

## The influence of glucose added urine on the *in vitro* antimicrobial activity of various antibiotics

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**Background & objectives:** Factors associated with the medium, including calcium and magnesium ion concentration and pH have been shown to affect the results of susceptibility testing but very little is known about glycosuria and the effect of glucose on the antimicrobial effect of antibiotics. In this study we assessed the influence of glucose added urine on the *in vitro* activities of various antibiotics by the microbroth dilution method.

**Methods:** Sixteen *Escherichia coli* isolates from patients with urinary infections were used in this study. Nine antibiotics were tested for their antimicrobial activity. Minimum inhibitory concentrations (MICs) were performed by the microbroth dilution method parallel in Mueller Hinton broth and glucose added urine.

**Results:** MICs of nearly all antibiotics were higher in glucose added urine than MICs in broth. MIC<sub>90</sub> against ampicillin was 32-fold higher in glucose added urine than MIC<sub>90</sub> in broth. MIC<sub>90</sub>s against ampicillin-sulbactam, cephalothin, cefuroxime, ceftriaxone and ciprofloxacin in glucose added urine were significantly ( $P<0.05$ ) higher than MIC<sub>90</sub> in broth. Equal MIC<sub>90</sub> in glucose added urine and broth were obtained for amikacin, sulphamethoxazole and trimethoprim.

**Interpretation & conclusions:** Our findings demonstrated that MICs of antibiotics are influenced by the glucose added urine.

**Key words** *Escherichia coli* - glucose added urine - MIC

Urinary tract infections (UTI) are one of the most prevalent infections of adults for which antimicrobials are used. Uncomplicated UTIs that occur most often in young healthy adult women are easy to treat<sup>1</sup>. However, UTIs often tend to have a more complicated course in patients with diabetes mellitus (DM)<sup>2</sup>.

Besides organ complications as retinopathy, neuropathy, nephropathy, *etc.*, patients with diabetes have an increased risk of infection which tend to be more severe. The increased frequency of UTIs

in diabetic patients is likely due to several factors including glycosuria, abnormal neutrophil function and increased adherence to uroepithelial cells. Glycosuria is not the sole reason but may play a major role in UTIs in diabetic patients<sup>1</sup>. *In vitro* studies have shown that glycosuria enhances the growth of different *Escherichia coli* strains<sup>3</sup>, however, this was not confirmed by *in vivo* studies which failed to show a higher prevalence of bacteriuria among diabetic patients with glycosuria compared with patients without glycosuria<sup>4</sup>.

Certain antimicrobials are less active in urine, an effect not explained by pH. Other solutes in urine likely mediate some of this effect<sup>5</sup>. We have recently reported that the minimum inhibitory concentrations (MICs) of antibiotics are generally higher in the urine<sup>6</sup>. Very little is known about glycosuria and the effect of glucose on the antimicrobial activity of antibiotics. We therefore undertook this study to assess the effect of glucose content (simulate glycosuria) on the antimicrobial activity of some antibiotics *in vitro*.

### Material & Methods

The study was done between June-September 2004 at the Department of Microbiology and Clinical Microbiology of Istanbul Faculty of Medicine, Istanbul University, Turkey.

**Bacterial isolates:** Sixteen *E.coli* isolates obtained from patients with urinary infections were used in this study. Isolates had a beta-lactam susceptible phenotype.

**Antibiotics:** The antibiotics used were: ampicillin, sulphamethoxazole and trimethoprim (Fako, Istanbul, Turkey), ampicillin+sulbactam (Bilim, Istanbul, Turkey), cephalothin (Lilly, Istanbul, Turkey), cefuroxime (GlaxoSmith Kline, Istanbul, Turkey), ceftriaxone (Deva, Istanbul, Turkey), amikacin (Mustafa Nevzat, Istanbul, Turkey) and ciprofloxacin (Bayer, Istanbul, Turkey).

**MIC determinations:** MICs were determined by the microbroth dilution method as described by Clinical and Laboratory Standards Institute (CLSI)<sup>7</sup>. Serial two-fold dilutions of antibiotics ranging from 1024 to 0.002 µg/ml were prepared in cation-adjusted Mueller Hinton broth (Oxoid, Hemakim, Istanbul, Turkey) and glucose (440 mg/dl) added urine (g-urine). Human pooled antibiotic-free urine with a pH of 6.4 was sterilized by passage through a 0.2 µm filter. *Escherichia coli* ATCC 25922 (American Type Culture Collection, University of Boulevard, USA) was used as a control strain. Exponential-phase organisms ( $5 \times 10^5$  cfu/well) were inoculated into each well containing 100 µl medium. The MIC was defined as the lowest concentration of each antibiotic giving complete inhibition of visible growth following incubation of the microtitre trays in air at 35°C for 16-24 h.

**Statistical analysis:** Data was analysed using the Kruskal-Wallis test. Multiple comparisons were performed with Dunn's method.

### Results & Discussion

MICs of nearly all antibiotics were higher in glucose added urine than MICs in broth. MIC<sub>90</sub>

against ampicillin was 32-fold higher in glucose added urine than MIC<sub>90</sub> in broth. MIC<sub>90</sub>s against ampicillin-sulbactam, cephalothin, cefuroxime, ceftriaxone and ciprofloxacin in glucose added urine were significantly ( $P < 0.05$ ) higher than MIC<sub>90</sub> in broth. Equal MIC<sub>90</sub> in glucose added urine and broth were obtained for amikacin, sulphamethoxazole and trimethoprim.(Table)

Routine susceptibility testing provides essential data, but has its limitations because it does not take into account the effect of urine or the content of urine on the outcome of the test. Factors associated with the medium, including calcium and magnesium ion concentrations and pH have been shown previously to affect the results of susceptibility testing<sup>8</sup>. Also many antibiotics are concentrated in urine and concentration of many antibiotics may exceed serum levels to which the organism is not generally considered susceptible<sup>8</sup>.

The purpose of this study was to assess the influence of glucose content of urine on the *in vitro* activities of antibiotics commonly used in the therapy of urinary tract infections. In a previous study we found a decrease in activity of the antibiotics studied except cephalotin in urine<sup>6</sup>. This study demonstrated that the MICs of some antibiotics are influenced by the human urine and that the MIC determination performed in broth may overestimate the activities of some principal antibiotics used in the treatment of urinary infections<sup>6</sup>.

**Table.** MIC range, MIC<sub>90</sub> and susceptibility rates of various antibiotics tested

Antibiotics	Media	MIC range (µg/ml)	MIC <sub>90</sub>	%R	%I	%S
Ampicillin	Broth	0.25-4	2			100
	G-urine	2-64	64			
Ampicillin + sulbactam	Broth	0.12-4	4			100
	G-urine	4-128	64			
Cephalothin	Broth	0.25-8	8			100
	G-urine	4-16	16			
Cefuroxime	Broth	0.5-4	4			100
	G-urine	2-16	16			
Ceftriaxone	Broth	0.004-0.12	0.12			100
	G-urine	0.015-2	2			
Amikacin	Broth	4-16	16			100
	G-urine	1-16	16			
Ciprofloxacin	Broth	<0.002-64	64	25	6.2	68.8
	G-urine	0.015-128	128			
Sulphamethoxazole	Broth	32≥1024	>1024	75		25
	G-urine	256≥1024	>1024			
Trimethoprim	Broth	<0.002-512	512	68.7		31.3
	G-urine	0.03-512	512			

R, resistant; I, intermediate; S, susceptible

In a study on therapeutic efficacy of different quinolones against a strain of *Serratia marcescens* in a model of urinary tract infections of normal and diabetic mice no difference was found between normal and diabetic mice in serum and kidney concentrations. These observations suggested that quinolones may be effective drugs in the treatment of urinary tract infections in diabetic state<sup>9</sup>.

Minuth *et al*<sup>10</sup> studied the antibacterial effect of gentamicin against *E.coli* and *Pseudomonas aeruginosa* in urine and showed that urine has an inhibitory effect that is dependent upon the acidity and osmolality as well as upon the presence of individual solutes of the urine. Up to 40 times as much gentamicin was needed to prevent the growth of *E. coli* and *P. aeruginosa* in human urine as is required in broth.

The *in vitro* effect of pH and glucose concentration on the antibacterial activity of norfloxacin in urine showed that norfloxacin inhibited effectively the growth of Gram-negative pathogens in urine *in vitro* at pH values of 6.0, 7.0, and 8.0<sup>11</sup>. Glucose at concentrations of 200 and 400 mg/dl (simulating glucosuria of diabetes) did not significantly affect the antibacterial activity of norfloxacin against clinical isolates of *E.coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *P. aeruginosa*<sup>11</sup>.

The effect of sucrose (s) and glucose (g) on the susceptibility of *Streptococcus mutans* to cephalosporins was evaluated in isolates grown in excess of sucrose and glucose<sup>12</sup>. About 21 per cent of the isolates were sensitive in the presence of sucrose and resistant in the presence of glucose. Only 3 per cent isolates showed the opposite situation. The mean values of the s-MICs of the four cephalosporins were significantly lower than those the g-MICs. The authors explained that in excess of sucrose insoluble glucan is synthesized by *S. mutans* which increases the interbacterial distance and promotes antibiotic diffusion<sup>12</sup>.

In conclusion, we found higher MICs of various antimicrobials tested against *E. coli* in glucose added urine compared to the MICs in broth. That certain

antibiotics are influenced by the content of human urine should be taken into account while prescribing these antibiotics for the treatment of urinary infections.

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