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Inoculum Effect on the Efficacies of Amoxicillin-Clavulanate, Piperacillin-Tazobactam, and Imipenem against Extended-Spectrum β-Lactamase (ESBL)-Producing and Non-ESBL-Producing *Escherichia coli* in an Experimental Murine Sepsis Model

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Escherichia coli* is commonly involved in infections with a heavy bacterial burden. Piperacillin-tazobactam and carbapenems are among the recommended empirical treatments for health care-associated complicated intra-abdominal infections. In contrast to amoxicillin-clavulanate, both have reduced *in vitro* activity in the presence of high concentrations of extended-spectrum β-lactamase (ESBL)-producing and non-ESBL-producing *E. coli* bacteria. Our goal was to compare the efficacy of these antimicrobials against different concentrations of two clinical *E. coli* strains, one an ESBL-producer and the other a non-ESBL-producer, in a murine sepsis model. An experimental sepsis model {~5.5 $\log_{10}$ CFU/g [low inoculum concentration (LI)] or ~7.5 $\log_{10}$ CFU/g [high inoculum concentration (HI)]} using *E. coli* strains ATCC 25922 (non-ESBL producer) and Ec1062 (CTX-M-14 producer), which are susceptible to the three antimicrobials, was used. Amoxicillin-clavulanate (50/12.5 mg/kg given intramuscularly [i.m.]), piperacillin-tazobactam (25/3.125 mg/kg given intraperitoneally [i.p.]), and imipenem (30 mg/kg i.m.) were used. Piperacillin-tazobactam and imipenem reduced spleen ATCC 25922 strain concentrations (−2.53 and −2.14 $\log_{10}$ CFU/g [$P < 0.05$, respectively]) in the HI versus LI groups, while amoxicillin-clavulanate maintained its efficacy (−1.01 $\log_{10}$ CFU/g [no statistically significant difference]). Regarding the Ec1062 strain, the antimicrobials showed lower efficacy in the HI than in the LI groups: −0.73, −1.89, and −1.62 $\log_{10}$ CFU/g ($P < 0.05$, for piperacillin-tazobactam, imipenem, and amoxicillin-clavulanate, respectively, although imipenem and amoxicillin-clavulanate were more efficacious than piperacillin-tazobactam). An adapted imipenem treatment (based on the time for which the serum drug concentration remained above the MIC obtained with a HI of the ATCC 25922 strain) improved its efficacy to −1.67 $\log_{10}$ CFU/g ($P < 0.05$). These results suggest that amoxicillin-clavulanate could be an alternative to imipenem treatment of infections caused by ESBL- and non-ESBL-producing *E. coli* strains in patients with therapeutic failure with piperacillin-tazobactam.

Escherichia coli is a common etiologic agent of intra-abdominal infections (IAI) (1) associated with a heavy bacterial burden (2) and of urinary tract infections and bacteremia (3–5), among others. The recommendations for empirical antimicrobial therapy for high-risk, severe, community-acquired, extrabiliary complicated IAI and health care-associated complicated IAI include piperacillin-tazobactam or carbapenems (6). Recently, data from an observational study suggested that amoxicillin-clavulanate and piperacillin-tazobactam are as effective as carbapenems in patients with bacteremia due to extended-spectrum β-lactamase (ESBL)-producing *E. coli*; however, most of the patients in that study had urinary or biliary tract infections and the number of patients with heavy bacterial loads was probably low (7). In this context, it is important to note that, in some areas, many ESBL-producing and non-ESBL-producing isolates of *Klebsiella pneumoniae* and *E. coli* are susceptible *in vitro* to piperacillin-tazobactam and imipenem, but the MICs may increase substantially if the susceptibility tests are performed with an inoculum concentration higher than the standard one (8–11). Recently, we have demonstrated, using a microdilution method and time-kill assays, that the reduction of *in vitro* activity at a high inoculum concentration does not occur with the amoxicillin-clavulanate combination against clinical non-ESBL-producing and ESBL-producing *E. coli* strains (9). Previously, when several *Enterobacteriaceae* species produced complex beta-lactamase patterns or ESBL (12, 13), both piperacillin-tazobactam and carbapenem compounds showed increased MICs when inoculum concentrations higher than the standard one are used. On the other hand, some authors have raised doubts about the existence of an inoculum effect (14), but in fact, little is known about the real *in vivo* impact of this effect. Thus, the objective of this study was to compare the *in vivo* efficacies of amoxicillin-clavulanate, piperacillin-tazobactam, and imipenem against different tissue bacterial concentrations of two clinical *E. coli* strains in an experimental murine sepsis model.

MATERIALS AND METHODS

Bacterial strains. Two *E. coli* strains were analyzed: ATCC 25922, which lacks any resistance determinant and is a non-ESBL producer, and Ec1062 (CTX-M-14-producing strain), which was selected from a previous study (9). The MICs obtained with the standard low inoculum concentration (LI) and a high inoculum concentrations (HI) in the previous study are shown in the Table 1 (9). Beta-lactamase production by the original

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TABLE 1 MIC of amoxicillin-clavulanate, piperacillin-tazobactam, and imipenem for the ATCC 25922 and Ec1062 strains using standard and high bacterial inoculum concentrations

Antibacterial and inoculum concentration[a]	MIC (μg/ml)	
	ATCC 25922	Ec1062[b]
Amoxicillin-clavulanate		
LI	2/1	4/2
HI	4/2	8/4
Piperacillin-tazobactam		
LI	2/4	2/4
HI	>256/4	>256/4
Imipenem		
LI	0.06	0.06
HI	0.5	0.25

[a] LI, low inoculum concentration (5×10^5 CFU/ml); HI, high inoculum concentration (10^7 CFU/ml).
[b] CTX-M-14 producer.

Ec1062 strain and a transconjugant was characterized as previously described by Hernández et al., by isoelectric focusing (IEF) (15). The ESBL-encoding gene was further characterized by PCR and sequenced in the original and transconjugant isolates. Only one band by IEF and one PCR product were detected. According to the results obtained, CTX-M-14 was the only beta-lactamase present in strain Ec1062.

Animals. Female C57BL/6 mice weighting 16 to 20 g (University of Seville Facility, Seville, Spain) were used. Animals were housed in regulation cages and given free access to food and water. This study was approved by the Ethics Committee of the University Hospitals Virgen del Rocío (Seville, Spain).

Experimental sepsis model characterization. In order to assess the *in vivo* inoculum size effect on antimicrobial activity, an experimental sepsis model was used (16). To achieve high (HI) and low (LI) spleen bacterial concentrations at the beginning of treatment, two different intraperitoneal (i.p.) challenges with the ATCC 25922 and Ec1062 strains were used. Four groups of 10 mice were i.p. inoculated with a volume of 250 μl of $\sim 10^7$ CFU/ml as a high inoculum concentration (HI) or $\sim 10^5$ CFU/ml as a low inoculum concentration (LI). Bacterial inocula were mixed 1:1 with 10% porcine mucin (Sigma-Aldrich, Madrid, Spain). Mice were then sacrificed by i.p. administration of a lethal dose of sodium thiopental (B. Braun Medical S.A. Rubi, Barcelona, Spain) 4 h after inoculation, at the time of the first treatment point. Immediately after death, laparotomy was carried out and spleens were extracted for homogenization in 2 ml of sterile saline solution (Stomacher 80; Tekmar Co., Cincinnati, OH). Finally, 10-fold dilutions were plated on sheep blood agar (SBA) plates for quantitative cultures.

Pharmacokinetics/pharmacodynamics. Serum antibiotic concentrations were determined in groups of 21 healthy mice after a single administration of amoxicillin-clavulanate (Laboratorios Normon, Madrid, Spain) at 50/12.5 mg/kg given intramuscularly (i.m.), piperacillin-tazobactam (Pfizer, Alcobendas, Spain) at 25/3.125 mg/kg given i.p., or imipenem (MSD, Madrid, Spain) at 30 mg/kg given i.m. Blood samples from sets of three anesthetized mice were obtained from the periorbital plexus at 10, 15, 30, 60, 90, 120, and 150 min after drug administration.

Serum amoxicillin, clavulanate, and imipenem concentrations were determined by using an agar diffusion bioassay. *Kocuria rhizophila* ATCC 9341 was the control strain for amoxicillin and imipenem, and *K. pneumoniae* ATCC 29665 was the control strain for clavulanate (17, 18). Serum piperacillin-tazobactam levels were quantified by high-performance liquid chromatography as previously described (19). A protein binding value of 20% for piperacillin and tazobactam was used (20). Because the method used for the determination of serum amoxicillin, clavulanate, and imipenem concentrations was a microbiological bioassay based on antimicrobial activity, specifically, the active fraction present in serum, we assumed that the entire antibiotic measured by this technique is free of protein binding.

Antibiotic assays were performed in triplicate. The maximum serum drug concentration (C_{max} in μg/ml) and elimination half-life ($t_{1/2}$ in hours) were obtained by a computer-assisted method (PK functions for Microsoft Excel; J. L. Usansky, A. Desai, and D. Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA [http://www.boomer.org/pkin/soft.html]). The time (fT_{MIC}, hours) and the percentage of time (%fT_{MIC}) for which the serum drug concentration remained above the MIC between two doses were extrapolated from the regression line of the serum drug concentration. The intraday and interday variations of the assays were also calculated.

The dosages of amoxicillin-clavulanate, piperacillin-tazobactam, and imipenem were those needed to obtain the optimal %fT_{MIC} of 20 to 40% established for β-lactams (21).

Therapeutic experimental sepsis model groups. Groups of 15 mice infected with a HI or LI of strain ATCC 25922 or Ec1062 were randomly designated controls (no treatment) or assigned to amoxicillin-clavulanate, piperacillin-tazobactam, and imipenem treatment groups. Animals received a total daily amoxicillin-clavulanate dose of 400/100 mg/kg i.m. in eight doses, a total daily piperacillin-tazobactam dose of 200/25 mg/kg i.p. in eight doses, or a total daily imipenem dose of 120 mg/kg i.m. in four doses. Mice were observed for death for 24 h, and the surviving animals were sacrificed 3 h (amoxicillin-clavulanate and piperacillin-tazobactam groups) and 6 h (imipenem groups) after the last dose by the i.p. administration of a lethal dose of sodium thiopental. Immediately after death, spleens were extracted and processed as previously described. Through cardiac puncture, blood samples were taken and 100 μl was plated on SBA for qualitative cultures.

To assess the benefits of a correction in a treatment based on the different MICs obtained with the different inocula, the %fT_{MIC} of imipenem was recalculated by using the MIC obtained with a HI of ATCC 25922. The dosage of imipenem was then adjusted to obtain a %fT_{MIC} of 30 to 40% in the interval between doses, and an additional experimental group was infected with a HI of strain ATCC 25922 and treated with a total daily imipenem dosage of 180 mg/kg i.m. (administered in six doses).

Statistical analysis. The variables analyzed were percent mortality, spleen bacterial concentration (mean $\log_{10}$ CFU/g of tissue ± standard deviation), and percent positive blood cultures. The two-tailed Fisher test, analysis of variance, and Dunnett and Tukey *post hoc* tests were used. A P value of <0.05 was considered significant.

RESULTS

Experimental model characterization. The spleen bacterial concentrations obtained 4 h after HI and LI bacterial challenges were as follows: ATCC 25922 HI, 7.5 ± 0.44 $\log_{10}$ CFU/g; ATCC 25922 LI, 5.5 ± 0.3 $\log_{10}$ CFU/g; Ec1062 HI, 7.4 ± 0.57; Ec1062 LI, 5.4 ± 0.21. The spleen bacterial burdens were similar in both groups, showing a difference of ~100-fold between the LI and HI groups, similar to that used for the *in vitro* experiments.

Pharmacokinetics/pharmacodynamics. The mouse serum pharmacokinetic and pharmacodynamic parameters of each antimicrobial are shown in Table 2. The intraday and interday variations of the assays were 3.01% ± 3.21% and 1.89% ± 4.22% for amoxicillin, 1.33% ± 1.44% and 1.09% ± 2.23% for clavulanate, 3.7% ± 0.42% and 4.35% ± 0.49% for piperacillin, 4% ± 1.7% and 3.6% ± 1.41% for tazobactam, and 2.62% ± 2.44% and 3.22% ± 1.91% for imipenem. The linearities (r^2) of the assays were 0.98 ± 0.01 (amoxicillin), 0.99 ± 0.01 (clavulanate, piperacillin, and tazobactam), and 0.97 ± 0.02 (imipenem). The lower limits of detection were 0.01 μg/ml (amoxicillin), 0.12 μg/ml (clavulanate), 1 μg/ml (piperacillin), 1 μg/ml (tazobactam), and 0.01

TABLE 2 Serum pharmacokinetic/pharmacodynamic parameters of amoxicillin-clavulanate, piperacillin-tazobactam, and imipenem in mice

Drug[a]	Doses (mg/kg)	C_{max} (μg/ml)	$t_{1/2}$ (h)	MIC (μg/ml)	fT_{MIC} (h)	% fT_{MIC}	Time between doses (h)
AMC	50/12.5	17.9/5.2	0.40/0.22	2	1.13	37.7	3
				4	0.84	33.6	3
TZP	25/3.125	26.19/5.48	0.46/0.41	2	1.13	37.6	3
IPM	30	23.25	0.26	0.06	2.5	41.7	6

[a] AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam; IMP, imipenem.

μg/ml (imipenem). On the basis of the pharmacokinetic results, the antimicrobial dosages for the efficacy studies were adjusted to maintain concentrations above the MIC during 20 to 40% of the intervals between doses.

Therapeutic experimental sepsis model groups. The spleen bacterial concentrations, percentages of positive blood cultures, and mortality percentages obtained are shown in Table 3.

(i) LI infection groups. All of the antimicrobials reduced the spleen ATCC 25922 or Ec1062 bacterial concentrations below those of the controls ($P < 0.05$). Also, the percentages of mortality and positive blood cultures were reduced by all of the treatments with respect to those of the controls ($P < 0.05$), with the exception of the positive blood culture percentage in mice infected with the Ec1062 strain and treated with piperacillin-tazobactam. No significant differences were found in the spleen bacterial concentrations among the treatments in the groups of mice infected with the ATCC 25922 strain. It must be noted that in the Ec1062-infected groups, amoxicillin-clavulanate and imipenem were better than piperacillin-tazobactam ($P < 0.05$) and imipenem was better than amoxicillin-clavulanate in reducing the bacterial burden in the spleen ($P < 0.05$). In relation to the other variables, imipenem produced a lower percentage of positive blood cultures ($P < 0.05$) than piperacillin-tazobactam did in the groups infected with the Ec1062 strain, and amoxicillin-clavulanate and imipenem produced lower percentages of mortality than did piperacillin-tazobactam in the groups infected with the ATCC 25922 strain.

(ii) HI infection groups. On the other hand, all of the treatments reduced the concentrations of both bacterial strains in the HI groups in relation to those of the control groups ($P < 0.05$). It is important to note that amoxicillin-clavulanate and imipenem were more efficacious than piperacillin-tazobactam ($P < 0.05$) in reducing the spleen bacterial concentrations of both strains and the mortality rate in the groups infected with the ATCC 25922 strain. Moreover, in the groups infected with the ATCC 25922 strain, the efficacy of amoxicillin-clavulanate in reducing the spleen bacterial concentrations was higher than that observed with imipenem ($P < 0.05$).

(iii) LI versus HI groups. Comparing the efficacies of the antimicrobials in the LI and HI groups with respect to spleen bacterial concentrations, in the ATCC 25922 strain-infected groups, piperacillin-tazobactam and imipenem showed diminished efficacy when a HI was used. Amoxicillin-clavulanate maintained its efficacy in both the HI and LI groups (3.89 ± 1.71 versus 2.88 ± 1.21 $\log_{10}$ CFU/g [no statistically significant difference]).

Regarding ESBL-producing strain Ec1062, all of the antimicrobials had lower efficacy in the HI than in the LI group in relation to the spleen bacterial concentrations, with significant reductions of −0.73 $\log_{10}$ CFU/g for piperacillin-tazobactam, −1.89 $\log_{10}$ CFU/g for imipenem, and −1.62 $\log_{10}$ CFU/g for amoxicillin-clavulanate.

Finally, when the imipenem treatment was adapted according to the MIC obtained with the HI of the ATCC 25922 strain (180 mg/kg i.m. administered in six doses), the efficacy of imipenem improved, decreasing the spleen bacterial concentration from

TABLE 3 *In vivo* spleen bacterial concentrations, percentages of positive blood cultures, and mortality rates in controls and after treatment with amoxicillin-clavulanate, piperacillin-tazobactam, and imipenem in mice infected with a high or low concentration of strain ATCC 25922 or Ec1062 bacteria

Strain and treatment[f]	Low inoculum concentration			High inoculum concentration		
	Bacterial concn in spleen ($\log_{10}$ CFU/g)	% of blood cultures positive	% Mortality	Bacterial concn in spleen ($\log_{10}$ CFU/g)	% of blood cultures positive	% Mortality
ATCC 25922						
Control	8.68 ± 0.35	100	100	8.02 ± 0.16	100	100
AMC	2.88 ± 1.21[a]	40[a]	0[a,b]	3.89 ± 1.71[a,b,c]	60[a]	0[a,b]
TZP	4.1 ± 2.69[a,e]	60[a]	53.3[a,e]	6.63 ± 0.36[a]	85.7	100
IPM	3.46 ± 1.46[a,e]	33.3[a]	0[a,b]	5.6 ± 1.1[a,b]	73.3	6.7[a,b]
Ec1062						
Control	8.57 ± 0.33	100	68.8[e]	8.19 ± 0.87	100	100
AMC	4.49 ± 0.18[a,b,e]	66.7[a]	0[a]	6.11 ± 0.2[a,b]	73.3	0[a]
TZP	6.26 ± 0.84[a,e]	86.7	6.7[a]	6.99 ± 0.99[a]	80	26.7[a]
IPM	4.08 ± 0.37[a,b,d,e]	26.7[a,b]	0[a]	5.97 ± 0.16[a,b]	66.7[a]	0[a]

[a] $P < 0.05$ versus control.
[b] $P < 0.05$ versus piperacillin-tazobactam.
[c] $P < 0.05$ versus imipenem.
[d] $P < 0.05$ versus amoxicillin-clavulanate.
[e] $P < 0.05$ versus high inoculum concentration.
[f] AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam; IMP, imipenem.

TABLE 4 *In vivo* results of treatment with an imipenem dosage adjusted to the MIC obtained with a high inoculum concentration of strain ATCC 25922

Treatment (no. of doses)	Time between doses (h)	Low inoculum: Mean spleen bacterial concn ± SD (log_{10} CFU/g)	Low inoculum: $\%fT_{MIC}$	High inoculum: Mean spleen bacterial concn ± SD (log_{10} CFU/g)	High inoculum: $\%fT_{MIC}$
Control		8.68 ± 0.35		8.02 ± 0.16	
Imipenem (6)	4	ND[d]	ND	3.93 ± 2.17[a,b]	32.5
Imipenem (4)	6	3.46 ± 1.46 [a,c]	41.7	5.6 ± 1.1[a]	21.67

[a] $P < 0.05$ versus control.
[b] $P < 0.05$ versus four doses of imipenem.
[c] $P < 0.05$ versus high inoculum concentration.
[d] ND, not done.

5.6 ± 1.1 to 3.93 ± 2.17 log_{10} CFU/g ($P < 0.05$). These results were similar to those obtained with animals infected with the LI and treated with 120 mg/kg i.m. administered in four doses, 3.93 ± 2.17 versus 3.46 ± 1.46 log_{10} CFU/g (no statistically significant difference) (Table 4).

DISCUSSION

There is increased interest in the potential utility of amoxicillin-clavulanate and piperacillin-tazobactam as alternatives to carbapenems in the treatment of invasive infections due to susceptible ESBL-producing *E. coli* (7). However, clinical data on the efficacy of these compounds in infections with heavy bacterial loads are still scarce. The present work analyzed the inoculum size effect on the *in vivo* efficacies of two beta-lactam–beta-lactamase inhibitor combinations (amoxicillin-clavulanate and piperacillin-tazobactam) and a carbapenem (imipenem) in an experimental murine model of sepsis caused by two *E. coli* strains, one a non-ESBL producer and the other an ESBL producer.

Regarding the groups infected with the non-ESBL-producing ATCC 25922 strain, it is noteworthy that the efficacy of amoxicillin-clavulanate, in terms of the bacterial burden in the spleen, was the same independently of the initial tissue bacterial concentration; on the contrary, an increase in the initial bacterial burden in the spleen led to a failure of piperacillin-tazobactam and imipenem to reduce the spleen bacterial concentration. It is important to note that treatment with imipenem at a dosage that took into account the MIC obtained with the HI of the ATCC 25922 strain to calculate a favorable $\%fT_{MIC}$ improved its efficacy significantly.

Moreover, in the groups infected with the ESBL-producing Ec1062 strain, all three antimicrobials had lower efficacies against an infection with a high tissue bacterial concentration than in that with a lower bacterial concentration, although imipenem and amoxicillin-clavulanate were more efficacious than piperacillin-tazobactam in the high inoculum concentration infection.

Many *in vitro* studies have shown the inoculum effect of piperacillin-tazobactam, amoxicillin-clavulanate, or carbapenems (8, 9, 12, 22), but limited data exist about the *in vivo* role of the inoculum size effect in the treatment of infections due to Gram-negative bacilli and on how optimized dosing may overcome the impact of this phenomenon.

With respect to the activity of piperacillin-tazobactam, there seems to be a good correlation between the previous *in vitro* and the present *in vivo* results with diminished efficacy against the heavier bacterial burden (9). This lack of efficacy was also observed by Vimont et al. in an experimental model of peritonitis caused by *E. coli* producing plasmid-mediated AmpC-type beta-lactamases, where the 50% effective doses (ED_{50}) of piperacillin-tazobactam ranged from 8.53 to 624 mg/kg, underscoring the lack of efficacy of this combination in this mouse model of infection (23). Whether the inoculum effect translates into clinical failure is controversial; even though failure of piperacillin-tazobactam in patients with intra-abdominal infections caused by susceptible isolates seems to be rare, the impact of the inoculum effect might be underestimated since surgery rapidly and drastically reduces the bacterial burden in such infections.

In accordance with our results with imipenem, Mizunaga et al. also observed an increase in the ED_{50} in a murine systemic infection model, showing a reduction in the efficacy of carbapenems (imipenem, panipenem, and meropenem) against a clinical *Pseudomonas aeruginosa* strain associated with a rise in the inoculum concentration from 10^5 to 10^7 CFU (24).

DeRyke et al., in a neutropenic mouse model of thigh infections caused by three clinical ESBL-producing *E. coli* strains, observed approximately 2- to 3.5-log_{10} decreases in thigh bacterial concentrations with meropenem and ertapenem, respectively, using a 10^7-CFU/ml inoculum (25). This increase in the bacterial load, from 10^5 to 10^7 CFU/ml, led to a change in the pharmacodynamic parameter $\%fT_{MIC}$ for the three strains from 85 to 93% to 30 to 45% for meropenem and from 77 to 90% to 20 to 30% for ertapenem. Our results are in agreement with that observation, because we obtained a spleen bacterial count reduction of approximately 2.5 to 4 log_{10} CFU/g with a $\%fT_{MIC}$ of 21 to 32.5%, depending on the imipenem dosage used.

To our knowledge, this is the first time that the *in vivo* inoculum size effect on amoxicillin-clavulanate has been assessed. Our previous *in vitro* studies showed that the bactericidal activity of amoxicillin-clavulanate against ESBL and non-ESBL producing strains is not modified by the presence of a heavy bacterial burden, in contrast to that of piperacillin-tazobactam (9). The present *in vivo* results are in agreement with the *in vitro* data in relation to the non-ESBL-producing ATCC 25922 strain (9), where amoxicillin-clavulanate maintained its efficacy, in terms of bacterial concentrations in the spleen, in the LI and HI groups. However, the presence of the beta-lactamase CTX-M-14 reduced the efficacy of amoxicillin-clavulanate in infections with a high bacterial concentration in tissues, in relation to those with a lower bacterial concentration. With similar C_{max}s and $t_{1/2}$s of amoxicillin (17.9 μg/ml and 0.4 h) and piperacillin (26.19 μg/ml and 0.46 h), this discrepancy could be explained by the shorter $t_{1/2}$ of clavulanate (0.22 h) than of tazobactam (0.41 h), which permits greater hydrolysis of amoxicillin than of piperacillin and consequently a little regrowth of the Ec1062 strain.

In summary, the results of this study support the hypothesis that the inoculum effect might be the basis for the reported clinical failures of piperacillin-tazobactam in spite of its good *in vitro* activity against ESBL-producing *Enterobacteriaceae* when a high bacterial concentration is present (26, 27). In addition, the results suggest that amoxicillin-clavulanate could be an alternative to imipenem treatment of infections caused by ESBL- and non-ESBL-producing *E. coli* strains in patients with therapeutic failure with piperacillin-tazobactam. Clinical studies need to be designed to validate these results in clinical practice.

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