

Plasmid Determined Beta-Lactamases in Carbenicillin-Resistant *Pseudomonas aeruginosa* and Cross-Resistance to Other Beta-Lactam Antibiotics

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Abstract

Beta-lactamases from 209 carbenicillin-resistant clinical isolates of *Pseudomonas aeruginosa* were identified. Each strain was isolated within a one-year period at different times and from different wards to exclude using the same strains being tested. Crude beta-lactamase preparation was obtained by sonication and centrifugation. These enzymes were identified by isoelectric focusing, using known enzymes as standard markers. Sensitivity to ticarcillin, piperacillin, cefotaxime, cefoperazone, ceftazidime and imipenem was performed by the disc method. Amongst the plasmid-mediated enzymes found in these strains were PSE 1 (65.4%), PSE 2 (3.9%), PSE 3 (1.5%) and TEM 1 (0.5%). 48.5% of the strains contained detectable chromosomal enzyme, either alone or in combination with the plasmid enzymes. 28.7% of strains produced chromosomal enzyme only. Imipenem was very active against these strains although 3.6% were resistant. 82.5% of strains were susceptible to ceftazidime, 56.1% to cefotaxime, 31.8% to piperacillin, 25.6% to cefoperazone and 10.9% to ticarcillin. Strains which were resistant to these antibiotics also showed varying degrees of cross resistance to beta-lactam antibiotics tested. (*J Infect Dis Antimicrob Agents* 1994;11:137-40.)

Key words : |Beta-lactamases, *P. aeruginosa*, antimicrobial resistance

เรื่องย่อ

เบต้าแลคตามเอสที่ผลิตจากเชื้อ *Pseudomonas aeruginosa* ที่ดื้อยา carbenicillin ซึ่งกำกับโดยพลาสมิด และการดื้อยาข้ามพวกในกลุ่มยาเบต้าแลคแทม

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ได้ศึกษาเบต้าแลคตามเอสจากเชื้อ *P. aeruginosa* จำนวน 209 สายพันธุ์ที่ดื้อยา carbenicillin แต่ละสายพันธุ์แยกมาจากผู้ป่วยในเวลาและหอผู้ป่วยที่แตกต่างกัน เพื่อหลีกเลี่ยงการใช้สายพันธุ์เดียวกันมาทดสอบ ได้ศึกษาความไวของเชื้อตื้อยา ticarcillin, piperacillin, cefotaxime, cefoperazone, ceftazidime และ imipenem. พบว่าเบต้าแลคตามเอสที่แยกได้ร้อยละ 65.4 เป็น PSE 1, ร้อยละ 3.9 เป็น PSE 2, ร้อยละ 1.5 เป็น PSE 3 และร้อยละ 0.5 เป็น TEM 1. ร้อยละ 48.5 ของสายพันธุ์ที่ทดสอบนี้ตรวจพบเบต้าแลคตามเอสที่กำกับการสร้างโดยโครโมโซม ซึ่งบางสายพันธุ์มีการสร้างเบต้าแลคตามเอสซึ่งกำกับโดยพลาสมิดร่วมด้วย มีเพียงร้อยละ 28.7 ที่สร้างเบต้าแลคตามเอสซึ่งกำกับโดยโครโมโซมเท่านั้น การทดสอบความไวพบว่าร้อยละ 82.5, 56.1, 31.8,

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25.6 และ 10.9 ของสายพันธุ์กลุ่มนี้ไวต่อยา imipenem, ceftazidime, cefotaxime, piperacillin, cefoperazone และ ticarcillin ตามลำดับ นอกจากนี้ยังมีการดื้อยาข้ามพวกกันในระหว่างยาที่ทดสอบด้วย (วารสารโรคติดเชื้อ และยาต้านจุลชีพ 2537:11:137-40.)

INTRODUCTION

Pseudomonas aeruginosa strains are normally susceptible to the carboxypenicillins and the ureidopenicillins amongst the penicillins. As such, these antibiotics are often used in the therapy of infections caused by these organisms. Resistance, however, may occur and is mediated by either enzymes or by non-enzymatic mechanisms (1) or by a combination of these mechanisms (2).

Carbenicillin is stable to the chromosomal enzyme of *Pseudomonas aeruginosa*. Decreased permeability as a cause of resistance to carbenicillin has been found in about 8% of clinical isolates and that caused by plasmid-mediated enzymes in 2% of isolates (3).

Strains of *Pseudomonas aeruginosa* resistant to carbenicillin were selected to assess the types of plasmid mediated enzymes present. The knowledge of the prevalence of these enzymes in these strains may enable the clinician to make a good choice of an alternative antibiotic in the management of infections caused by such organisms.

MATERIALS AND METHODS

Organisms : 209 strains of *Pseudomonas aeruginosa* resistant to carbenicillin (tested by standard disc susceptibility method) were selected. These strains were isolated from clinical specimens in the General Hospital, Kuala Lumpur over a one year period and only one isolate from a patient was included.

Identification of beta-lactamases : The organisms were grown in overnight cultures and the cells collected by centrifugation. Crude extracts of enzymes were obtained through sonication of these cells and a final centrifugation in an Eppendorf centrifuge. The supernatant was used in the identification of enzymes. Identification of the beta-lactamases was performed by isoelectric focusing (4) using nitrocefin as the indicator and known beta-lactamases used as standards and for comparison.

Susceptibility to beta-lactam antibiotics : The method used was the disc diffusion method, with Mueller-Hinton agar and using standardised inoculum (5). Ticarcillin (75 µg), piperacillin (100 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoperazone (75 µg) and imipenem (10 µg) were tested against the organisms. *Pseudomonas aeruginosa* NCTC 10662 was used as control.

RESULTS

The susceptibility of the strains tested against various beta-lactam compounds is shown in Figure 1. The strains were most susceptible to imipenem (96.4%). Ceftazidime managed to kill 82.5% of strains, cefotaxime - 56.1% and piperacillin - 31.8%. Cefoperazone and ticarcillin (another carboxy-penicillin) were less active; resistance to these antibiotics was 74.4% and 89.1% respectively.

The types of enzymes produced by the various strains is shown in Figure 2. Detectable chromosomal enzyme were found in 47.8% of strains. The chromosomal enzymes were produced alone (28.7%) or together with plasmid-mediated enzymes of types PSE 1, PSE 2 and PSE 3. Plasmid-mediated enzymes were present in 71.3% of strains. They were of types PSE 1 to 3 and TEM enzymes. PSE 1 enzyme was produced by 65.4%, PSE 2 by 3.9% and PSE 3 by 1.5% of strains. PSE 1 alone was produced by 47.3% of strains. TEM 1 was produced alone in 1 (0.5%) of strains.

Cross resistance of these carbenicillin-resistant *Pseudomonas aeruginosa* to other beta-lactams is shown in Table 1. There was great variation of cross resistance shown. Strains resistant to cefoperazone were also most resistant to piperacillin (88.5%). In these strains (cefoperazone/piperacillin-resistant) cefotaxime was not active against 40%-42% of the strains. Cefotaxime resistant strains (n = 98) were also resistant to ceftazidime in 36.7% of strains, although ceftazidime-resistant strains (n = 39) showed 92.3% cross resistance to cefotaxime. There were eight strains resistant to imipenem with six

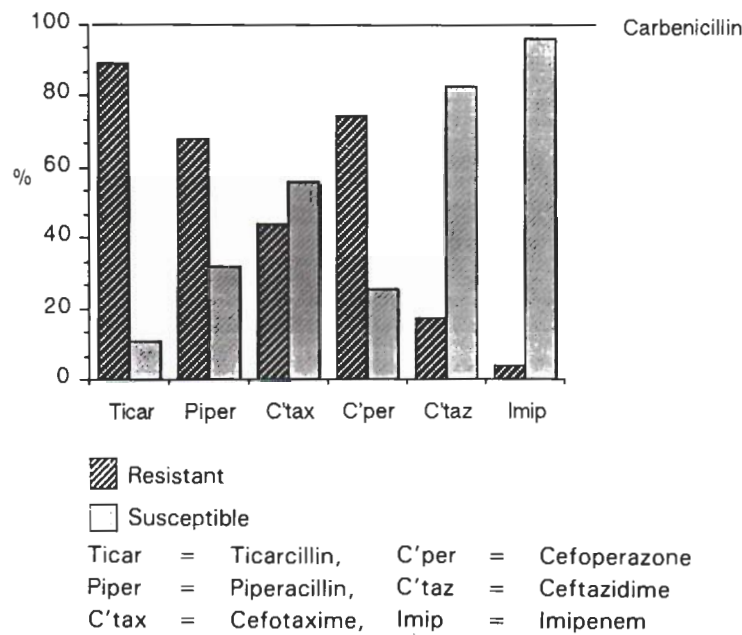


Figure 1. Susceptibility of carbenicillin-resistant strains of *Pseudomonas aeruginosa* against beta-lactams.

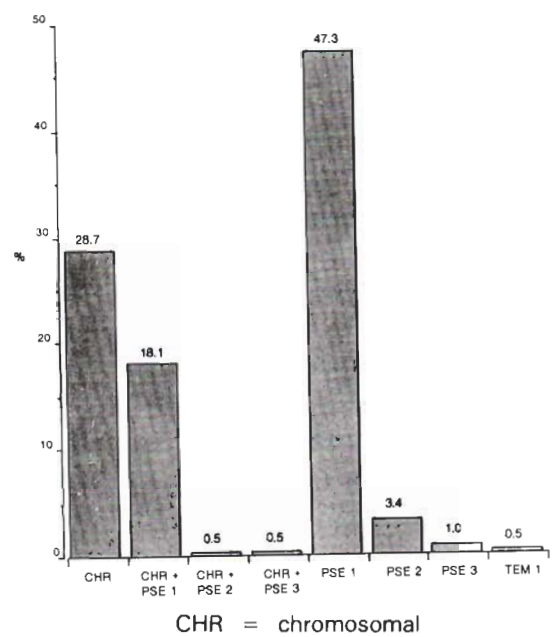


Figure 2. Types and frequency of beta-lactamases found in carbenicillin-resistant *Pseudomonas aeruginosa*.

Table 1. Antibiotic cross resistance shown by carbenicillin-resistant *Pseudomonas aeruginosa*

Main resistance (number of strains)	Cefoperazone	Piperacillin	Cefotaxime	Ceftazidime	Imipenem
Cefoperazone (166)	—	88.5%	42.8%	19.9%	3.0%
Piperacillin (152)	96.7%	—	40.7%	18.4%	2.0%
Cefotaxime (98)	72.4%	63.2%	—	36.7%	6.1%
Ceftazidime (39)	84.6%	71.8%	92.3%	—	2.6%
Imipenem (8)	62.5%	37.5%	75.0%	12.5%	—

strains also being resistant to cefotaxime, five strains resistant to cefoperazone, three strains resistant to piperacillin and one strain resistant to ceftazidime.

DISCUSSION

Resistance to the carboxy group of penicillins by *Pseudomonas aeruginosa* is usually caused by plasmid-mediated beta-lactamases (6). Carbenicillin is stable to chromosomal enzymes. In this study however 28.7% of strains producing these chromosomal enzymes were resistant to carbenicillin. The mechanism for this resistance may probably be due to impermeation and this form of resistance had been reported by Rodriguez-Tebar et al. (7). It was the main mechanism of resistance of isolates to carbenicillin in England in a study conducted in 1984 (3). An altered target mechanism may also be possible. Further studies on these strains should be

conducted to obtain exact mechanisms.

Amongst the plasmid-mediated enzymes found in these strains are TEM 1, PSE 1, PSE 2, and PSE 3. Other enzymes which may also be produced by *Pseudomonas aeruginosa* such as OXA and other TEM enzymes were not present. PSE 4 was also not present. In the United Kingdom, PSE 4 was the most common enzyme present (51.4%) (3). In this study however PSE 1 was the most common (65.4%), followed by PSE 2 (3.9%), PSE 3 (1.5%) and TEM 1 (0.5%). PSE 1 enzyme was also the most common present in strains found in Spain (49.3%) and France (73%) (8,9).

The prevalence of resistance of *Pseudomonas aeruginosa* to beta-lactams varies from place to place and from country to country. Data obtained from this small study cannot be compared directly with published data as the strains in this study comprised of carbenicillin-resistant strains only. Neu (10) reported that 30% of

carbenicillin-resistant strains were also resistant to cefoperazone. This study found that 74.7% of strains were resistant to cefoperazone. Neu however, did not mention the types of enzymes found.

The presence of plasmid-mediated enzymes will affect the susceptibility of organisms to piperacillin and cefsulodin, but not against cefotaxime or ceftazidime. The derepressed state of the organism with the production of high levels of chromosomal enzyme can however inactivate cefotaxime and ceftazidime (11). This study showed that 74.4% and 68.2% of strains were resistant to cefoperazone and piperacillin respectively, which may be due to plasmid-mediated enzymes present in 71.3% of strains; compared to 43.9% of strains being resistant to cefotaxime or 17.5% to ceftazidime due to the presence of chromosomal enzymes in 47.8% strains.

3.6% of strains were resistant to imipenem. Impermeation caused by deficiency in D2 porin (12) or enzymes which hydrolyse imipenem slowly, found also in *Pseudomonas aeruginosa* (13) may be the possible mechanisms of resistance. Such enzymes, the carbapenemases, have also been found in *Serratia marcescens*, *Xantomonas maltophilia*, *Aeromonas* sp., *Legionella* and *Flavobacterium* sp. (14,15,16) and the anaerobic *Bacteroides* (17,18).

Most of the strains tested were resistant to cefoperazone and piperacillin (74.4% and 68.2% respectively). Of these strains about 40% were also resistant to cefotaxime; and about 37% of cefotaxime strains were resistant to ceftazidime. This implies that neither of these antibiotics (cefoperazone, piperacillin and cefotaxime) should be alternates against carbenicillin-resistant *Pseudomonas aeruginosa* strains. When strains are resistant to ceftazidime, only imipenem seemed to be the alternate beta-lactam, although about 2.6% will still be resistant. On the other hand, of the imipenem resistant strains, some (87.5%) will still be susceptible to ceftazidime. It will therefore be crucial to test *Pseudomonas aeruginosa* isolates against a battery of beta-lactam compounds : piperacillin, cefoperazone, ceftazidime and imipenem, as dictated by antibiotic usage within the hospital setup to ascertain choice for treatment and also to monitor resistance.

It will also be of interest if a beta-lactamase-inhibitor combination, such as clavulanate-ticarcillin, sulbactam-cefoperazone or tazobactam-piperacillin should be used against these strains. In this way perhaps, the prevalence of such plasmid-mediated enzymes shall be made to decrease.

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