, for his expert analysis of the manuscript and his useful critical contributions

## REFERENCES

- Ambler RP (1980). The structure of  $\beta$ -lactamases. Philos. Trans. R. Soc. London Ser. B 289 : 321-331
- Bush K, Jacoby GA, Medeiros AA (1995). A functional Classification Scheme for  $\beta$ -lactamase and Its correlation with Molecular structure 39(6): 1211-1233.
- Datta N, Kontomichalou P (1965). Penicillinase synthesis controlled by infectious R Factors in Enterobacteriaceae. Nature 208 : 239-244.
- Hanson N D, Sanders CC (1999). Regulation of inducible AmpC  $\beta$ -lactamase expression among Enterobacteriaceae. Curr. Pharm. Des. 5: 881-894.
- Joris B, Ledent P, Diderberg O, Fonzé E, Lamotte-Brasseur J, Kelly JA, Ghuysen J-M, Frère J-M (1991). Comparison of the sequences of classe A  $\beta$ -lactamases and the secondary structure elements of penicillin-recognizing proteins. Antimicrob. Agents Chemother. 35: 2294-2301.
- Kliebe C, Nies BA, Meyer JF, Tolxdorff-Neutzling RM, Wiedemann B (1985) Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob. Agents Chemother. 28: 302-307.
- Pitout DD, Thomson KS, Hanson ND, Ehrhardt AF, Moland ES, Sanders CC (1998).  $\beta$ -Lactamases Responsible for Resistance to Expanded-Spectrum Cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* Isolates Recovered in South Africa. Antimicrobial Agents and Chemotherapy. 42(6): 1350-1354.
- Poirel L, Girlich D, Naas T, Nordmann P (2001). OXA-28, An Extended -Spectrum Variant of OXA-10  $\beta$ -actamase from *Pseudomonas aeruginosa* and Its Plasmid- and Integron-located Gene. Antimicrob. Agents and Chemother. 45 (2): 447-453
- Shaokat S, Ouellette M, Sirot D, Joly B, Cluzel R (1987). Spread of SHV-1 $\beta$ -lactamase in *Escherichia coli* Isolated from fecal Samples in Africa. Antimicrob. Agents and Chemother. 31: 943-945.
- Webb EC (Ed.) (1984) Enzyme Nomenclature. pp. 366-374. Academic press Inc. (London Ltd London).
- Zeba B, Simporé J, Nacoulma OG, Frere JM (2004). Prevalence of *bla*<sub>SHV</sub> genes in clinical isolates of *Klebsiella pneumoniae* at Saint Camille Hospital Center in Ouagadougou. Isolation of *bla*<sub>SHV11</sub> like gene. African journal of Biotechnology. 3 (9): 477-480.
- Zeba B, Simporé J, Nacoulma OG, Frere JM (2005). Identification of metallo- $\beta$ -lactamase from a clinical isolate at Saint Camille Hospital Center of Ouagadougou/ Burkina Faso. Afr. J. Biotechnol. 4(3): 286-289.