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Urinary Bactericidal Activity of Oral Antibiotics against Common Urinary Tract Pathogens in an ex vivo Model

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Key Words

Bactericidal activity, urinary · β-Lactam antibiotics · Fluoroquinolones · Linezolid

Abstract

Background: In this investigation, the urine samples obtained in a single oral-dose pharmacokinetic study were examined for their bactericidal activity against a range of relevant urinary tract pathogens. ***Methods:*** Six healthy volunteers received a single oral dose of ten oral antibiotics available in Croatia. Urine samples were taken every 2 h during the whole dosing interval of the particular antibiotic. The urinary bactericidal activity was tested by determination of urinary bactericidal titers. ***Results:*** All antibiotics showed a significant urinary bactericidal activity against non-extended spectrum β-lactamase *Escherichia coli* and *Proteus mirabilis*. Fluoroquinolones displayed high and persisting levels of urinary bactericidal activity against all gram-negative bacteria and *Staphylococcus saprophyticus*. ***Conclusions:*** Average urinary bactericidal activity can be predicted from in vitro susceptibility testing, but we expect that there will be patients with a low level of urinary bactericidal activity.

Introduction

In the assessment of an antibiotic, the bactericidal activity of plasma, urine and other body fluids is a relevant pharmacodynamic parameter, being an integration of pharmacokinetic properties with in vitro activity [1, 2]. In routine bacteriological laboratories the antibacterial activity of antibiotics is determined by in vitro testing, usually by the disk diffusion test. However, in vitro antibiotic susceptibility tests cannot reflect the situation in vivo. In vitro the bacteria are exposed to fixed concentrations of antibiotics whereas in vivo there is a more gradual decrease in antibiotic levels depending on the elimination half-life of the antibiotic. In this investigation, the urine samples obtained in a single oral-dose pharmacokinetic study were examined for their bactericidal activity against a range of relevant urinary tract pathogens. In vitro susceptibilities of urinary tract pathogens to oral antibiotics have been extensively studied, but there are no published reports on ex vivo urinary bactericidal activity of most oral antibiotics except fluoroquinolones [3] and linezolid [4].

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Methods

Volunteers

Ten oral antibiotics available on the Croatian market were tested: amoxycillin, amoxycillin/clavulanate (co-amoxiclav), cephalexin, cefuroxime, cefadroxil, ceftibuten, norfloxacin, ciprofloxacin, sulfamethoxazole/trimethoprim (co-trimoxazole) and linezolid. Six healthy volunteers (females, age range 40–60 years) received a single oral dose of amoxycillin/clavulanate (Klavocin) 875/125 mg, cephalexin (Ceporex) 500 mg, cefuroxime (Novocef) 500 mg, cefadroxil (Duracef) 500 mg, ceftibuten (Cedax) 400 mg, norfloxacin (Nolicin) 400 mg, ciprofloxacin (Cipromed) 500 mg, co-trimoxazole (sulphamethoxazole/trimethoprim) (Sinersul) 400/80 mg and linezolid (Zyvoxid) 600 mg, respectively. The dosing of each drug was recommended by the manufacturer.

Bacteria

Experiments were performed on bacteria isolated from urinary tract infections in 2002–2003 at the Zagreb Clinical Hospital Center: *Escherichia coli* 2080/79 non-extended spectrum β-lactamase (non-ESBL), *E. coli* 4199/198 ESBL (TEM type), *Klebsiella pneumoniae* 287/286 non-ESBL, *K. pneumoniae* 1951 ESBL (SHV-5), *Proteus mirabilis* 4335/334, *Serratia marcescens* 4920/9199, *Enterobacter cloacae* 1211, *Acinetobacter baumannii* 4473/472, *Pseudomonas aeruginosa* 3579/578, *Enterococcus faecalis* 2252/251, *Enterococcus faecium* 162/161 and *Staphylococcus saprophyticus* 582. The isolates originated from the hospitalized patients with diagnosed urinary tract infection. *P. aeruginosa*, *A. baumanii* and ESBL producers were isolated from nosocomial infections. Reference ATCC strains of the respective species were used as the quality control strains.

Susceptibility Testing

Disk diffusion and broth microdilution tests were performed according to the NCCLS guidelines [5, 6].

Determination of Urinary Bactericidal Titers

Urinary bactericidal titers (UBTs) of oral antibiotics were determined by the method described previously [7]. Urine samples containing antibiotics were double diluted in urine taken from the same volunteer before antibiotic administration, from 1:2 to 1:2,048 in 96-well microtiter trays. Plates were incubated at 37°C for 24 h before examination. The number of colonies was counted, and the urine dilution which demonstrated 99.9% killing was taken as the bactericidal dilution. A titer of ≥1:8 was taken as clinically relevant since it has been shown to predict a successful therapeutic outcome for the fluoroquinolones [7]. There is no recommendation in the references for the β-lactam antibiotics which are time-dependent antibiotics in contrast to the fluoroquinolones which are concentration dependent.

Statistical Analysis

UBTs obtained for different time-dependent antibiotics (β-lactams) were compared (for example, ceftibuten vs. cephalexin), as well as those obtained for concentration-dependent antibiotics (fluoroquinolones), which were also compared (ciprofloxacin vs. norfloxacin). UBTs of various antibiotics for particular bacterial species were compared using the paired t test. For computing reasons the UBTs had been previously transformed into ordinal data using a scale from 1 (UBT = 0) to 12 (UBT ≥2,048).

Results

In vitro Susceptibility Testing

E. coli non-ESBL was susceptible to all antibiotics tested, and *K. pneumoniae* non-ESBL to all except amoxycillin. ESBL-positive strains were resistant to all β-lactams except ceftibuten. *P. mirabilis* was resistant to co-trimoxazole. *A. baumanii* was resistant to all antibiotics and *P. aeruginosa* to all apart from fluoroquinolones. *E. cloacae* and *S. marcescens* were susceptible to fluoroquinolones and ceftibuten. Enterococci were resistant to all cephalosporins as expected and co-trimoxazole. *E. faecalis* was susceptible to amoxycillin, co-amoxiclav, ciprofloxacin and to co-trimoxazole in contrast to *E. faecium*, but both were susceptible to linezolid.

Determination of UBTs

UBTs are shown in figures 1 and 2. Significant differences in UBTs between amoxycillin and co-amoxiclav were found for ESBL-producing *E. coli* and *K. pneumoniae* during the whole dosing interval. UBTs for ceftibuten were significantly higher than for the older cephalosporins for both ESBL-positive and ESBL-negative *E. coli* and *K. pneumoniae* in the later time intervals (after 6 h). Ceftibuten showed markedly higher UBTs for *S. marcescens* and *E. cloacae* as well. *P. aeruginosa* displayed significantly higher UBTs for ciprofloxacin in comparison to norfloxacin.

Discussion

According to the results of ex vivo experiments, amoxycillin could be recommended only for the therapy of infections caused by non-ESBL *E. coli*, *P. mirabilis* and *E. faecalis* strains. Amoxycillin combined with clavulanate showed a broader spectrum and could be considered as an option for the therapy of infections caused by ESBL-negative *E. coli*, *K. pneumoniae*, *P. mirabilis*, *S. saprophyticus* and *E. faecalis*. Older cephalosporins had high titers against non-ESBL *E. coli*, *K. pneumoniae*, *P. mirabilis* and *S. saprohyticus* strains in the first 4–6 h as expected since they achieve high concentrations in urine. Their main drawback is short $t_{1/2}$ in urine resulting in a rapid decrease of UBTs. Ceftibuten as third generation cephalosporin was the only β-lactam displaying high and persistent activity against ESBL-producing *E. coli* and *K. pneumoniae* in urine during the whole dosing interval (24 h) due to its long elimination half-life in urine. Fluoroquinolones are the antibiotics of choice for the treat-

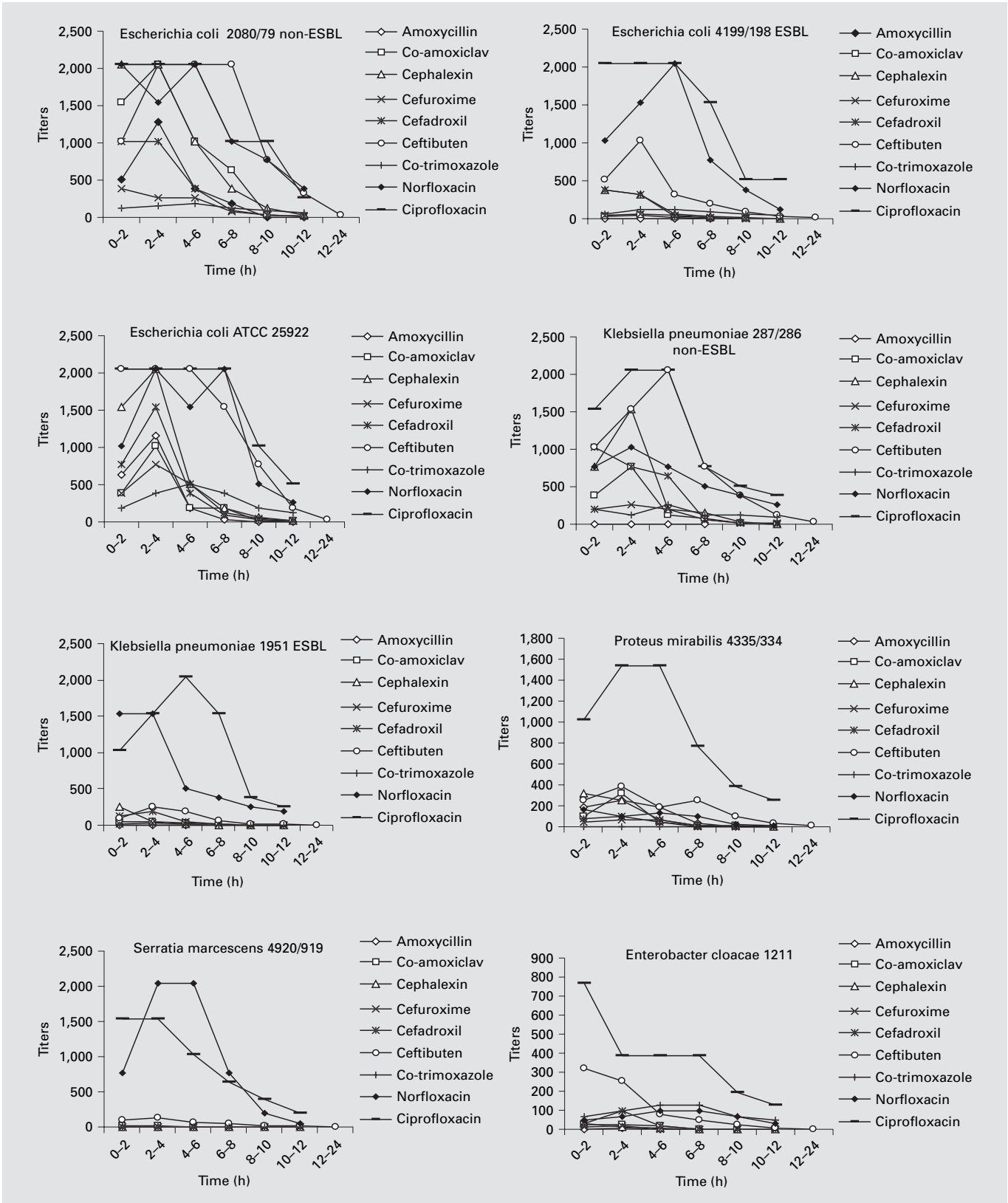

Fig. 1. Median UBTs of various antibiotics against Enterobacteriaceae.

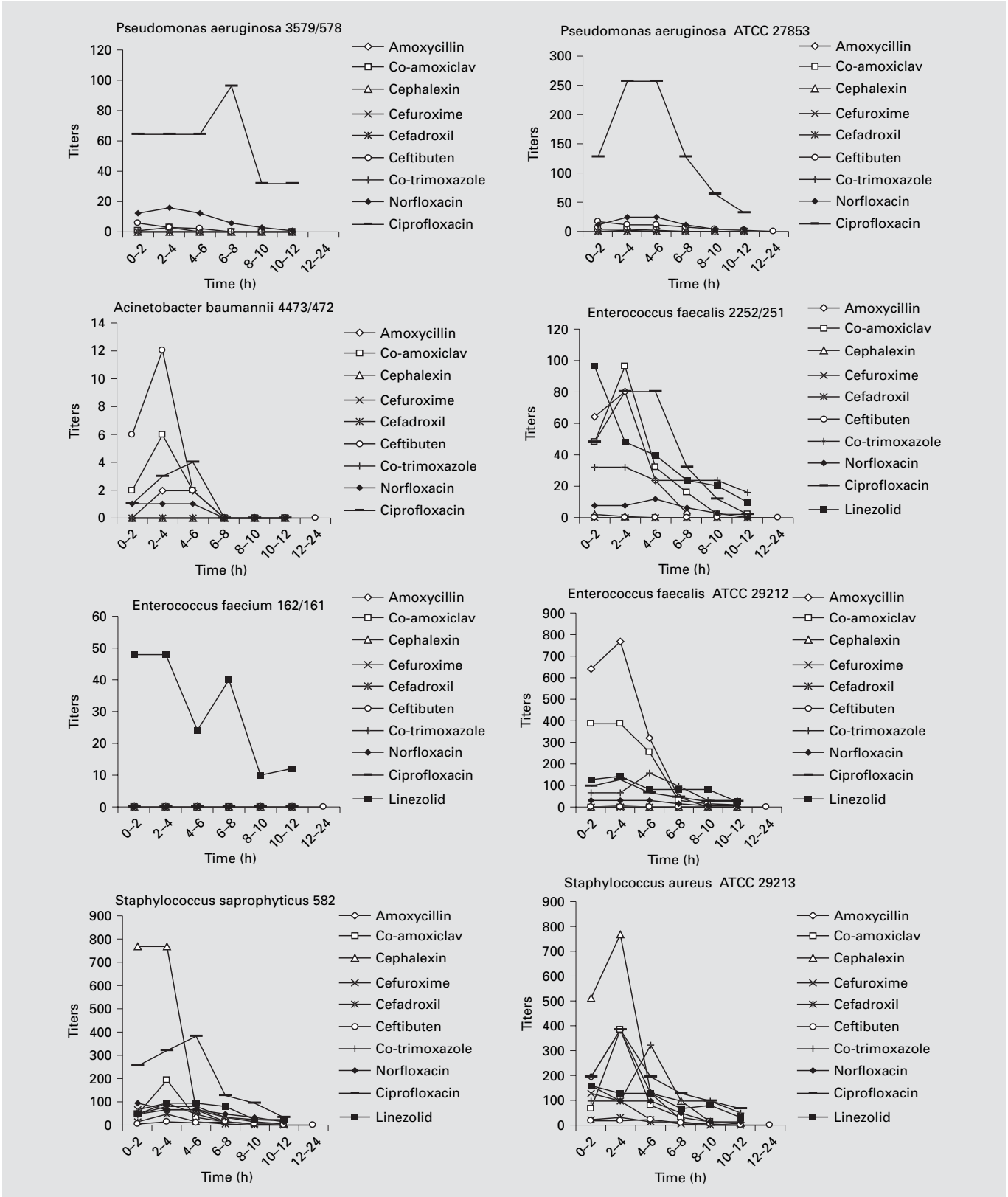

Fig. 2. Median reciprocal UBTs of various antibiotics against nonfermentative and gram-positive bacteria.

ment of hospital-acquired urinary tract infections when *P. aeruginosa*, *E. cloacae*, *S. marcescens*, or ESBL-positive Enterobacteriaceae are isolated from urine as pathogens because they demonstrate excellent in vitro and ex vivo activity and achieve high concentrations in urine [8]. Their high titers persisted throughout the whole dosing interval, which can be attributed to their long elimination half-life in urine. Co-trimoxazole had good activity against non-ESBL *E. coli* and *K. pneumoniae*, ESBL-positive *E. coli*, *E. cloacae*, *E. faecalis* and staphylococci. Its UBTs did not change significantly during the dosing interval due to the long $t_{1/2}$ in urine of both components. Linezolid had good urinary bactericidal activity against all gram-positive cocci including *E. faecium* resistant to amoxycillin and fluoroquinolones. Urine itself has a significant effect on the bactericidal activity of antibiotics. MICs are usually elevated if they are determined in urine instead of the standard medium [9, 10]. The susceptible bacteria had significantly higher titers than resistant bacteria. Discrepancies were found when ESBL-producing *K. pneumoniae* and *E. coli* were exposed to older cephalosporins and co-amoxiclav. In spite of the fact that in vitro tests revealed a resistance to these antibiotics, high UBTs were observed for cephalosporins and moderate UBTs for co-amoxiclav particularly at the beginning of the dosing interval. Furthermore, a high range of UBTs (data not shown) was observed, which is attributable to a variable urinary antibiotic concentration. In spite of the fact that median UBT was above 1:8 during the whole dosing interval for most susceptible strains, in some urine samples it dropped bellow this value before the end of the dosing interval. As a consequence, in most of the patients a positive therapeutic outcome can be predicted if in vitro tests show susceptibility, but some who demonstrate low titers and thus probably achieve lower urinary antibiotic concentrations are likely to have therapeutic failure.

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