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Short Communication

Identification of metallo-β-lactamase from a clinical isolate at Saint Camillle medical Center of Ouagadougou, Burkina Faso

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A metallo-β-lactamase was identified from a clinical sample. The host bacteria was identified to be *Chryseobacterium indologenes*. This preliminary characterization of the enzyme is reported here.

Key words: β-lactamase , metallo-β-lactamase,*Chyseobacterium indologenes*, bacterial resistance, antibiotics.

INTRODUCTION

Bacteria escape the action of β-lactam antibiotics by producing hydrolytic enzymes, β- lactamases (Abraham and Chain, 1940). These enzymes are able to hydrolyse the β-lactam ring and give products which are harmless to bacteria. There are four classes of β-lactamases on the basis of molecular structure (Ambler, 1975, 1980, Ambler and Scott,1978) or functional characteristics (Bush,1988, 1989 a, b). Among them, metallo-β-lactamases (MBL) which belong to the molecular class B constitute a distinct family of β-lactamases (MBL). Metallo-β-lactamases from most pathogenic bacteria are active with two Zn(II) ions bound to their active site (Ullah et al ., 1998;Wang et al, 1998). These enzymes are mainly reported in Asia and Europe. During our survey of β-lactamase incidence in bacterial resistance to β-lactam antibiotics we encountered a strain producing metallo-β-lactamase. To the best of our knowledge, this is the first time that the metallo-β-lactamase has been reported on the African continent. The aim of this simple communication is to inform the scientific community, before undertaking further studies.

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MATERIALS AND METHODS

Isolation and identification of the MBL producing strain

The strain which was isolated belongs to non enterobacteriaceae. It was collected from a patient's urine at Saint Camille Hospital of Ouagadougou and identified by the Api 20 E system (Bio Merieux France). A solid medium for obtention of pure colonies on plates is obtained from Tryptic Soy Agar.

An overnight preculture is generally performed by inoculating 5 ml Luria Bertani (LB) liquid medium with pure colonies of the interesting strain. 300 ml of the preculture was then used to inoculate 10 ml of liquid medium of LB, and allowed to grow on a shaker (250 rpm) at 37°C for 4 h.

After growing, the cells were harvested by centrifugation at 8246×g for 15 min. The pellet was redissolved in 500 µl of phosphate buffer 100 mM pH 7 and the periplasmic content was released by subjecting cells to five cycles of freeze-thaw (Simpson and James, 1982). Then the debris of cells are discarded by centrifugation at 14000×g for15 min. The supernatant contains crude β-lactamase activity which was stored for further use. The amount of protein of the extract was evaluated using bicinchoninic acid (BCA) protein assay kit (Pierce). The specific activity of the crude extract was also estimated (Woodford et al, 2000; Rice et al, 2000). The β-lactamins used as substrate in this study was obtained from Sigma.

Using specific inhibitor such as EDTA (Bush,1988) and substrate profiles allow for the identification of the enzyme molecular class.1 nM EDTA (the metal trapping agent) completely suppress the enzyme's activity. Moreover, the preparation is active on most β-lactams.

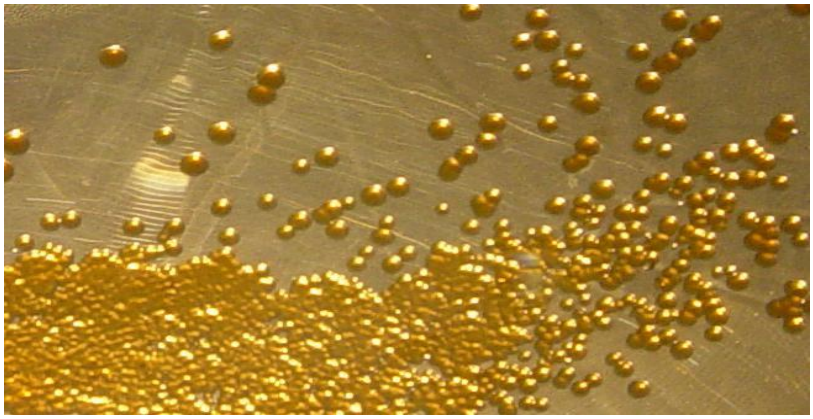

Figure 1. Colonies of clinical *Chryseobacterium indologenes* strain producing metallo-β-lactamase in Ouagadougou Saint Camille Hospital Center.

Table1. Substrate profiles of β-lactamines hydrolysed by the preparation.

β-lactams	Kinetic parameters			
	Km(µM)	Specific activity[1]	Relative activity[2]	Efficiency[3] of hydrolysis
benzylpenicillin	33,85 ±1,35	411,5 ±19,17	100	12,15
ampicillin	40,86 ± 4,10	421,9 ±21,36	103	10,32
cefalotin	39,47 ± 2,22	315,61 ±18,63	76,69	7,99
Cefuroxime	18,41 ± 0,23	70,68 ±2,19	17,17	3,83
cefotaxime	33,55 ± 1,08	66,30 ±6,57	16,11	1,98
imipenem	12,34 ± 0,96	92,05 ±1,09	22,37	7,46

[1] µM of substrate hydrolysed per mn and per mg of protein
[2] expressed as percentage of the value of benzylpenicillin

[3] $\frac{\mu M\, mn^{-1} mg^{-1} of\ protein}{Km}$

Table 2. Kinetic parameters of some β-lactams for the current preparation compared to those of IND-1 and IND-2 (Bellais et al., 1999 and 2000).

Substrate	Preparation		IND-1		IND-2	
	Km µM	Relative Vmax	Km µM	Relative Vmax	Km µM	Relative Vmax
Benzylpenicillin	33,85	100	26,4	100	70	100
Cefuroxime	18,41	17,17	∞	0	∞	0
cefotaxime	33,55	16,11	60	19	10	2,8
Ceftazidime	∞	0	765	15	440	0,7
Imipenem	12,34	22,37	198	90	170	30

Hydrolysis of β -lactams are monitored with the crude extracts in 50 nM phosphate buffer pH 7 at 25^{O}C and recorded on Uvikon 923 double beam spectrophotometer at fixed wavelength (235nm for benzylpenicillin and ampicillin, 273 nm for cefalotine, 260 nM for cefuroxime and cefotaxime, and 300 nM for iminipenem).

The initial maximal velocity (Vmax) used to determine specific

activity defined as µM of substrate hydrolysed per min and per mg of protein.

RESULTS AND DISCUSSION.

The API 20E system (bioMerieux France) successfully identified the bacteria as *Chyseobacterium* (previously *Flavobacterium*) *indologenes.* The major characteristic of this strain is the production of yellow colonies on plates (Figure 1) or in liquid culture. When the culture reaches the exponential phase, a strong fruity odour is emitted.

The preparation exhibited a broad substrate spectrum, hydrolysing successfully most of the β-lactam antibiotics (Table 1)

The inhibition by EDTA and the ability to hydrolyse carbapenem establishes the enzyme as a metallo -β-lactamase. This enzyme is able to hydrolyse common β-lactam antibiotics including benzylpenicillin, ampicillin, amoxicillin cefalotin and cephaloridin,as well as powerful antibiotics such as cefotaxime, cefuroxime and imipenem (Table 1). But it is unable to act on ceftazidime another third generation β-lactamin and cefalexine , a second generation cephalosporin. Its affinity for imipenem is higher than that of the other substrates studied. The comparison of kinetic parameters of this enzyme to those of two other metallo- -lactamase is shown in IND-1 and IND-2 (Table2).. It is interesting to note that IND-1 and IND-2 are active on ceftazidime,while the enzyme preparation does not hydrolyse this antibiotic.

The existence of metallo -β-lactamase from an extract of clinical isolate at the Saint Camille Hospital in Ouagadougou is a major revelation in epidemiology of bacterial resistance in our country. The background of local antibiotherapy can not explain the existence of metallo-β-lactamase, because carbapenem are rarely used.. Further investigations may be carried out for precise genetic identification of enzyme.

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