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Cefepime, Piperacillin-Tazobactam, and the Inoculum Effect in Tests with Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae*

KENNETH S. THOMSON* AND ELLEN SMITH MOLAND

Center for Research in Antiinfectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska 68178

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There is little information about the clinical effectiveness of cefepime and piperacillin-tazobactam in the treatment of infections caused by extended-spectrum β-lactamase (ESBL)-producing pathogens. Some inferences have been drawn from laboratory studies, which have usually involved only one or a few strains of ESBL-producing *Klebsiella pneumoniae* or *Escherichia coli* that produced only a limited range of ESBLs. Such studies are indirect, sometimes conflicting, indicators of efficacy. To extend previous laboratory findings, a study was designed to investigate organism-drug interactions by determining the in vitro activities of eight parenteral β-lactam agents against 82 clinical and laboratory strains of *Klebsiella*, *Escherichia*, *Enterobacter*, *Citrobacter*, *Serratia*, *Morganella*, and *Proteus* species that produced 22 different ESBLs, alone or in combination with other β-lactamases. Activities were determined in broth microdilution MIC tests using standard and 100-fold-higher inocula. An inoculum effect, defined as an eightfold or greater MIC increase on testing with the higher inoculum, was most consistently detected with cefepime, cefotaxime, and ceftriaxone and least frequently detected with meropenem and cefoteten. Piperacillin-tazobactam was intermediate between these two groups of agents. Although the inoculum effect is an in vitro laboratory phenomenon, if it has any predictive value in identifying increased risk of therapeutic failure in serious infections, these results support suggestions that cefepime may be a less-than-reliable agent for therapy of infections caused by ESBL-producing strains.

Extended-spectrum β-lactamases (ESBLs) are novel β-lactamases produced by a variety of gram-negative bacilli. The distinguishing feature of these enzymes is that compared to the broad-spectrum β-lactamases, such as TEM-1, TEM-2, SHV-1, and others, ESBLs have extended substrate profiles which permit hydrolysis of aztreonam and expanded-spectrum cephalosporins such as cefotaxime, ceftriaxone, ceftazidime, cefepime, and others. To date, ESBLs are most commonly produced by isolates of *Klebsiella pneumoniae* and, to a lesser extent, *Escherichia coli* (8, 9, 12, 27, 30, 40, 44), but infections, colonization, and nosocomial spread involving other ESBL-producing organisms (such as *Morganella morganii*; *Serratia marcescens*; *Shigella dysenteriae*; several species of *Enterobacter*, *Salmonella*, *Proteus*, and *Citrobacter*; *Pseudomonas aeruginosa*; *Burkholderia cepacia*; and *Capnocytophaga ochracea*) have been reported (45). A major problem with ESBLs is their capacity to cause therapeutic failures with cephalosporins and aztreonam when the host organism appears to be susceptible to these agents in laboratory tests (5, 13, 15, 27, 30, 44, 47). In response to this problem, the National Committee for Clinical Laboratory Standards (NCCLS) recommends that laboratories should report ESBL-producing isolates of *E. coli* or *Klebsiella* spp. as resistant to all penicillins, cephalosporins (including cefepime and cefpirome), and aztreonam irrespective of in the vitro test results (23). This recommendation does not extend to ESBL-producing organisms of other genera.

Cefepime and cefpirome are often substantially more active in vitro than earlier cephalosporins against ESBL-producing pathogens (38, 46). This probably stems from their greater intrinsic potency due to more rapid permeation through the outer membrane (37). Clinical data determining the efficacy of these agents in ESBL-associated infections are lacking. In vitro studies with high inocula show that the MICs of cefepime (14, 19, 43) and cefpirome (19) for ESBL-producing isolates of *K. pneumoniae* and *E. coli* are often greatly elevated, suggesting that these agents are inactivated by ESBLs. Animal models of infection with these organisms have produced both successful and unsuccessful therapeutic outcomes with cefepime (34, 43). The in vitro and animal studies have been somewhat limited in scope, comprising investigations with only two bacterial species and with only a few types of ESBLs. It is unknown if the findings of these studies can be extended to other types of ESBLs and to other ESBL-producing pathogens. Indeed, it has been suggested that there is great variability among ESBLs in their interactions with cefepime, with SHV-derived ESBLs exhibiting more of a tendency to decrease cefepime activity than TEM-derived ESBLs (38).

There are also questions about whether β-lactamase inhibitor combinations should be used for therapy of infections caused by ESBL-producing pathogens. Current data are incomplete and sometimes conflicting. Many ESBL-producing isolates of *K. pneumoniae* or *E. coli* are susceptible in vitro to piperacillin-tazobactam (3, 7, 11, 24), but MICs may increase substantially in tests with a higher-than-standard inoculum (10, 14). Efficacy has been reported in animal models of infection

* Corresponding author. Mailing address: Center for Research in Antiinfectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, NE 68178. Phone: (402) 280-2921. Fax: (402) 280-1225. E-mail: kstaac@creighton.edu.

TABLE 1. Representative isogenic *E. coli* strains: standard- and high-inoculum MICs

Strain	Enzyme	MIC (μg/ml) of drug at indicated inoculum (CFU/ml)[a]															
		MEM		CTT		CTX		CAZ		CRO		FEP		ATM		TZP	
		10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7
PAB-C14	SHV-2	0.03	0.03	0.25	0.5	16	512	16	32	32	512	8	>128	8	64	4	256
PAB-CS7	SHV-7	0.03	0.03	0.25	1	32	>1,024	256	1,024	64	>1,024	8	>128	1,024	1,024	2	64
PAB-C43	TEM-43	0.03	0.06	0.25	0.25	0.5	8	64	1,024	1	32	1	32	16	128	2	4
PAB-C12	TEM-12	0.03	0.06	0.25	0.25	0.25	8	32	512	0.25	8	4	>128	2	16	2	8
PAB-C10	TEM-10	0.03	0.03	0.25	1	2	32	256	>1,024	4	64	4	>128	128	512	2	2
PAB-C-4	TEM-4	0.03	0.03	1	1	32	>1,024	32	128	32	>1,024	4	>128	32	64	2	4
PAB-C3	TEM-3	0.03	0.06	0.5	0.5	16	>1,024	32	256	16	>1,024	4	>128	16	32	2	4

[a] Abbreviations: MEM, meropenem; CTT, cefoteten; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam.

(17, 21, 32, 33, 43), but clinical failure was reported in a patient with spontaneous bacterial peritonitis who was awaiting liver transplantation for end-stage liver disease (28). In some of these reports the presence of ESBLs was inferred from indirect tests and the actual enzymes involved were not identified (14, 28). This precludes analysis of which particular ESBLs were associated with favorable or unfavorable outcomes. In addition, minimal and only presumptive characterization of resistance mechanisms may not take into account the possibility that other resistance mechanisms may have contributed to the findings.

The present study was designed to investigate further the activity of cefepime and piperacillin-tazobactam against ESBL-producing strains by determining the activities of these two agents and six other parenteral β-lactam agents in standard- and high-inoculum MIC tests against nine species of *Enterobacteriaceae* that produced 22 different TEM-, SHV-, or Toho-type ESBLs. To represent the more recent clinical isolates, which seem to be continually evolving, often acquiring additional β-lactamases, the study included ESBL-producing isolates that produced multiple (up to five) other β-lactamases.

MATERIALS AND METHODS

Strains. Tests were performed with a panel of 82 ESBL-producing clinical and laboratory strains including *E. coli* ($n = 35$), *K. pneumoniae* ($n = 19$), *Klebsiella oxytoca* ($n = 3$), *Proteus mirabilis* ($n = 1$), *Citrobacter koseri* ($n = 1$), *Citrobacter freundii* ($n = 2$), *Enterobacter cloacae* ($n = 4$), *Enterobacter aerogenes* ($n = 10$), *M. morganii* ($n = 1$), and *S. marcescens* ($n = 6$). The strains were chosen to provide a wide variety of ESBLs (produced either alone or in combination with one or more other β-lactamases) and were collected from multiple centers across North America, Europe, Africa, and Asia. The panel included 13 genetically constructed, isogenic *E. coli* C600N strains that produced the enzymes TEM-3, TEM-4, TEM-5, TEM-7, TEM-8, TEM-10, TEM-12, TEM-28, TEM-43, SHV-2, SHV-3, SHV-4, and SHV-7 (4). Other ESBLs included in the study were TEM-6, TEM-9, TEM-16, TEM-24, TEM-26, TEM-43, TEM-50, SHV-5, Toho-1, and Toho-2 and several as yet unidentified ESBLs. Other (non-ESBL) β-lactamases included in the study for comparison with the ESBLs were TEM-1, SHV-1, SHV-10, AmpC, and K1. All β-lactamase identifications were confirmed in our laboratory by the appropriate biochemical or molecular procedures, such as isoelectric focusing (20, 39), substrate profile (1, 25), inhibitor profile (39), plasmid isolation, recombinant DNA techniques, and transformations (36).

Quality control strains used were *E. coli* ATCC 25922, *E. coli* ATCC 35218 (TEM-1), *E. coli* MISC 128 (SHV-1), *K. pneumoniae* 156 (SHV-1), and *E. coli* PAB-C15 (SHV-3).

Susceptibility tests. Antibiotic susceptibilities were determined by microdilution MIC methodology using inocula that differed 100-fold in density. The inocula comprised approximately 10^5 (standard inoculum) and 10^7 CFU/ml suspended in Mueller-Hinton broth (catalog no. CM405; Oxoid, Basingstoke, United Kingdom). The standard-inoculum tests were by NCCLS methodology (22). An inoculum effect was defined as an eightfold or greater increase in MIC on testing with the higher inoculum. The microdilution panels, prepared in-house and stored frozen at −70°C, contained doubling dilutions of meropenem, cefoteten, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam, and piperacillin in combination with tazobactam (4 μg/ml). The antibiotic powders were kindly provided by their manufacturers.

RESULTS

Tests with isogenic *E. coli* C600N strains. Testing with the *E. coli* C600N host strain provided a genetically more uniform background by eliminating the confounding influences that may arise with more heterogeneous clinical strains. In these tests, cefotaxime, ceftriaxone, and cefepime were the agents most frequently associated with inoculum effects. All ESBLs except SHV-4 were associated with an inoculum effect with these three agents (Table 1 shows representative results). Ceftazidime was affected by fewer ESBLs but was associated with inoculum effects with organisms producing TEM-3, TEM-5, TEM-7, TEM-8, TEM-10, TEM-12, TEM-28, TEM-43, or SHV-3 ESBLs. Aztreonam was associated with inoculum effects in tests with strains producing TEM-5, TEM-7, TEM-8, TEM-12, TEM-28, TEM-43, and SHV-2. Piperacillin-tazobactam was only associated with inoculum effects in tests with strains producing SHV-derived ESBLs, and not in tests with strains producing TEM-derived ESBLs. There were no inoculum effects in tests with meropenem and cefoteten.

Tests with species lacking an inducible chromosomal AmpC β-lactamase. (i) *K. pneumoniae*, *K. oxytoca*, *E. coli*, *C. koseri*, and *P. mirabilis*. Being obtained from diverse clinical sources, the isolates of these five species were more heterogeneous and produced a greater range of β-lactamases than the *E. coli* C600 panel. Many of the *K. pneumoniae* isolates produced multiple β-lactamases, sometimes including two ESBLs. Although the *K. pneumoniae* strains tended to be somewhat more resistant overall than the *E. coli* strains (Tables 2 and 3), probably reflecting the greater incidence of multiple β-lactamase production, the results for all the species within this group appeared to be generally similar.

Cefepime was associated with inoculum effects in 100% of evaluable tests with *K. pneumoniae* and *E. coli* (i.e., excluding those which could not be evaluated because of off-scale MICs), making it slightly more affected than ceftriaxone and cefotaxime (both had inoculum effects in 97% of tests) (Tables 2 and 3). The propensity for inoculum effects with these drugs was reflected in comparisons of the percentages of isolates of

TABLE 2. MIC and susceptibility data for non-isogenic *E. coli* isolates[a]

Inoculum (CFU/ml) (*n*) and antibiotic	MIC[e] (μg/ml)			% Susceptible[b,c]
	Range	50%	90%	
10^5 (19)				
Meropenem	≤0.015–0.06	≤0.015	0.03	100
Cefoteten	0.06–2	0.25	1	100
Cefotaxime	0.25–512	2	64	79
Ceftazidime	1–1,024	32	256	32
Ceftriaxone	0.25–1,024	4	128	79
Cefepime	0.25–128	2	16	79
Aztreonam	0.5–128	16	128	47
Pip-Tazo[d]	1–32	2	8	95
10^7 (19)				
Meropenem	0.03–0.5	0.06	0.12	100 (1/19)
Cefoteten	0.12–16	1	4	100 (4/19)
Cefotaxime	2–>1,024	256	>1,024	21 (17/18)
Ceftazidime	4–>1,024	>1,024	>1,024	5 (11/16)
Ceftriaxone	8–>1,024	1,024	>1,024	5 (18/19)
Cefepime	4–>128	>128	>128	5 (18/18)
Aztreonam	4–>1,024	>1,024	>1,024	16 (16/19)
Pip-Tazo	1–1,024	8	1,024	58 (8/19)

[a] Excludes isogenic panel of *E. coli* C600N strains.
[b] Susceptibility based on the percentage of strains inhibited at the NCCLS susceptible breakpoint concentration of each agent. (The breakpoints are only validated for the tests with the 10^5 CFU/ml inoculum.)
[c] For the inoculum of 10^7 CFU/ml, in addition to percent susceptibility, the following is shown parenthetically: number of strains showing inoculum effect/number of strains evaluable. (Not all tests were evaluable. For some MICs out of the test range it was impossible to determine if there was an eightfold increase in MIC.)
[d] Pip-Tazo, piperacillin-tazobactam.
[e] 50 and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

E. coli and *K. pneumoniae* susceptible to a concentration of each agent of 8 μg/ml in the lower- and higher-inoculum tests (Tables 2 and 3). For example, 79% of the *E. coli* isolates were susceptible to a concentration of cefepime and ceftriaxone of 8 μg/ml in the standard-inoculum tests, but only 5% of isolates were susceptible to each agent in the higher-inoculum tests.

In the tests which could be evaluated, the Toho-1 and Toho-2 enzymes were associated with inoculum effects with cefotaxime, ceftriaxone, cefepime, and aztreonam, but not with ceftazidime. Only Toho-2 was associated with an inoculum effect with piperacillin-tazobactam (data not shown). SHV-10 was associated with an inoculum effect only with ceftazidime

TABLE 3. MIC and susceptibility data for *K. pneumoniae*

Inoculum (CFU/ml) (*n*) and antibiotic	MIC[c] (μg/ml)			% Susceptible[a,b]
	Range	50%	90%	
10^5 (18)				
Meropenem	≤0.015–0.12	0.03	0.06	100
Cefoteten	0.06–2	0.25	1	100
Cefotaxime	0.5–64	4	32	67
Ceftazidime	1–>1,024	256	1,024	11
Ceftriaxone	1–128	4	64	56
Cefepime	0.5–16	4	16	89
Aztreonam	0.5–>1,024	64	512	22
Pip-Tazo	2–1,024	8	1,024	67
10^7 (18)				
Meropenem	0.03–4	0.125	4	100 (6/18)
Cefoteten	0.06–32	2	16	90 (6/18)
Cefotaxime	8–>1,024	256	>1,024	5 (18/18)
Ceftazidime	8–>1,024	>1,024	>1,024	5 (6/8)
Ceftriaxone	128–>1,024	>1,024	>1,024	0 (18/18)
Cefepime	>128	>128	>128	0 (18/18)
Aztreonam	4–>1,024	>1,024	>1,024	11 (8/12)
Pip-Tazo	4–>1,024	1,024	>1,024	22 (8/14)

[a] Susceptibility based on the percentage of strains inhibited at the NCCLS susceptible breakpoint concentration of each agent. (The breakpoints are only validated for the tests with the 10^5 CFU/ml inoculum.)
[b] For the inoculum of 10^7 CFU/ml, in addition to percent susceptibility, the following is shown parenthetically: number of strains showing inoculum effect/number of strains evaluable. (Not all tests were evaluable. For some MICs out of the test range it was impossible to determine if there was an eightfold increase in MIC.)
[c] 50 and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

TABLE 4. Representative strains with low MICs of ESBL screening agents: standard- and high-inoculum MICs

Strain (enzyme)	MIC (μg/ml) of drug at the indicated inoculum (CFU/ml)[a]															
	MEM		CTT		CTX		CAZ		CRO		FEP		ATM		TZP	
	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7
P. mirabilis 177 (TEM-10)	0.06	0.06	0.25	0.25	0.12	1	4	256	0.5	4	2	64	0.25	32	2	8
E. coli MISC 377 (SHV-10)	≤0.015	≤0.015	0.12	≤0.03	≤0.06	≤0.06	≤0.25	4	≤0.06	≤0.06	0.12	0.25	0.12	≤0.06	4	8
K. pneumoniae 98 (TEM-10, TEM-1, SHV-1)	0.03	4	0.25	1	1	128	512	>1,024	4	128	4	>128	256	>1,024	1,024	>1,024
K. pneumoniae 221 (TEM-12-like)	0.06	0.12	0.12	0.25	0.5	8	128	>1,024	1	512	8	>128	8	>1,024	8	16
K. pneumoniae 222 (SHV-3, SHV-1 or -2[b])	0.03	0.03	0.06	0.06	2	128	1	8	4	512	1	>128	0.5	4	4	8
C. freundii M421b (SHV-3-like)	0.03	0.06	0.12	1	2	256	1	32	4	512	0.5	>128	0.5	32	2	16
E. cloacae 154 (SHV-3)	0.06	0.12	1	64	8	>1,024	4	512	4	>1,024	1	>128	2	>1,024	2	>1,024
M. morganii M518b (SHV-4)	0.12	1	1	4	2	64	2	128	1	128	0.06	4	4	32	0.5	64

[a] Abbreviations: MEM, meropenem; CTT, cefoteten; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam.
[b] Additional testing required to identify this enzyme.

(Table 4). Three isolates of *K. pneumoniae* (strains 98, 221, and 222) were highly susceptible to at least one of cefotaxime, ceftriaxone, ceftazidime, or aztreonam with standard-inoculum MICs of these drugs below the NCCLS ESBL screening concentration of 2 μg/ml (Table 4).

Piperacillin-tazobactam inhibited 95% of *E. coli* isolates at the susceptible breakpoint of 16 and 4 μg/ml (for piperacillin and tazobactam, respectively) in the standard-inoculum tests and inhibited 58% of isolates at this concentration in the higher-inoculum tests (Table 2). It was less active in tests with *K. pneumoniae*, inhibiting 67 and 22% of isolates, respectively, at this concentration. In contrast to the results with the isogenic *E. coli* panel, piperacillin-tazobactam was subject to inoculum effects in the presence of certain TEM-derived ESBLs. In tests with *E. coli*, inoculum effects were associated with production of TEM-9, TEM-26, TEM-50, and also with the combination of TEM-12 and TEM-1 (data not shown). Of these enzymes, only TEM-12 was present in the isogenic *E. coli* C600N panel and then was produced alone. This suggests that the coproduction of TEM-1 may have contributed to the inoculum effect in the isolate that produced both enzymes. In tests with *K. pneumoniae*, inoculum effects were associated with TEM-8 and TEM-16 (data not shown). TEM-8 was present in the isogenic *E. coli* C600N panel. TEM-16 was not.

Meropenem at 0.12 and at 4 μg/ml inhibited all isolates in the standard-inoculum tests and the higher-inoculum tests, respectively, and cefoteten at 2 and at 32 μg/ml inhibited all isolates in the standard-inoculum tests and the higher-inoculum tests, respectively (Tables 2 and 3). Some *K. pneumoniae* isolates, such as strain 98 in Table 4, show an inoculum effect in tests with meropenem (six strains) and/or cefoteten (seven strains).

(ii) *C. freundii*, *E. aerogenes*, *E. cloacae*, *M. morganii*, and *S. marcescens*. In addition to their chromosomally encoded AmpC β-lactamases, the 23 isolates of *C. freundii*, *E. aerogenes*, *E. cloacae*, *M. morganii*, and *S. marcescens* produced at least one of the ESBLs TEM-3, SHV-2, SHV-3, SHV-4, and SHV-5 or an unidentified TEM-derived ESBL. Five isolates produced three β-lactamases.

In standard-inoculum tests, all isolates were susceptible to meropenem, with cefepime being the next most active agent (96% susceptible) (Table 5). Fourteen isolates (61%) were susceptible to at least one of the cephalosporins, cefotaxime, ceftriaxone, or ceftazidime. Some cephalosporin MICs were very low: e.g., 0.06 μg/ml for cefepime, 1 μg/ml for ceftazidime and ceftriaxone, and 2 μg/ml for cefotaxime (Table 4). In the higher-inoculum tests, inoculum effects occurred with all agents, reflecting the presence of the AmpC-mediated β-lactamase. Again, the inoculum effects occurred most frequently in tests with cefepime, cefotaxime, and ceftriaxone (Table 5). Cefepime was the agent most dramatically affected, with susceptibility decreasing from 96% in the standard-inoculum tests to only 8% of isolates inhibited by 8 μg/ml in the higher-inoculum tests. Inoculum effects with meropenem and/or cefoteten occurred in tests with more than 50% of these strains (17 of 23 isolates for meropenem and 11 of 19 isolates for cefoteten). Even so, the high-inoculum MICs of meropenem exceeded 4 μg/ml for only three isolates. Representative results are shown in Table 4.

DISCUSSION

A pronounced inoculum effect may occur when a bacterium produces an enzyme capable of destroying an antibiotic. When bacterial killing and destruction of the antibiotic occur simultaneously, liberated enzyme from dead cells reduces the external concentration of the antibiotic (42). In an in vitro test the ability of the inoculum to influence the antibacterial activity of a β-lactam drug is determined primarily by two factors—the intrinsic activity of the drug against the test organism and the

TABLE 5. MIC and susceptibility data: species producing a chromosomal AmpC β-lactamase[a]

Inoculum (CFU/ml) (*n*) and antibiotic	MIC[d] (μg/ml)			% Susceptible[b,c]
	Range	50%	90%	
10^5 (23)				
Meropenem	0.03–4	0.03	0.06	100
Cefoteten	0.12–>64	4	>64	79
Cefotaxime	0.12–256	8	64	54
Ceftazidime	1–1,024	32	512	29
Ceftriaxone	0.5–256	8	128	50
Cefepime	0.06–32	2	8	96
Aztreonam	0.25–1,024	16	256	38
Pip-Tazo	0.5–256	4	128	75
10^7 (23)				
Meropenem	0.06–>64	1	16	79 (17/23)
Cefoteten	0.25–>64	32	>64	25 (11/19)
Cefotaxime	1–>1,024	>1,024	>1,024	4 (22/23)
Ceftazidime	16–>1,024	>1,024	>1,024	0 (17/20)
Ceftriaxone	4–>1,024	>1,024	>1,024	4 (21/22)
Cefepime	0.25–>128	>128	>128	8 (22/23)
Aztreonam	8–>1,024	512	>1,024	4 (10/21)
Pip-Tazo	4–>1,024	64	>1,024	42 (13/22)

[a] The strains comprised *C. freundii* (*n* = 2), *E. cloacae* (*n* = 4), *E. aerogenes* (*n* = 10), *M. morganii* (*n* = 1), and *Serratia marcescens* (*n* = 6). All produced a TEM- or SHV-derived ESBL. Three isolates produced an additional β-lactamase. An *E. cloacae* and an *S. marcescens* strain each also produced TEM-1, and one *S. marcescens* strain also produced TEM-2.

[b] Susceptibility based on the percentage of strains inhibited at the NCCLS susceptible breakpoint concentration of each agent. (The breakpoints are only validated for the tests with the 10^5 CFU/ml inoculum.)

[c] For the inoculum of 10^7 CFU/ml, in addition to percent susceptibility, the following is shown parenthetically: number of strains showing inoculum effect/number of strains evaluable. (Not all tests were evaluable. For some MICs out of the test range it was impossible to determine if there was an eightfold increase in MIC.)

[d] 50 and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

susceptibility of the drug to hydrolysis by the β-lactamase(s) of the organism. In general, the larger the inoculum effect is, the more susceptible the drug is to hydrolysis by the organism's β-lactamase(s).

High inocula occur in endocarditis, meningitis, septic arthritis, osteomyelitis, abscesses, and other deep-seated infections. In addition, stationary-phase growth, which favors β-lactamase activity and is detrimental to β-lactam drug efficacy, also occurs in these infections (41). As examples of high inocula, colony counts of 10^9 to 10^{10} CFU per g of tissue in infective endocarditis vegetations (16) and 10^9 CFU per ml of cerebrospinal fluid in meningitis (2) have been reported, and these values significantly exceed the more modest 10^7 CFU per ml used in the present study. In this context, it is pertinent to consider the relevance of inoculum effects and of MICs determined with higher-than-standard inocula. In support of their utility, animal models of pneumonia have shown that antibiotics not associated with an inoculum effect are more efficacious than those exhibiting an inoculum effect (29). Balanced against this is the caution that it is unwise to overlook the fact that data from animal models are subject to variables such as the host status (e.g., neutropenic versus normal) and the challenge organism (29, 35). Despite such concerns, inoculum effects are accepted as relevant by some investigators, at least to the extent that it has been proposed that "a major inoculum effect for a compound contraindicates its use in serious infections caused by the pathogen" (18).

In this study, the inoculum effect was most pronounced in tests with the cephalosporins cefepime, cefotaxime, and ceftriaxone, with cefepime being at least as affected as cefotaxime and ceftriaxone. The inoculum effect was smallest and least common in tests with meropenem and cefoteten. These findings are consistent with previous reports that carbapenems and cephamycins are not inactivated by Bush group 2be β-lactamases while cephalosporins are (6). Cefepime has been described in some reports as less prone than other cephalosporins to hydrolysis by ESBLs (26, 31). However, the inoculum effects consistently observed with cefepime suggest that it is at least as prone to inactivation by an extensive range of ESBLs as cefotaxime and ceftriaxone, and more so than ceftazidime and aztreonam. This finding adds support to and extends findings of other investigators (14, 19, 43). To our knowledge, the clinical implications of an inoculum effect with cefepime have not been evaluated. Therefore, until there are reliable clinical data to resolve this issue, it might be prudent for clinicians who are considering using cefepime for therapy of serious infections caused by ESBL-producing pathogens to be prepared to monitor patients closely for signs of treatment failure.

Piperacillin-tazobactam was less subject to an inoculum effect than the cephalosporins and aztreonam. Inoculum effects with this agent occurred consistently in tests with strains producing SHV-derived ESBLs but occurred only occasionally in tests with strains producing TEM-derived ESBLs, and only with certain TEM-derived ESBLs. As with cefepime, clinical data are required to determine the relevance of these findings. It is possible that piperacillin-tazobactam might have better efficacy against pathogens that produce the TEM-derived ESBLs that are not associated with an inoculum effect than against those that produce other types of ESBLs. However, this issue is currently moot because it is beyond the capability of clinical laboratories to routinely identify the types of ESBLs encountered in patient isolates.

In standard-inoculum tests 61% (14 of 23) of the ESBL-producing isolates from species known to produce an inducible AmpC β-lactamase were susceptible to one or more of the expanded-spectrum cephalosporins, and 96% (22 of 23) were susceptible to cefepime. With cephalosporin MICs as low as 0.06 to 2 μg/ml for some of the ESBL-producing isolates, but significantly higher in ESBL-negative, derepressed mutants (internal data, Center for Research in Antiinfectives and Biotechnology), it is clearly impossible to devise a reliable ESBL screen for inducible AmpC-producing organisms based on susceptibility to cephalosporins. Aztreonam is similarly inappropriate as an indicator drug for ESBL detection in these organisms. Therefore, other approaches, such as inhibitor-based tests, must be used to detect ESBLs in these organisms. In view of reports of the increasing occurrence of ESBLs in *Enterobacter* spp. and other AmpC-producing species (8; T. Gottlieb and C. Wolfson, Letter, J. Antimicrob Chemother. **46:**330–331, 2000), the development of such tests must be a priority.

Laboratories using NCCLS guidelines are currently required to report susceptibility if the MICs of expanded-spectrum cephalosporin or aztreonam for ESBL-producing isolates of species other than *E. coli* or *Klebsiella* spp. are 8 μg/ml or less. However, ESBL-producing *E. coli* and *Klebsiella* spp. are reported as resistant to these agents irrespective of MIC. (β-Lactamase inhibitor combinations and cephamycins are excluded from this recommendation.) Unless there is evidence that ESBLs are clinically less significant in organisms other than *E. coli* and *Klebsiella* spp., it would seem prudent to extend the reporting practice to all ESBL-producing isolates, i.e., report them all as resistant to all penicillins, cephalosporins, and aztreonam, irrespective of their identity.

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REFERENCES

1. **Bauernfeind, A., H. Grimm, and S. Schweighart.** 1990. A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli.* Infection **18:**294–298.
2. **Bingen, E., N. Lambert-Zechovsky, P. Mariani-Kurkdjian, C. Doit, Y. Aujard, F. Fournerie, and H. Mathieu.** 1990. Bacterial counts in cerebrospinal fluid of children with meningitis. Eur J. Clin. Microbiol. Infect. Dis. **9:**278–281.
3. **Bradford, P. A., C. E. Cherubin, V. Idemyor, B. A. Rasmussen, and K. Bush.** 1994. Multiply resistant *Klebsiella pneumoniae* strains from two Chicago hospitals: identification of the extended-spectrum TEM-12 and TEM-10 ceftazidime-hydrolyzing β-lactamases in a single isolate. Antimicrob. Agents Chemother. **38:**761–766.
4. **Bradford, P. A., and C. C. Sanders.** 1995. Development of test panel of β-lactamases expressed in a common *Escherichia coli* host background for evaluation of new β-lactam antibiotics. Antimicrob. Agents Chemother. **39:** 308–313.
5. **Brun-Buisson, C., P. Legrand, A. Philippon, F. Montravers, M. Ansquer, and J. Duval.** 1987. Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. Lancet **ii:**302–306.
6. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
7. **Chanal, C. M., D. L. Sirot, A. Petit, R. Labia, A. Morand, J. L. Sirot, and R. A. Cluzel.** 1989. Multiplicity of TEM-derived beta-lactamases from *Klebsiella pneumoniae* strains isolated at the same hospital and relationships between the responsible plasmids. Antimicrob. Agents Chemother. **33:**1915–1920.
8. **De Champs, C., D. Sirot, C. Chanal, R. Bonnet, J. Sirot, and The French Study Group.** 2000. A 1998 survey of extended-spectrum β-lactamases in *Enterobacteriaceae* in France. Antimicrob. Agents Chemother. **44:**3177–3179.
9. **DuBois, S. K., M. S. Marriott, and S. G. B. Amyes.** 1995. TEM- and SHV-derived extended-spectrum β-lactamases: relationship between selection, structure and function. J. Antimicrob. Chemother. **35:**7–22.
10. **French, G. L., K. P. Shannon, and N. Simmons.** 1996. Hospital outbreak of *Klebsiella pneumoniae* resistant to broad-spectrum cephalosporins and β-lactam–β-lactamase inhibitor combinations by hyperproduction of SHV-5 beta-lactamase. J. Clin. Microbiol. **34:**358–363.
11. **Jacoby, G. A., and I. Carreras.** 1990. Activities of β-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **34:**858–862.
12. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
13. **Jarlier, V., M.-H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
14. **Jett, B. D., D. J. Ritchie, R. Reichley, T. C. Bailey, and D. F. Sahm.** 1995. In vitro activities of various β-lactam antimicrobial agents against clinical isolates of *Escherichia coli* and *Klebsiella* spp. resistant to oxyimino cephalosporins. Antimicrob. Agents Chemother. **39:**1187–1190.
15. **Karas, J. A., D. G. Pillay, D. Muckart, and A. W. Sturm.** 1996. Treatment failure due to extended spectrum β-lactamase. J. Antimicrob. Chemother. **37:**203–204.
16. **Korzeniowski, O., and D. Kaye.** 1998. Endocarditis, p. 663–674. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), Infectious diseases, 2nd ed. W. B. Saunders Company, Philadelphia, Pa.
17. **Leleu, G., M. D. Kitzis, J. M. Vallois, L. Gutmann, and J. M. Decazes.** 1994. Different ratios of the piperacillin-tazobactam combination for treatment of experimental meningitis due to *Klebsiella pneumoniae* producing the TEM-3 extended-spectrum β-lactamase. Antimicrob. Agents Chemother. **38:**195–199.
18. **Livermore, D. M.** 1998. β-Lactamase-mediated resistance and opportunities for its control. J. Antimicrob. Chemother. **41**(Suppl. D)**:**25–41.
19. **Martinez-Martinez, L., A. Pascual, S. Hernandez-Alles, D. Alvarez-Diaz, A. I. Suarez, J. Tran, V. J. Benedi, and G. A. Jacoby.** 1999. Roles of β-lactamases and porins in activities of carbapenems and cephalosporins against *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **43:**1669–1673.
20. **Matthew, M. A., A. M. Harris, M. J. Marshall, and G. W. Ross.** 1975. The use of analytical isoelectric focusing for detection and identification of β-lactamases. J. Gen. Microbiol. **88:**169–178.
21. **Mentec, H., J. M. Vallois, A. Bure, A. Saleh-Mghir, F. Jehl, and C. Carbon.** 1992. Piperacillin, tazobactam, and gentamicin alone or combined in an endocarditis model of infection by a TEM-3-producing strain of *Klebsiella pneumoniae* or its susceptible variant. Antimicrob. Agents Chemother. **36:** 1883–1889.
22. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
23. **National Committee for Clinical Laboratory Standards.** 2000. Performance standards for antimicrobial susceptibility testing; tenth informational supplement (aerobic dilution) M100-S10. NCCLS, Wayne, Pa.
24. **Neu, H. C., N. X. Chin, and H. B. Huang.** 1993. In vitro activity and beta-lactamase stability of FK-037, a parenteral cephalosporin. Antimicrob. Agents Chemother. **37:**566–573.
25. **O'Callaghan, C. H., P. W. Muggleton, and G. W. Ross.** 1968. Effects of beta-lactamase from gram-negative organisms on cephalosporins and penicillins. Antimicrob. Agents Chemother. **8:**57–63.
26. **Paterson, D. L.** 2000. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). Clin. Microbiol. Infect. **6:**460–463.
27. **Paterson, D. L., W.-C. Ko, A. Von Gottberg, J. M. Casellas, L. Mulazimoglu, K. P. Klugman, R. A. Bonomo, L. B. Rice, J. G. McCormack, and V. L. Yu.** 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. J. Clin. Microbiol. **39:**2206–2212.
28. **Paterson, D. L., N. Singh, T. Gayowski, and I. R. Marino.** 1999. Fatal infection due to extended-spectrum beta-lactamase-producing *Escherichia coli*: implications for antibiotic choice for spontaneous bacterial peritonitis. Clin. Infect. Dis. **28:**683–684.
29. **Pennington, J. E.** 1985. Animal models of pneumonia for evaluation of antimicrobial therapy. J. Antimicrob. Chemother. **16:**1–6.
30. **Philippon, A., S. B. Redjeb, G. Fournier, and A. B. Hassen.** 1989. Epidemiology of extended spectrum β-lactamases. Infection **17:**347–354.
31. **Rahal, J. J.** 2000. Extended-spectrum beta-lactamases: how big is the problem? Clin. Microbiol. Infect. **2**(Suppl. 2)**:**2–6.
32. **Rice, L. B., L. L. Carias, and D. M. Shlaes.** 1994. In vivo efficacies of β-lactam–β-lactamase inhibitor combinations against a TEM-26-producing

strain of *Klebsiella pneumoniae.* Antimicrob. Agents Chemother. **38:**2663–2664.
33. **Rice, L. B., E. C. Eckstein, J. DeVente, and D. M. Shlaes.** 1996. Ceftazidime-resistant *Klebsiella pneumoniae* isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. Clin. Infect. Dis. **23:**118–124.
34. **Rice, L. B., S. H. Willey, G. A. Papanicolaou, A. A. Medeiros, G. M. Eliopoulos, J. Robert C. Moellering, and G. A. Jacoby.** 1990. Outbreak of ceftazidime resistance caused by extended-spectrum β-lactamases at a Massachusetts chronic-care facility. Antimicrob. Agents Chemother. **34:**2193–2199.
35. **Roosendaal, R., I. A. Bakker-Woudenberg, M. van den Berghe-van Raffe, and M. F. Michel.** 1986. Continuous versus intermittent administration of ceftazidime in experimental *Klebsiella pneumoniae* pneumonia in normal and leukopenic rats. Antimicrob. Agents Chemother. **30:**403–408.
36. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
37. **Sanders, C. C.** 1993. Cefepime: the next generation? Clin. Infect. Dis. **17:** 369–379.
38. **Sanders, C. C.** 1996. In vitro activity of fourth generation cephalosporins against enterobacteriaceae producing extended-spectrum beta-lactamases. J. Chemother. **8**(Suppl. 2)**:**57–62.
39. **Sanders, C. C., W. E. Sanders, Jr., and E. S. Moland.** 1986. Characterization of β-lactamases in situ on polyacrylamide gels. Antimicrob. Agents Chemother. **30:**951–952.
40. **Sirot, D.** 1995. Extended-spectrum plasmid-mediated β-lactamases. J. Antimicrob. Chemother. **36:**19–34.
41. **Stratton, C. W.** 1991. In vitro testing: correlations between bacterial susceptibility, body fluid levels and effectiveness of antibacterial therapy, p. 849–879. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. Williams & Wilkins, Baltimore, Md.
42. **Sykes, R. B., and M. Matthew.** 1976. The beta-lactamases of gram-negative bacteria and their role in resistance to beta-lactam antibiotics. J. Antimicrob. Chemother. **2:**115–157.
43. **Thauvin-Eliopoulos, C., M. F. Tripodi, R. C. Moellering, Jr., and G. M. Eliopoulos.** 1997. Efficacies of piperacillin-tazobactam and cefepime in rats with experimental intra-abdominal abscesses due to an extended-spectrum beta-lactamase-producing strain of *Klebsiella pneumoniae.* Antimicrob. Agents Chemother. **41:**1053–1057.
44. **Thomson, K. S., A. M. Prevan, and C. C. Sanders.** 1996. Novel plasmid-mediated β-lactamases in Enterobacteriaceae: emerging problems for new β-lactam antibiotics, p. 151–163. *In* J. S. Remington and M. N. Swartz (ed.), Current clinical topics in infectious diseases, vol. 16. Blackwell Science, Inc., Cambridge, Mass.
45. **Thomson, K. S., and E. Smith Moland.** 2000. Version 2000: the new beta-lactamases of Gram-negative bacteria at the dawn of the new millennium. Microbes Infect. **2:**1225–1235.
46. **Tzouvelekis, L. S., E. Tzelepi, A. F. Mentis, and N. J. Legakis.** 1995. In vitro activity of cefpirome against selected clinical enterobacterial isolates with beta-lactamase-mediated resistance. Infection **23:**384–387.
47. **Venezia, R. A., F. J. Scarano, K. E. Preston, L. M. Steele, T. P. Root, R. Limberger, W. Archinal, and M. A. Kacica.** 1995. Molecular epidemiology of an SHV-5 extended-spectrum beta-lactamase in Enterobacteriaceae isolated from infants in a neonatal intensive care unit. Clin. Infect. Dis. **21:**915–923.