# Broad-Spectrum β-Lactam Antibiotics for Treating Experimental Peritonitis in Mice Due to *Klebsiella pneumoniae* Producing the Carbapenemase OXA-48

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A lethal peritonitis model was induced in mice with a *Klebsiella pneumoniae* isolate producing the carbapenemase OXA-48. Administration of a single dose (up to 100 mg/kg) of the antibiotic piperacillin-tazobactam, imipenem-cilastatin, ertapenem, or cefotaxime had little or no impact on lethality. Ceftazidime had the highest efficacy *in vivo*, which mirrored its *in vitro* activity; this was not the case for carbapenems. Therefore, ceftazidime may be recommended for the treatment of infections due to OXA-48 producers if they do not coproduce an extended-spectrum β-lactamase or a plasmid-mediated AmpC cephalosporinase.

Among the β-lactam antibiotics, carbapenems possess the broadest spectrum of activity against Gram-negative rods (1). However, resistance to carbapenems, while still rare, is emerging and represents a significant threat to the management of multidrug-resistant isolates in many areas of the world (9). In *Enterobacteriaceae*, this resistance trait is mediated mostly by metallo-β-lactamases (IMP, VIM, and NDM), by plasmid-mediated β-lactamases (KPC), and by the Ambler class D β-lactamase OXA-48 (10). Single isolates and outbreaks of OXA-48-producing enterobacterial isolates were described initially in Turkey (2, 10) and then in North African countries (Morocco, Algeria, Tunisia, Libya, and Egypt), Europe (France, Spain, Belgium, The Netherlands, Italy, the United Kingdom, Denmark, Sweden, Norway, and Germany), and Senegal (2, 4, 10). OXA-48 producers are multidrug-resistant isolates (10). They either produce OXA-48 alone or coproduce a clavulanic-acid-inhibited, extended-spectrum β-lactamase (ESBL; mostly of the CTX-M type) and very rarely a plasmid-mediated cephalosporinase (2, 10). Biochemical analyses showed that OXA-48 hydrolyzes penicillins (including amoxicillin, ticarcillin, and piperacillin), is resistant to inhibition by β-lactamase inhibitors (clavulanate, tazobactam), and hydrolyzes carbapenems at a moderate level (12). Weak (cefotaxime) or no (ceftazidime) hydrolysis activity of broad-spectrum cephalosporins by OXA-48 has been reported (12). Consequently, many OXA-48 producers that do not coproduce any ESBL may be categorized as susceptible *in vitro* to broad-spectrum cephalosporins according to the CLSI guidelines (3, 10). However, it is unknown whether the results obtained *in vitro* can be translated to the *in vivo* setting. Clinical reports of the treatment of infections due to OXA-48 producers indeed remain scarce (2, 4, 10).

Taking into account the paucity of therapeutic options for the treatment of infections due to OXA-48 producers, we have tested the efficacy of five broad-spectrum β-lactam antibiotics against an OXA-48 producer (that did not coproduce an ESBL) using an experimental model of peritonitis in mice (15). The OXA-48 producer was a clinically significant *Klebsiella pneumoniae* isolate that was resistant to penicillins, including amoxicillin, ticarcillin, amoxicillin-clavulanate, piperacillin, and piperacillin-tazobactam, as described elsewhere (8). The MICs of imipenem and ertapenem were 0.5 and 1 µg/ml, respectively, still in the susceptible

range for imipenem (MIC susceptibility breakpoint, ≤1 µg/ml) but resistant to ertapenem (MIC susceptibility breakpoint, ≤0.25 µg/ml) according to the updated CLSI guidelines (3). The strain producing OXA-48 was susceptible to ceftazidime and cefotaxime, with MICs of 0.25 and 1 µg/ml, respectively (MIC susceptibility breakpoint, ≤4 µg/ml). Peritonitis was induced in animals by the intraperitoneal injection of 0.5 ml of a sterile talcum-saline solution (125 mg/ml) containing the bacterial inocula via a 26-gauge syringe. Talcum was added to the bacterial inoculum to enhance infection. The lack of a lethal effect of talcum alone has been shown previously (15). All experiments were done in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85-23, revised 1985).

Initially, the inoculum that was able to kill 100% of the animals infected over 72 h (100% lethal dose [$LD_{100}$]) was determined by the Probit method (5). Groups of six mice were injected intraperitoneally with 0.5-ml volumes of a sterile talcum-saline solution containing inocula of $5 \times 10^2$, $1 \times 10^4$, $1 \times 10^6$, $1 \times 10^7$, $5 \times 10^7$, $1 \times 10^8$, and $5 \times 10^8$ CFU, respectively. Mouse deaths were recorded at regular intervals of time for 72 h. The $LD_{100}$ was established at $1.6 \times 10^8$ CFU.

The effects of piperacillin-tazobactam, cefotaxime, ceftazidime, ertapenem, and imipenem-cilastatin were then studied in the mouse model of peritonitis with a bacterial suspension of OXA-48-producing *K. pneumoniae* corresponding to the $LD_{100}$. Antibiotic treatment was given as a single dose of 0.2 ml of each β-lactam by subcutaneous injection in the neck 2 h after infection. The 50% effective dose ($ED_{50}$; the single dose giving protection to 50% of the mice) was determined from two trials by the Probit method (5). In the first trial, drugs were given in 10-fold dilutions of 0.1 to 100 mg/kg to six mice in each treatment group (7, 14, 15).

**TABLE 1** Number of dead mice 72 h after antibiotic treatment and derived $ED_{50}$ estimates

| Antibiotic | No. of mice dead after receiving a dose (mg/kg)[a] of: | | | | | | | | $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|
| | 0.1 | 1 | 10 | 20 | 40 | 60 | 80 | 100 | |
| Ertapenem | 6 | 6 | 6 | 6 | 5 | 6 | 4 | 3 | 100 |
| Imipenem-cilastatin | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 4 | >100 |
| Ceftazidime | 6 | 5 | 5 | 1 | 1 | 0 | 1 | 0 | 24 |
| Cefotaxime | 6 | 6 | 6 | 6 | 5 | 5 | 6 | 5 | >100 |
| Piperacillin-tazobactam | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | >100 |

[a] There were six mice per dose group.

The observation period was 72 h, a time after which no further deaths occurred. In a second trial, intermediate doses (20, 40, 60, and 80 mg/kg) were tested. Mortality in nontreated animals was 100%. As expected from results obtained *in vitro*, piperacillin-tazobactam did not modify animal mortality (Table 1). The efficacy of ertapenem and imipenem-cilastatin was low, and the $ED_{50}$ of ertapenem could only be estimated. The efficacy of cefotaxime was also limited and much lower than that of ceftazidime, which exhibited the highest efficacy *in vivo*, with an estimated $ED_{50}$ of 24 mg/kg. Differences in therapeutic efficacy between the two expanded-spectrum cephalosporins cannot be explained by differences in their pharmacokinetic behavior in mice, since their volumes of distribution, elimination half-lives, and binding to proteins are similar (6, 7, 14). However, the MIC of cefotaxime was 4-fold higher than that of ceftazidime, which may explain these findings, at least in part. The most interesting finding of this study is that ceftazidime appears to be much more active than any of the other molecules tested, including the carbapenems, for the treatment of an infection due to an OXA-48 producer. The lack of efficacy of carbapenems here, despite their low MICs, is a cause for concern. If these results were translated to the treatment of human infections, the MICs of carbapenems should not be reported just as found. Testing for carbapenemase activity may become mandatory for the safe treatment of patients when an enterobacterial isolate shows less susceptibility to any carbapenem than the wild type.

This is the first study reporting an analysis in an animal model of the efficacy of antibiotics for the treatment of an infection caused by an OXA-48 producer. These results could possibly be extended to many other OXA-48 producers (mostly *Escherichia coli* and *K. pneumoniae*) and producers of OXA-181, which is a point mutant analogue of OXA-48 that has been reported mostly in India and shows the same spectrum of activity as OXA-48 (13). The level of biosynthesis of OXA-48 may likely be the same in other OXA-48 producers, since the $bla_{OXA-48}$ gene is always located on the same ca. 62-kb (previously identified as a ca. 70-kb) plasmid and is bracketed by the same insertion sequences as a source of promoter sequences (11). Therefore, ceftazidime could be a reliable molecule for the treatment of infections caused by OXA-48 producers if they do not produce an ESBL or a plasmid-mediated AmpC cephalosporinase (ca. 25% of the cases [P. Nordmann, unpublished data]). That finding may be of particular interest in countries that are facing a rapid spread of OXA-48 producers, such as, at least, the strains found in Western Europe, North Africa, Egypt, and Turkey (10).

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