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Bactericidal activity of oral β-lactam antibiotics in plasma and urine versus isogenic *Escherichia coli* strains producing broad- and extended-spectrum β-lactamases

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Abstract

Bacteria harbouring extended-spectrum β-lactamases (ESBLs), derived by mutation from TEM-1, TEM-2 or SHV-1 β-lactamases, have been described world-wide. The in vitro activities of these enzymes against β-lactam antibiotics, including oral cephalosporins, are well recognised. The aim of this investigation was to assess the bactericidal activity of oral β-lactam antibiotics available in Croatia (amoxicillin/clavulanate, cephalexin, cefuroxime, cefadroxil and ceftibuten), in biological fluids against isogenic *Escherichia coli* strains producing broad-spectrum (TEM-1, TEM-2 and SHV-1) and extended-spectrum β-lactamases (SHV-2, SHV-3, SHV-4, SHV-5, SHV-12). Bactericidal activity of oral β-lactams in plasma and urine was tested in time-kill experiments and by determining bactericidal titres at different time intervals post-dose. The killing rate of antibiotics in urine was slower than in plasma, but faster than in Mueller–Hinton broth. High bactericidal titres in urine were only maintained throughout the whole dosing interval by ceftibuten against strains producing broad-, SHV-2 and SHV-3 β-lactamases. The older generation cephalosporins can be considered for the therapy of urinary tract infections caused by *E. coli* harbouring TEM-1, TEM-2 and SHV-1 β-lactamases but a shorter dosing interval is needed. Ceftibuten can be recommended with caution in ESBL producing *E. coli* except those producing SHV-4, SHV-5 and SHV-12 that confer resistance to it. If these enzymes are produced, fluoroquinolones or carbapenems could be considered.

Keywords: β-Lactam antibiotics; Cephalosporins; Extended-spectrum β-lactamases; Resistance

1. Introduction

β-Lactamases continue to be the leading cause of resistance to β-lactam antibiotics among Gram-negative bacteria. In recent years, there has been increased incidence and prevalence of extended-spectrum β-lactamases (ESBLs), causing resistance to oxymino-cephalosporins and aztreonam [1]. The majority of ESBLs are derived from widespread broad-spectrum β-lactamases such as TEM-1, TEM-2 and SHV-1, but new families of ESBLs such as AmpC, CTX-M and OXA enzymes are also recognised (http://www.lahey.org/studies/). The in vitro activities of these enzymes against β-lactam antibiotics including oral cephalosporins, have been studied [2–4]. However, the results of in vitro studies are not always a predictor of therapeutic efficacy of a particular antibiotic. Bactericidal components of serum and other body fluids such as complement and basic polypeptides have additive or synergistic effect with antibiotics and may potentiate their activity [5]. In the assessment of an antimicrobial agent, the bactericidal activity of plasma and other body fluids is a relevant pharmacodynamic parameter. Additionally, there is a gradual decrease of antibiotic concentrations in body fluids during the dosing interval depending on the elimination half-life that is not possible to mimic in standard in vitro testing. The antibiotic concentration may fall below the

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minimum inhibitory concentration (MIC), which enables bacteria to regrow and this may result in therapeutic failure. The aim of this investigation was to assess the bactericidal activity of oral β-lactams in biological fluids against isogenic *Escherichia coli* strains producing broad-spectrum (TEM-1, TEM-2 and SHV-1) and extended-spectrum β-lactamases in ex vivo model. It was hypothesised that ceftibuten would demonstrate stronger activity against β-lactamase producers than older compounds. β-Lactam antibiotics except for carbapenems are not recommended for the therapy of infections caused by ESBL producing organisms because therapeutic failures can occur even when in vitro tests show susceptibility [6]. However, some in vivo experiments on animal models have shown that other β-lactams might be effective in the therapy of such infections [7]. Furthermore, ESBL producing Enterobacteriaceae, which are predominantly hospital pathogens, are isolated from community-acquired infections with increasing frequency [8]. In community-acquired infections, therapy is often prescribed empirically prior to laboratory identification including ESBL production. In this study, serum and urine samples obtained from a single oral dose pharmacokinetic study were examined for their bactericidal activity against isogenic *E. coli* hosts producing broad- and extended-spectrum β-lactamases in ex vivo model. There are no bibliographical data on the bactericidal activity of cephalosporins in plasma and urine against *E. coli* strains with well-defined resistance mechanisms.

2. Materials and methods

2.1. Collection of samples

Six healthy volunteers (females, age range 40–55 years) received a single oral dose of amoxicillin/clavulanate—875/125 mg, cephalexin—500 mg, cefadroxil—500 mg, cefuroxime axetil—500 mg and ceftibuten—400 mg, respectively. Cefuroxime was administered as prodrug cefuroxime axetil (1-acetoxyethyl ester of cefuroxime). The wash-out period between receiving two different antibiotic regimens was at least 4 weeks. Strenuous physical activity, smoking and alcohol intake were prohibited 24 h previous to and until 24 h after drug administration. The test drug was taken after fasting for 12 h and the first meal was allowed 2 h after drug administration. Blood samples (10 ml) were collected immediately before and at 2, 4, 6, 8, 10, 12 h (for all antibiotics) and 24 h (for ceftibuten) after dosing, depending on the dosing interval of the particular antibiotic. The samples were allowed to clot at room temperature for 30 min and were subsequently centrifuged at $1300 \times g$ for 10 min. The serum was reserved for use in the experiments. Urine was collected before dosing and at 0–2, 2–4, 4–6, 6–8, 8–10, 10–12 h (for all antibiotics) and 12–24 h (only for ceftibuten) after dosing, depending on the dosing interval of the particular antibiotic. Urine samples were sterilised by filtration. Dosing intervals of the antibiotics used in this study followed the manufacturer's recommendations: amoxicillin/clavulanate, cephalexin, cefuroxime and cefadroxil—12 h and ceftibuten—24 h. The volunteers participated after written informed consent had been obtained, after physical examination and when haematological, biochemical, ECG and EEG tests were found to be within normal limits. Exclusion criteria were the regular use of medication, alcohol abuse, symptoms of significant illness within 3 months before the study period, history of gastrointestinal, liver or kidney disease potentially interfering with absorption, metabolism or excretion of drugs, history of central nervous system disorders, allergy or hypersensitivity to β-lactam antibiotics, participation in a clinical trial within 3 months before the study period and pregnancy. The study was approved by the Ethical Committee of the Medical School of Zagreb University. No serious adverse effects were observed. One volunteer reported abdominal cramps and diarrhoea after amoxicillin/clavulanate and cefuroxime and one after cephalexin administration. Vaginal candidosis was detected in one volunteer following amoxicillin/clavulanate administration.

Antibiotics for oral administration in volunteers were provided by the following manufacturers: amoxicillin/clavulanate (Klavocin), cephalexin (Ceporex) and cefuroxime (Novocef)—Pliva, Zagreb, Croatia; cefadroxil (Duracef)—Bristol Myers Squibb; ceftibuten (Cedax)—Schering Plough Corporation.

2.2. Bacteria

The following bacterial strains were used: plasmid-mediated broad-spectrum β-lactamase producers: *E. coli* A 15 R^+ TEM-1, *E. coli* A 15 R^+ TEM-2 and *E. coli* A 15 R^+ SHV-1; plasmid-mediated ESBL producers: *E. coli* A 15 R^+ SHV-2, *E. coli* A 15 R^+ SHV-3, *E. coli* A 15 R^+ SHV-4, *E. coli* A 15 R^+ SHV-5 and *E. coli* A 15 R^+ SHV-12. The strains were obtained by transconjugation with *Klebsiella pneumoniae* strains harbouring the particular β-lactamases. *E. coli* strain A 15 R^-, free of plasmids and resistant to rifampicin, was used as recipient. β-Lactamases were identified by sequencing of bla_{TEM} and bla_{SHV} genes. The recipient strain and transconjugants producing TEM-1, TEM-2, SHV-3 and SHV-4 β-lactamases were kindly provided by Professor Adolf Bauernfeind (Max von Pettenkofer Institut, Munich, Germany).

2.3. Determination of minimum inhibitory concentrations and minimum bactericidal concentrations (MIC and MBC)

MICs of amoxicillin/clavulanate, cefprozil, cephalexin, cefadroxil, cefuroxime, ceftibuten and cefetamet were determined by the broth microdilution method according to NCCLS guidelines [6]. The standard inoculum of 5×10^5 CFU/ml (colony forming units) was used. Clavulanic acid was added to amoxicillin in a fixed concentra-

tion of 4 mg/l. The MIC was defined as the lowest concentration of antibiotic inhibiting visible growth of bacteria. Ten microlitres of sample from all wells with no visible growth, was subcultured onto Mueller–Hinton (MH) agar for determination of the MBC. The MBC was defined as the lowest antibiotic concentration which demonstrated 99.9% reduction in CFU/ml when compared with the control well. Experiments were run in duplicate. Antibiotic powders for MIC determination were obtained from the following manufacturers: amoxicillin, clavulanate, cephalexin, cefuroxime, Pliva, Zagreb, Croatia; cefadroxil, cefprozil, Bristol Myers Squibb, Zagreb, Croatia; cefetamet Hoffman La Roche AG, Grenzach-Wyhlen, Germany. Ceftibuten powder was kindly provided by Dr. Ellen Stobbering, University Hospital Maastricht, Maastricht, The Netherlands.

2.4. Time-kill experiments in plasma and urine

Time-kill experiments were carried out by exposing test cultures of the strains to plasma and urine samples containing amoxicillin/clavulanate, cephalexin, cefadroxil, cefuroxime and ceftibuten. Blood and urine samples containing peak antibiotic concentrations according to the titres determined were used in the experiments. An overnight culture of the test strain was inoculated in plasma or urine to yield an inoculum of 10^5–10^6 CFU/ml. For determination of kill kinetics in plasma, the samples were diluted with fresh plasma without antibiotics, prewarmed to 37 °C, in the ratio 1:1 in the time intervals corresponding to the elimination half-life of a particular antibiotic in plasma, to mimic the normal pharmacokinetics of the antibiotics. Their elimination half-lives are as follows: amoxicillin, 1.3 h; clavulanic acid, 1.03 h [9,10]; cephalexin, 0.9 h [11,12]; cefadroxil, 1.5 h [11,12]; cefuroxime, 1.2 h [13,14]; ceftibuten, 2.4 h [14]. Antibiotic concentration in the samples obtained from the volunteers was not determined. For determination of kill kinetics in urine, urine samples containing peak antibiotic concentration (highest titres) were diluted with urine not containing antibiotics in the time intervals corresponding to the elimination half-life in urine, which was approximated from the changes in titres. In order to estimate $t_{1/2}$ in urine, titres were plotted against time and the time necessary for the titre to decrease for one dilution was determined. This corresponded to the time necessary for the antibiotic concentration to decrease approximately in the same manner. A control sample contained the strain inoculated in plasma and urine without any antibiotics. Bacterial counts at times 0, 2, 4, 6, 8, 10 and 24 h were determined by viable counting. The samples were diluted by factor of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} in saline, and 100 μl of undiluted cultures and each dilution were seeded on MH agar for CFU determination. The samples were spread on the whole agar surface to avoid any antibiotic carryover. Experiments were run in duplicate with urine samples obtained from two different volunteers randomly selected out of six. For cefuroxime and ceftibuten, experiments were repeated with another two urine samples obtained from volunteers showing the highest and the lowest urinary bactericidal titres for the respective antibiotics. Results were charted by plotting $\log_{10}$ CFU/ml against time.

2.5. Time-kill experiments in Mueller–Hinton broth

Time-kill experiments in MH broth were carried out by exposing the test cultures to antibiotics at concentrations corresponding to the peak values in plasma. The antibiotic concentrations applied were as follows: amoxicillin—10.2 mg/l, clavulanic acid—3.3 mg/l [9], cephalexin—5.8 mg/l [11], cefadroxil—9.5 mg/l [11], cefuroxime—6.3 mg/l [13] and ceftibuten—15 mg/l [14]. The samples were then diluted with broth not containing antibiotics, at a ratio of 1:1 in the time intervals corresponding to the elimination half-life in plasma of a particular antibiotic, and the bacterial numbers were determined by viable counting. Time-kill experiments were carried out twice for each antibiotic.

2.6. Determination of plasma bactericidal titres

Plasma bactericidal titres of oral β-lactams were determined by the method described previously [15]. Plasma samples containing antibiotics were double diluted in MH broth from 1:2 to 1:2048 in microtitre trays. The dilutions were made in broth to eliminate the effect of inherent bactericidal effect of human plasma. Each well contained 100 μl of the plasma sample dilution. Organisms were prepared by diluting an overnight culture of the test organism to yield an inoculum of 5×10^5 CFU/ml. One hundred microlitres of bacterial suspension was added to the wells of the microtitre trays. Viable counts were performed to check the final inoculum size. Plates were incubated at 35–37 °C for 18–20 h. Bactericidal levels were determined by subculturing 10 μl of sample from all wells with no visible growth onto MH agar. Plates were incubated at 37 °C for 24 h before examination. The number of colonies was counted and the plasma dilution which gave 99.9% killing was taken as the bactericidal dilution. A titre of at least 1:8 was taken as significant, as described previously [15] because this ensures that there is measurable bactericidal activity in a patient's serum for at least a further three half-lives.

2.7. Determination of urinary bactericidal titres

Urine samples were handled in a similar way as plasma. However, dilutions of urine were made in filtered human urine without antibiotics [15]. If the test strain grew from the first well, the titre was considered to be 0 and if it grew from the second well and 99.9% killing occurred in the first well, it was read as 2. The experiments were performed on six different urine samples obtained from six volunteers and the median value was calculated.

Table 1
Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of various oral β-lactam antibiotics for *E. coli* strains producing different types of broad- and extended-spectrum β-lactamases

	Amoxicillin/clavulanate		Cephalexin		Cefadroxil		Cefprozil		Cefuroxime		Ceftibuten		Cefetamet	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
TEM-1	64/4	64/4	8	16	8	8	8	8	8	8	0.12	0.12	0.12	0.25
TEM-2	128/4	128/4	8	8	8	8	8	16	8	8	0.12	0.25	0.25	0.25
SHV-1	32/4	32/4	8	8	8	16	16	16	16	16	0.5	0.5	0.5	0.5
SHV-2	8/4	8/4	16	16	8	16	8	8	16	16	0.5	0.5	0.5	0.5
SHV-3	16/4	16/4	256	256	64	64	32	32	32	32	1	2	1	1
SHV-4	32/4	64/4	64	64	64	64	64	64	32	32	8	8	2	2
SHV-5	32/4	64/4	128	256	128	128	64	128	64	64	8	8	2	2
SHV-12	64/4	64/4	64	128	64	64	128	128	16	16	4	4	4	4
E. coli A15 R^-	1/4	2/4	2	2	4	4	4	4	2	2	0.12	0.12	0.25	0.25

3. Results

3.1. Minimum inhibitory concentrations and minimum bactericidal concentrations

The MICs and MBCs of oral β-lactams for the different *E. coli* strains are shown in Table 1.

3.2. Time-kill curves in plasma

After exposure to serum containing antibiotics, a rapid bactericidal effect was detected with most strains with no surviving bacteria after 2 h. The killing rate was slower with cephalexin and cefadroxil. The time taken to kill all organisms producing SHV-3, SHV-4, SHV-5 and SHV-12 was 4 h.

3.3. Time-kill curves in urine

The bactericidal effect of oral β-lactams in urine was slower than in plasma. After exposure to amoxicillin/clavulanate in urine, TEM-1 and TEM-2 producers were completely killed after 4 h, SHV-1 producer after 6 h, SHV-2, SHV-4 and SHV-5 producers after 10 h, and SHV-3, within 24 h (Fig. 1).

Cephalexin showed weaker bactericidal activity compared with amoxicillin/clavulanate. The cultures containing TEM-1, TEM-2 and SHV-1 producers were sterilised within 24 h. For most ESBL-positive strains, a gradual decrease in viable counts was found after 2–8 h. SHV-2 and SHV-3 producers grew only a few colonies after 24 h while SHV-4, SHV-5 and SHV-12 producers regrew after overnight incubation. Cefadroxil was less efficient than cefuroxime against ESBL producers. With SHV-3, SHV-4, SHV-5 and SHV-12 producers, surviving bacteria were found at 24 h, whereas the SHV-1 producer was killed after 6 h, TEM-1 after 8 h, and TEM-2 and SHV-2 after 10 h. Cefuroxime exhibited strong and sustained bactericidal activity against strains producing broad-spectrum β-lactamases. In cultures containing TEM-1, TEM-2 and SHV-2, no surviving bacteria were detectable after 6 h, whereas cultures containing SHV-4, SHV-5 and SHV-12 producers regrew after 24 h.

Ceftibuten exhibited rapid bactericidal effect against strains possessing broad-spectrum β-lactamases, whereas strains harbouring ESBLs were more resistant to its antibacterial activity. With the SHV-2 producer, no growth was observed after 8 h (Fig. 1).

3.4. Time-kill curves in Mueller–Hinton broth

All antibiotics used in this study were significantly less bactericidal in artificial medium at the same concentrations present in plasma (Fig. 2). In most cases, there was only a moderate decrease in viable counts followed by regrowth after 24 h. Ceftibuten exerted the strongest antibacterial activity followed by cefuroxime. Amoxicillin/clavulanate was the least bactericidal in MH medium. Strains harbouring broad-spectrum β-lactamases were more susceptible to the killing effect of all antibiotics used in the study, than ESBL producing strains.

3.5. Growth kinetics of test strains in plasma, urine and Mueller–Hinton broth without antibiotics

When test strains were exposed to plasma without antibiotics, a strong killing effect was observed after 4–6 h due their serum sensitivity followed by regrowth after 24 h (Figs. 1 and 2). Growth kinetics of test strains in urine without antibiotics resembled those in MH broth without antibiotics.

3.6. Bactericidal titres in plasma

With amoxicillin/clavulanate high titres (≥1:8) were observed only in samples taken after 2 h against TEM-1, TEM-2, SHV-1 and SHV-2 β-lactamases as shown in Table 2 (based on median value). Bactericidal titres for cephalexin were detected for only 2 h and only for TEM-1 and SHV-1 producers. No plasma bactericidal activity was found against SHV-3, SHV-4, SHV-5 and SHV-12 producers with cephalexin. For cefadroxil significant titres were present after 2 h against SHV-1 and SHV-2, and up to 4 h for TEM-1 and TEM-2 producers (Table 2). Cefuroxime exhibited bactericidal titres

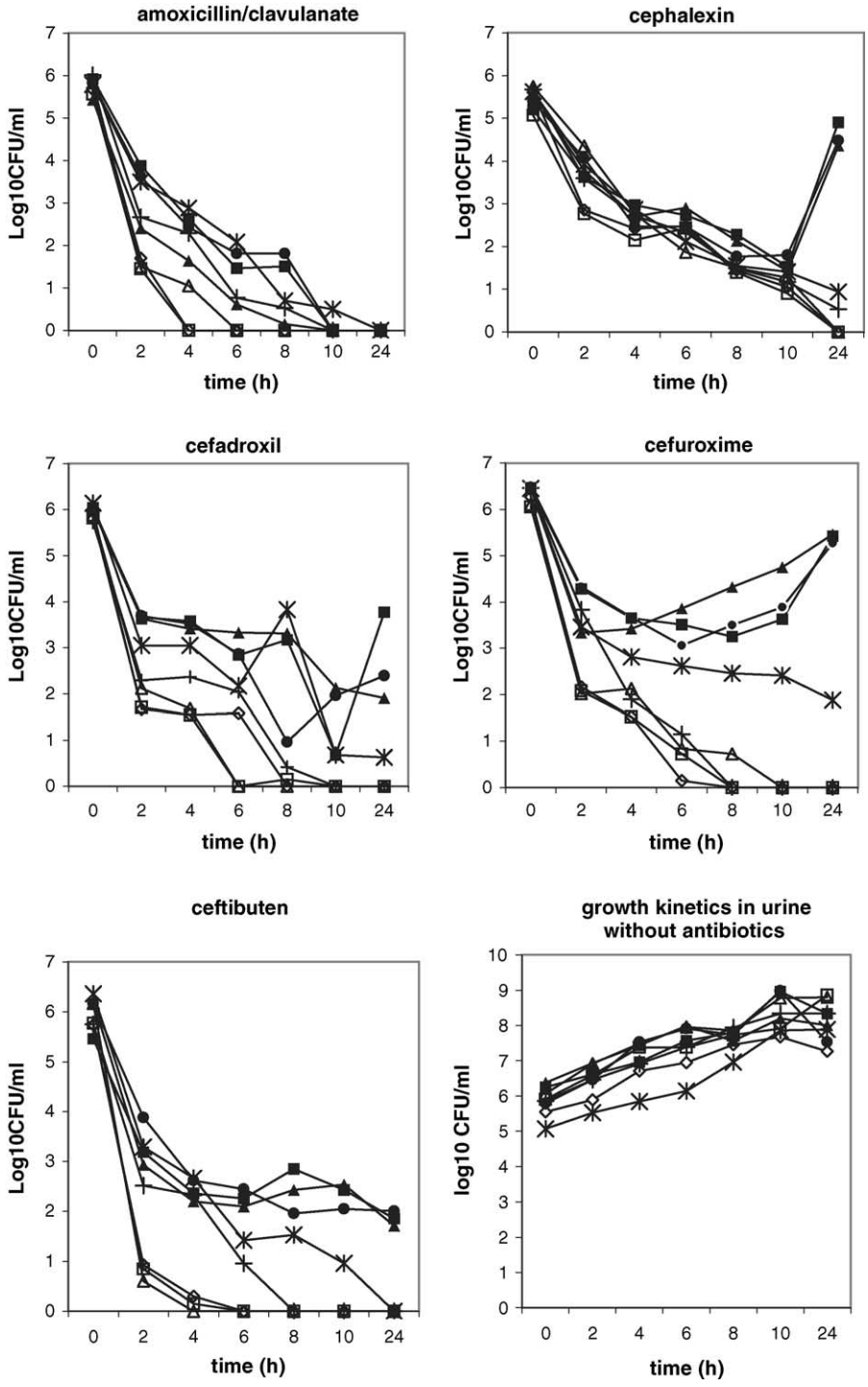

Fig. 1. Time-kill curves of oral β-lactam antibiotics in urine: (◊) TBM-1, (□) TBM-2, (△) SHV-1, (+) SHV-2, (*) SHV-3, (♦) SHV-4, (■) SHV-5, (▲) SHV-12.

for up to 4 h against TEM-1, TEM-2, SHV-1 and SHV-2 β-lactamases producers, and up to 4 h against SHV-4 producer. Ceftibuten maintained bactericidal titres against TEM-1, TEM-2, SHV-1 and SHV-2 producers for 10 h and against a SHV-3 producer in first 6 h. For other ESBL producers, the titres were below 1:8 (median) from the beginning of the dosing interval.

3.7. Bactericidal titres in urine

Unlike plasma, none of the urine samples without antibiotics (taken prior to antibiotic administration) showed detectable antibacterial activity against the tested strains. For amoxicillin/clavulanate titres of ≥1:8 (based on median) were seen with the SHV-2 producer for 10 h, with SHV-3

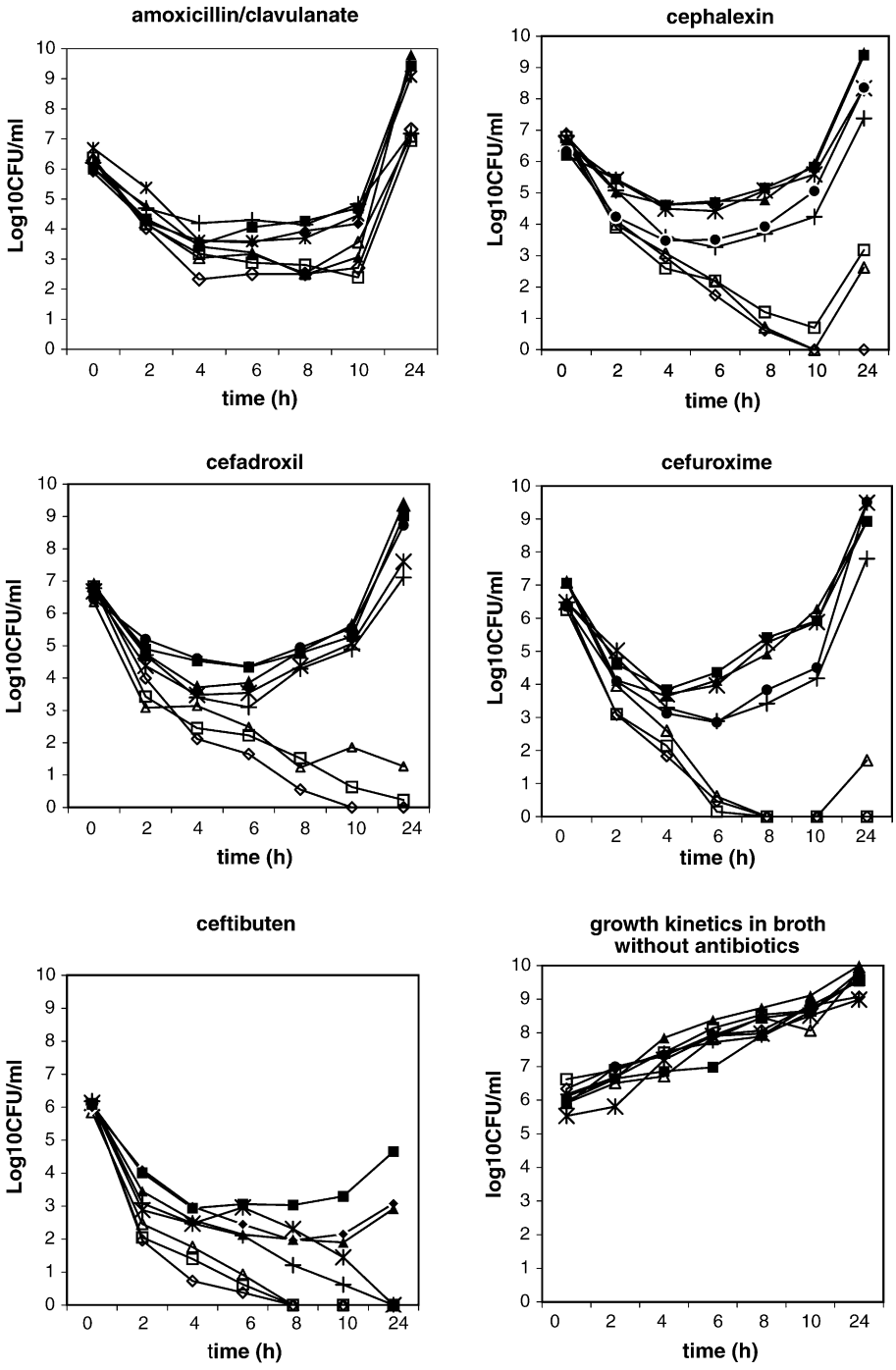

Fig. 2. Time-kill curves of oral antibiotics in Mueller–Hinton broth. (◊) TBM-1, (□) TBM-2, (△) SHV-1, (+) SHV-2, (*) SHV-3, (♦) SHV-4, (■) SHV-5, (▲) SHV-12.

and SHV-1 over 6 h, and with TEM-1 and SHV-12 over 4 h (Table 3). SHV-5 and TEM-1 producers had titres below 1:8 from the beginning of the dosing interval. For cephalexin titres of ≥1:8 were maintained for 10 h only against SHV-2 producer, for 8 h against TEM-1, TEM-2 and SHV-1 producers, for the first 4 h for SHV-4, SHV-5 and SHV-12 producers, and for 2 h for SHV-3 producer (Table 3). For cefadroxil, a titre of at least 1:8 was exceeded for 8 h by TEM-1 and SHV-2 producers, for 6 h by TEM-2, SHV-1 and SHV-4 producers, and for 4 h by SHV-3 and SHV-12 producers. The median titre for SHV-5 was below 1:8 throughout the whole dosing interval. With cefuroxime, titres of ≥1:8 were present almost during the whole dosing interval (10 h) for TEM-1 and SHV-2 producers. With TEM-2, SHV-1 and SHV-3 producers, the bactericidal titres were maintained for 8 h, for SHV-4 during first 6 h and for SHV-5 for up to 4 h. With SHV-12 producer, the titre was below the bactericidal level from the beginning.

Table 2
Median reciprocal plasma bactericidal titres of oral β-lactam antibiotics against *E. coli* strains producing various broad- and extended-spectrum β-lactamases

Time (h)	Titre							
	TEM-1	TEM-2	SHV-1	SHV-2	SHV-3	SHV-4	SHV-5	SHV-12
Amoxicillin/clavulanate (12 h)[a]								
0	0	0	0	0	0	0	0	0
2	12	8	8	12	3	4	2	2
4	3	4	2	6	2	0	2	2
6	2	2	0	4	0	0	0	0
8	0	0	0	0	0	0	0	0
Cephalexin (12 h)[a]								
0	0	0	0	0	0	0	0	0
2	8	6	8	4	0	0	0	0
4	4	4	3	2	0	0	0	0
6	0	0	2	0	0	0	0	0
Cefadroxil (12 h)[a]								
0	0	0	0	0	0	0	0	0
2	16	8	8	12	4	3	2	2
4	8	8	4	4	2	2	0	0
6	0	2	2	0	0	0	0	0
Cefuroxime (12 h)[a]								
0	0	0	0	0	0	0	0	0
2	12	24	12	16	6	8	4	4
4	8	12	8	12	3	2	2	1
6	3	3	2	3	2	0	0	0
8	0	0	0	0	0	0	0	0
Ceftibuten (24 h)[a]								
0	0	0	0	0	0	0	0	0
2	192	128	96	64	32	4	4	3
4	128	96	48	64	16	4	3	2
6	96	64	32	16	16	2	2	2
8	48	32	16	16	6	2	0	0
10	32	24	16	8	4	2	0	0
24	3	2	2	1	0	0	0	0

[a] Dosing interval is given in parentheses.

Ceftibuten consistently showed very high titres against broad-spectrum lactamases (TEM-1, TEM-2 and SHV-1) and SHV-2 producers during the whole dosing interval, whereas against the SHV-3 producerm high titres were shown in the first 10 h, against SHV-5 and SHV-12, within 8 h, and the SHV-4 producer for up to 6 h (Table 3).

4. Discussion

In order to assess the bactericidal activities of drugs during various points in time over a prolonged incubation period, time-kill studies were performed. The strains used in this study were strongly susceptible to the inherent bactericidal activity of plasma. The viable counts were significantly reduced in the presence of human plasma during the first 8 h, but there was regrowth after 24 h with all strains. However, when antibiotics were combined with plasma, the cultures were sterile after 2–4 h and there was no regrowth after overnight incubation. The results of the time-kill curves in plasma may have been different if clinical isolates which are usually not serum-sensitive were used instead of the laboratory *E. coli* strains which are not typical pathogens. However, our aim was to study the effect of antibiotics in the biological fluids against strains harbouring particular types of β-lactamases, without interference of other resistance mechanisms.

The bactericidal titres were performed to determine at what dilution plasma and urine samples exert bactericidal activity. Since the strains were serum-sensitive, the plasma dilutions were made in broth to eliminate the effect of the inherent bactericidal effect of plasma and to establish the effect of antibiotics alone on bacteria. A bactericidal titre of at least 1:8 in plasma was taken as significant because it was shown to predict a successful therapeutic outcome [15]. Titres in plasma were low from the beginning of the dosing interval for all antibiotics except for ceftibuten, as expected, since the peak plasma concentrations for most antibiotics [9–14] do not exceed the MICs of the tested strains. However, the titres were higher than expected from the MIC values because of the additive bactericidal effect of human serum in the initial dilutions. The bactericidal titres in urine were higher because all cephalosporins are primarily excreted through kidney by glomerular filtration and tubular secretion and therefore there is high urinary recovery. The bactericidal

Table 3
Median reciprocal urinary bactericidal titres of oral β-lactam antibiotics against *E. coli* strains producing broad- and extended-spectrum β-lactamases

Time (h)	Titre							
	TEM-1	TEM-2	SHV-1	SHV-2	SHV-3	SHV-4	SHV-5	SHV-12
Amoxicillin/clavulanate (12 h)[a]								
0–2	4	4	24	192	24	12	3	8
2–4	10	5	6	192	40	24	2	16
4–6	5	3	10	96	20	4	2	3
6–8	5	1	1	64	4	3	1	2
8–10	1	0	0	24	1	2	0	2
10–12	0	0	0	2	0	0	0	0
Cephalexin (12 h)[a]								
0–2	192	96	96	320	20	20	16	12
2–4	96	128	128	96	5	10	24	16
4–6	48	40	24	12	2	6	4	6
6–8	12	12	18	9	0	0	2	3
8–10	3	6	5	9	0	0	1	0
10–12	0	0	0	0	0	0	0	0
Cefadroxil (12 h)[a]								
0–2	16	20	24	24	12	6	3	12
2–4	128	48	32	96	8	6	4	8
4–6	96	48	32	80	6	12	4	6
6–8	8	6	4	12	1	0	0	1
8–10	4	3	2	1	0	0	0	0
10–12	0	0	0	0	0	0	0	0
Cefuroxime (12 h)[a]								
0–2	48	96	154	48	24	8	8	6
2–4	80	80	72	24	32	16	8	6
4–6	72	128	72	64	48	8	4	4
6–8	20	24	32	32	16	3	2	0
8–10	8	4	3	8	6	1	0	0
Ceftibuten (24 h)[a]								
0–2	768	768	1024	256	48	24	32	64
2–4	768	768	768	320	24	24	40	48
4–6	512	1024	640	384	12	8	12	32
6–8	96	320	512	80	16	4	8	12
8–10	144	128	256	128	8	2	2	4
10–12	48	48	96	32	4	0	0	0
12–24	24	24	24	12	1	0	0	0

[a] Dosing interval is given in parenthesis.

titres were only maintained throughout the whole dosing interval with ceftibuten for TEM-1, TEM-2, SHV-1 and SHV-2 producers. Urinary bactericidal titres agreed with the results of in vitro testing. Strains with lower MICs had higher titres. For amoxicillin/clavulanate, there was a discrepancy between strong and rapid bactericidal activity in the time-kill curves and low titres in the urine. In spite of the low titres, amoxicillin/clavulanate markedly reduced the viable counts in kill-kinetic studies. Amoxicillin/clavulanate against SHV-2 producing strain showed unexpectedly high titres in plasma and urine compared with other strains although the MIC and MBC for that antibiotic were not significantly lower than those of other test strains. High titres can be explained by low level of enzyme production by the particular strain. Unlike amoxicillin/clavulanate, ceftibuten showed lower activity in kill-kinetic experiment compared with high titres in urine. These discrepancies can be explained by the fact that kill-kinetic studies were performed on only two urine samples (obtained from two of six volunteers who were randomly selected) because such experiments are laborious and time-consuming, whereas titres were determined for all six different urine samples. There was a high range of urinary antibiotic titres probably reflecting variability of urinary antibiotic concentrations due individual pharmacokinetic differences between volunteers. Since humans have very variable urinary antibiotic concentrations, results obtained on two samples do not necessarily represent average time-killing effect of particular antibiotic.

In spite of the low titres in plasma, most of the compounds tested in this study have been shown to be efficient in the treatment of pyelonephritis and other systemic infections. It has been recommended that antibiotics are given in doses to achieve peak serum concentrations that exceed the MIC for the pathogen by a factor of 4–10 [16] which was not the case in this study. Clearly, there are other important issues such as intrinsic, pharmacodynamic and pharmacokinetic factors

as well as antibacterial activity, interference with the host factors and their interactions with the microorganism which my explain some therapeutic results [5,17].

According to the results of our study, older cephalosporins could be efficient for the therapy of infections caused by *E. coli* producing broad-spectrum β-lactamases such as TEM-1, TEM-2 or SHV-1. Cefuroxime, cephalexin and cefadroxil showed good activity in the urine against strains possessing TEM-1, TEM-2 and SHV-1 β-lactamases, and some ESBLs (SHV-2), but not throughout the whole dosing period. We can conclude that older generation cephalosporins may be considered for the therapy of urinary tract infections caused by *E. coli* harbouring only broad-spectrum and SHV-2 β-lactamases but with shorter dosing interval being used. A twice-daily dosing regimen would not be appropriate. As reported by other authors, the very high concentrations achieved in urine, decrease quickly due to their short $t_{1/2}$ in urine [18]. Ceftibuten could be recommended with caution for an ESBL-producing isolate, unless SHV-4, SHV-5 and SHV-12 are produced, which confer resistance to it. If these enzymes are produced, fluoroquinolones or carbapenems could be administered. However, it would be important to limit the administration of those antibiotics in order to preserve their activity. The detection of ESBLs in most laboratories is based only on the double disc synergy test and without characterising the β-lactamases; hence, it is not possible to identify which enzyme is produced by the isolate. Unfortunately, other third generation oral cephalosporins such as cefixime, cefpodoxime and cefetamet are not yet available in Croatia to test their activity against ESBL producers in biological fluids. The MICs of fluoroquinolones were low for all tested strains (data not shown) and therefore could be considered as therapeutic options. Their titres in urine, however, were not determined in this study, but results in the literature report excellent activity in urine against Enterobacteriaceae [19,20]. β-Lactams are still very often prescribed for empirical treatment of uncomplicated urinary tract infections in Croatia (personal communication) although they are not recommended as first-line therapy for urinary tract infections [21]. The main advantage of β-lactams over fluoroquinolones is that they cause fewer adverse effects and they may be used in pregnancy and childhood when quinolones should be avoided.

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