ANKEM Derg 11 (No.2):221-225(1997)

CARBAPENEMASES

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Introduction

Many consider the carbapenems to be the final defence against severe nosocomial infection. Although these antibiotics are extremely expensive, their use has been considered worthwhile because there was so little resistance to them. However, they are derived from natural products and thus microorganisms in the environment have been exposed to them for millions of years. Unfortunately, the procedures that we now employ in our intensive care units favours the emergence and spread of multi-antibiotic resistant bacteria; under the antibiotic blanket this permits the less virulent environmental bacteria to infect. These bacteria can often only be treated with the carbapenems but the presence of carbapenemases threatens this continued success.

Class B metallo-β-lactamases

β-lactamases can usually be divided up into four main structural class under the Ambler classification scheme based on structure alone(1). Class B enzymes have a metal ion, usually zinc, at the active site and are usually capable of hydrolysing imipenem. They are often naturally occuring β-lactamases that presumably have been selected by the natural presence of thienamycin-like compounds in the soil. They are usually not the only nor the major β-lactamase within the bacterial cell and may be accompanied by two other β-lactamases. Under the most recent Bush classification scheme(4), based on biochemical properties as well, these metallo-enzymes are listed as group 3. These enzymes are usually, easily identifiable by their inhibition with EDTA and their stability to clavulanic acid. The enzymes are sub-divided into three sub-groups, a - c. Sub-group 3a comprises the chromosomal β-lactamases of *Bacillus cereus* II, *Bacteroides fragilis* (CcrA) and *Stenotrophomonas maltophilia* (XM-A)(10,14,15). These enzymes have similar structures and there are some variations between similar enzymes isolated from strains isolated in different parts of the world. These enzymes are capable of hydrolysing penicillins more efficiently than carbapenems or cephalosporins. They appear to be penicillinases that have adapted to encompass carbapenems. It is often difficult to establish categorically that the presence of these enzymes accounts for carbapenem resistance. A sinister development within this class is the emergence of a plasmid-mediated β-lactamases in *Pseudomonas aeruginosa* strains isolated in Japan. In over 3000 clinical isolates, 123 were found to be resistant to imipenem. Fifteen of these possessed a new β-lactamase, IMP-1, which could be transferred from one *P.aeruginosa* strain to another. It has also been found in *Serratia* and *Klebsiella* isolates in Japan(18). It has been heralded as the looming threat to carbapenems. However, amongst the 15 original isolates, whereas some did posses an MIC of imipenem of around 128 mg/L, some were sensitive to as little as 8 mg/L suggesting that the presence of this β-lactamase was insufficient on its own to confer resistance and that the bacterium had to support this enzyme with a reduction in permeability or an alteration in the penicillin binding proteins. Examination of the

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IMP-1 β-lactamase itself does show that, for a group 3a, it is surprisingly more efficient against the carbapenems than the penicillins. If this enzyme is the great threat to the carbapenems, it does not yet seem to have disseminated and has only appeared in Japan despite the fact that higher incidences of imipenem resistance have been reported elsewhere.

The group 3b β-lactamases derive largely from the genus *Aeromonas* and are sometimes called the "true" carbapenemases because, unlike the group 3a enzymes, they have almost no hydrolytic effect on cephalosporins or penicillin. This makes them extremely difficult to detect as they do not appear with the application of nitrocephin. They are usually accompanied in *Aeromonas* spp. by two other β-lactamases and the host strains are usually sensitive to clinical levels of carbapenems. It is thus very difficult to demonstrate that these β-lactamase are capable of conferring resistance despite their presence(7,8).

There is only one member of group 3c, this is a β-lactamase in *Legionella gormannii.* This enzyme has a much broader spectrum of activity than the other two 3 sub-groups and is capable of hydrolysing most cephalosporins. It is a metallo-β-lactamase with an extended-spectrum(5).

Class A serine-β-lactamases

These are the most diverse and adaptable of all the β-lactamase classes. They appear to have derived originally from the chromosomal β-lactamases of Gram-positive bacteria. There is a sub-class of class A β-lactamases that has evolved to confer high-level-cefotaxime resistance. These enzymes epitomised by MEN-1 have demonstrated a flexibility in their capability to adapt to successive challenges by fast-penetrating cephalosporins(2). More recently a number of β-lactamases closely related in structure to the cefotaximases have been identified which are now capable of hydrolysing imipenem. The two prototype β-lactamases of this sub-class are Sme-1 identified in *Serratia marcescens*(20) and NMC-A in *Enterobacter cloacae*(12). The enzymes are related to each other, approximately 70% identity at the amino acids sequence. These enzymes have a preference for the hydrolysis of carbapenems though they retain a capability to hydrolyse penicillins and cephalosporins. They have particularly high velocity in the reaction against cephalosporins which suggests that they may have been derived from the MEN group of enzymes. More recently a third member, IMI-1, has been identified(16). This enzyme was identified in a clinical *E.cloacae* strain from the United States is closely related, but not identical to NMC-A. It is a better hydrolyser of benzyl-penicillin than NMC-A which may indicate the route of its evolutionary origin.

A strain of *Acinetobacter baumannii* was isolated from a blood culture in the Royal Infirmary Edinburgh in 1985. This strain unusually conferred resistance to imipenem, penicillin and cephaloridine but had intermediate sensitivity to the third generation cephalosporins, ceftazidime and cefotaxime(13). Its MIC of imipenem of 16 mg/L was particularly surprising because neither imipenem nor any other carbapenem had ever been used on this population and imipenem was not introduced until some years later. A cell-free extract was applied to a polyacrylamide IEF gel containing broad-range ampholines (pH 3.5 - 10.0) and the β-lactamases visualised with nitrocephin. Two β-lactamases were identified within the cell; one with considerable activity with a sharp focusing band at pI 6.65. The second,weaker β-lactamase focused poorly at a higher pI and was reminiscent of the cephalosporinases described above. The only other β-lactamase found in *Acinetobacter* with a similar but

not identical pI was CARB-5(9).

Both enzymes were partially purified and their sizes determined by gel filtration. The pI 6.65 enzyme had a molecular size of 23,000 daltons whereas the high pI enzyme was 58,000 daltons. Enzyme kinetic data showed that the pI 6.65 enzyme had a high affinity for penicillins and cephaloridine but that hydrolysis of these substrates was slow, particularly with cephaloridine which was only about a third of the rate of the penicillins. No hydrolysis could be demonstrated by the conventional spectrophotometric assays when imipenem, cefuroxime, cefotaxime and ceftazidime were used as substrates. Although spectrophotometric assays are convenient for β-lactamases, they may not always reveal hydrolysis. A modification of the microbiological assay of Masuda et al(11) was developed by Paton et al(13) and provides a more sensitive technique for determining hydrolytic activity. An Isosensitest agar plate is seeded with *Staphylococcus aureus*. Inhibitory zones, to the antibiotics to be tested, are determined and the radii measured. Filter disks containing 15 μL of the undiluted or diluted β-lactamase preparation are then applied to the seeded plates at the expected periphery of the zone of sensitivity. Inactivation of the antibiotic is shown by the growth of the indicator within the expected zone of inhibition. This technique clearly demonstrated the hydrolysis and destruction of both imipenem and azlocillin by the pI 6.65 β-lactamase. Cefuroxime, ceftazidime and cefotaxime were not inactivated and thus this enzyme was designated ARI-1 (*Acinetobacter* Resistant to Imipenem). When nitrocephin was used as the substrate, the ARI-1 β-lactamase was not readily inhibited by clavulanic acid, with and ID_{50} approximately 200-fold greater than TEM-1(13). ARI-1 was not inhibited by pCMB nor by high concentrations of EDTA, suggesting that metal ions play no role in the catalytic activity of this enzyme and thus it is *not a* metallo-β-lactamase. Furthermore when imipenem or meropenem were used as inhibitors, rather than substrates, both inhibited the action of ARI-1 but were without effect on the TEM-1 β-lactamase, demonstrating that the carbapenems were much more strongly bound by ARI-1. The inhibitor BRL42715 was a powerful inhibitor of ARI-1 with an ID_{50} of 1nM. This inhibitor binds to most serine-based active site β-lactamase, unlike clavulanic acid that binds preferentially to the class A enzymes. The results, coupled with the resistance to EDTA, suggest that ARI-1 is a class A serine-based β-lactamase.

Conjugation studies in *Acinetobacter* species are not easy(6); however the ARI-1 β-lactamase genes has found to be located on a 40 kb plasmid which can transfer to *Acinetobacter junii* from *A.baumannii*(17). This demonstrates that this class A β-lactamase has the capability to spread through the nosocomial population but they uniformly failed to demonstrate any transfer or mobilization of the ARI-1 β-lactamase gene.

There are now reports from all over the world about increasing resistance to imipenem in *Acinetobacter* spp. In Latin America, there have been reports of up to 35% resistance. In a collaborative project in Buenos Aires, we have shown that imipenem-resistant *A.baumannii* isolated from nosocomial isolates contain a new class A β-lactamase, which we call ARI-2. This enzyme has very similar properties to ARI-1 but must have a different structure as it has a different pI(3).

Class C serine-β-lactamases

Enterobacter aerogenes mutants with high-level resistance to imipenem have been found to be resistant because of stable hyper-production of a class-C β-lactamase. This enzyme, with a high pI of 8.2, exhibited high affinity toward imipenem and

hydrolysed the drug slowly. This was not the only mechanism of resistance as the carbapenem-resistant strains also lacked a major 43-kDa outer membrane protein and displayed decreased permeability to cephaloridine. However, the importance of the class C enzyme was proved by the introduction of a plasmid coding for the regulatory ampD gene which abolished β-lactamase hyper-production and rendered the mutants susceptible to imipenem(19). The large number of *Pseudomonas aeruginosa* strains from the Japanese study which did not possess IMP-1, were probably resistant because of a class C β-lactamase(18).

The diversity of β-lactamases capable of hydrolysing carbapenems augurs badly for the future of the carbapenems. We must wait and see which become the most predominant.

REFERENCES

1- Ambler RP: The structure of β-lactamases, *Philosophical Transactions of the Royal Society London, Series B289*:321 (1980).

2- Bauernfeind A, Stemplinger I, Jungwirth R, Ernst S, Casellas JM: Sequences of β-lactamases genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other β-lactamase, *Antimicrob Agents Chemother 40*:509 (1996).

3- Brown S, Bantar C, Young HK, Amyes SGB: An outbreak of imipenem resistance in *Acinetobacter* strains from Buenos Aires, Argentina, *Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy* C122 (1996).

4- Bush K, Jacoby GA, Medeiros AA: A functional classification scheme for β-lactamases and its correlation with molecular structure, *Antimicrob Agents Chemother 39*:1211 (1995).

5- Fuji T, Sato K, Miyata K, Inoue M, Mitsuhashi S: Biochemical properties of β-lactamase produced by Legionella gormannii, *Antimicrob Agents Chemother 29*: 925(1986).

6- Goldstein FW, Labigne-Roussel A, Gerbaud G, Carlier C, Collatz E, Courvalin P: Transferable plasmid mediated antibiotic resistance in Acinetobacter, *Plasmid 10*:138 (1983).

7- Hayes MV, Thomson CJ, Amyes SGB: Three β-lactamases isolated from Aeromonas salmonicida, including a carbapenemase, not detectable by conventional methods, *Eur J Clin Microbiol Infect Dis 13*:805 (1994).

8- Hayes MV, Thomson CJ, Amyes SGB: The "hidden" carbapenemase in Aeromonas hydrophila, *J Antimicrob Chemother 37*:33 (1996).

9- Joly-Guillou MJ, Vallee E, Bergogne-Berezin E, Philippon A: Distribution of β-lactamases and phenotype analysis in clinical strains of Acinetobacter calcoaceticus, *J Antimicrob Chemother 22*:597 (1988).

10- Kuwabara S, Abraham EP: Some properties of two extracellular β-lactamases from Bacillus cereus 569 H, *Biochem J 103*:27c (1967).

11- Masuda G, Tomioka S, Hasegawa M: Detection of β-lactamase production in gram-negative bacteria, *J Antibiotics 29*:662 (1976).

12- Nordmann P, Mariotte S, Naas T, Labia R, Nicolas MH: Biochemical properties of a carbapenem-hydrolyzing β-lactamase from Enterobacter cloacae and cloning of the gene into Escherichia coli, *Antimicrob Agents Chemother 37*:939 (1983).

13- Paton RH, Miles RS, Hood J, Amyes SGB: ARI-1: β-lactamase-mediated imipenem resistance in Acinetobacter baumannii, *Internal J Antimicrob Agents 2*:81 (1993).

14- Paton RH, Miles RS, Amyes SGB: Biochemical properties of inducible β-lactamases produced from Xanthomonas maltophilia, *Antimicrob Agents Chemother 38*:2143 (1994).

15- Rasmussen BA, Yang YJ, Jacobus N, Bush K: Contribution of enzymatic properties, cell permeability, and enzyme expression to microbiological activities of β-lactams in three Bacteroides fragilis isolates that harbor a metallo-β-lactamase gene, *Antimicrob Agents Chemother 38*:2116 (1994).

16- Rasmussen BA, Bush K, Keeney Y, Yang Y, Hare R, O'Gara C, Medeiros AA: Characterization of IMI-1 β-lactamase, a novel class A carbapenem-hydrolysing enzyme from Enterobacter cloacae, *Antimicrob Agents Chemother 40*:2080 (1996).

17- Scaife W, Young HK, Paton RH, Amyes SGB: Transferable imipenem-resistance in Acinetobacter species from a clinical source, *J Antimicrob Chemother 36*:585 (1995).
18- Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K, Kato N, Ohta M: Multifocal outbreaks of metallo-β-lactamase-producing Pseudomonas aeruginosa resistant to broad-spectrum β–lactams, including carbapenems, *Antimicrob Agents Chemother 40*:349 (1996).
19- Tzouvelekis LS, Tzelepi E, Mentis AF, Vatopoulos AC, Tsakris A: Imipenem resistance in Enterobacter aerogenes is associated with derepression of chromosomal cephalosporinases and impaired permeability, *FEMS Microbiol Lett 95*:195 (1992).
20- Yang YJ, Wu PJ, Livermore DM: Biochemical characterization of a beta-lactamase that hydrolyzes penems and carbapenems from two Serratia marcescens isolates, *Antimicrob Agents Chemother 34*:755 (1990).