

# Direct antimicrobial susceptibility testing from urine samples. Validation against EUCAST standard disk diffusion method.

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P 1550

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## Introduction

The majority of urinary tract infections (UTI) are caused by *Escherichia coli* and treated empirically. However, increased antibiotic resistance makes this strategy more cumbersome. To improve antibiotic treatment, a short turnover time for antimicrobial susceptibility testing and species identification is desirable. Matrix Assisted Laser Desorption/Ionisation Time of Flight (MALDI-TOF) can provide species identification faster than former routine methods. To decrease the time to report, several laboratories use direct antimicrobial susceptibility testing from urine samples. However, so far no validation against EUCAST methodology has been performed.

## Objective

To evaluate two different methods for Direct Susceptibility Testing (DST) from urine samples against EUCAST disk diffusion methodology for Enterobacteriaceae (EB).

## Materials and Methods

Urine samples (n=196) with  $\geq 10^5$  CFU/mL of EB, from the routine work at the Dept of Clinical Microbiology, Växjö, Sweden, were blinded to the primary investigator. Samples with more than one Gram-negative species were excluded. Antimicrobial susceptibility testing was performed directly from the urine (DST-U) or from a re-suspension in saline to 0.4 - 0.6 McFarland from a pellet obtained as described below (DST-P). Two mL urine was centrifuged at 2000 g for 30 s to remove cells and other large particles. The supernatant was centrifuged at 15 500 g for 5 min. The supernatant was discarded and the pellet was washed with 600  $\mu$ L de-ionised water. A final centrifugation at 15 500 g for 5 min was performed to achieve a working pellet of bacteria. Species identification was performed directly from the pellet by transfer to a MALDI-TOF target plate with a toothpick, followed by analysis on MicroflexTM/Biotyper 3.1 (Bruker Daltonics) using the mixed culture algorithm. Disk diffusion was performed on Mueller-Hinton (MH) agar according to EUCAST methodology (except for inoculum preparation) using mecillinam 5  $\mu$ g, cefadroxil 30  $\mu$ g, trimethoprim 5  $\mu$ g, nitrofurantoin 100  $\mu$ g and ciprofloxacin 5  $\mu$ g disks. The categorical agreements of DST were compared with the standard susceptibility testing according to EUCAST (standard AST) used at the laboratory.

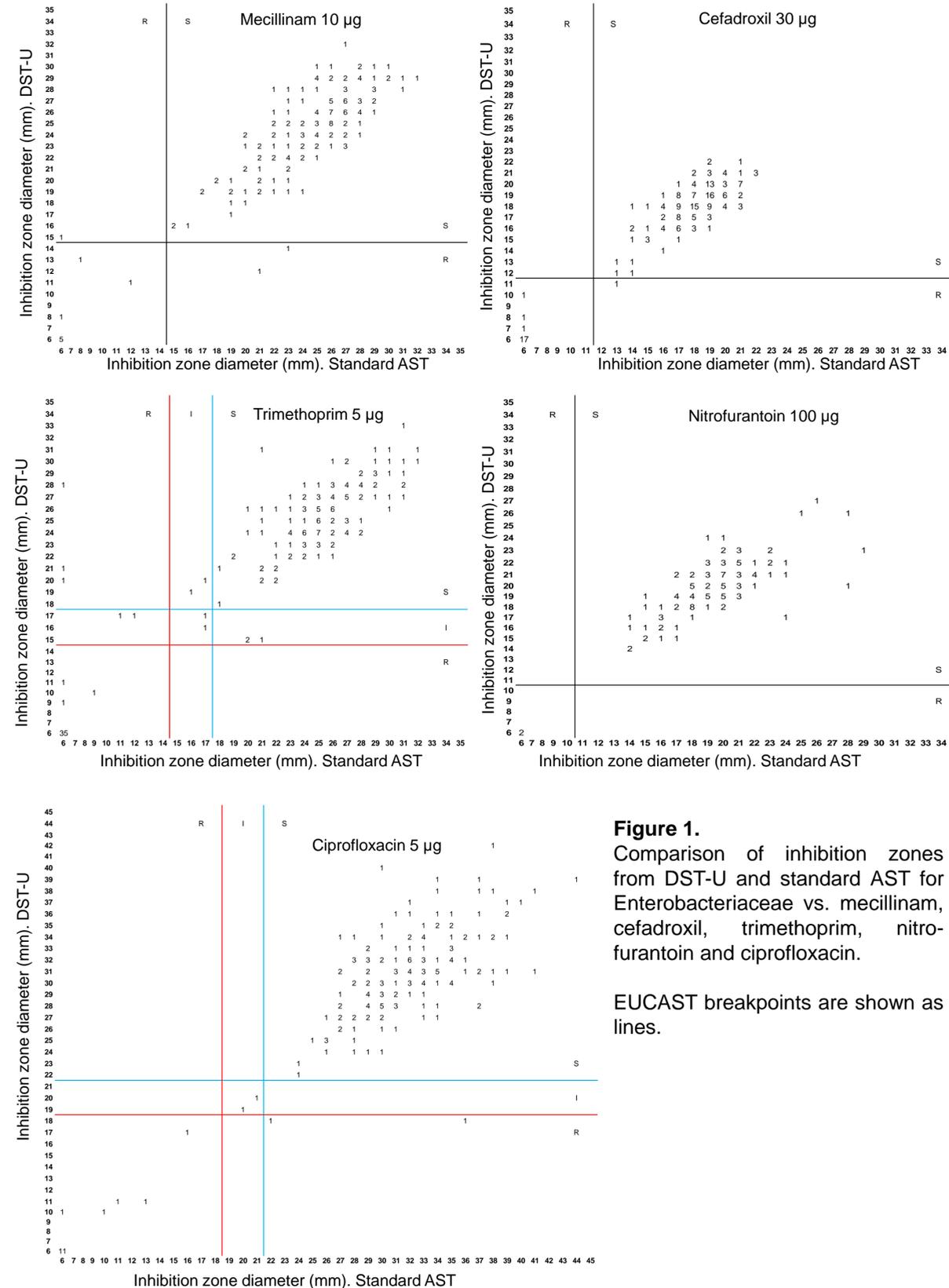
## Results and discussion

Correlations between inhibition zones for DST-U and standard AST are shown in **Figure 1**. For DST-P, AST results could not be registered for 58 of 196 isolates because there was either not sufficient amount of material in the pellet to make a suspension of McFarland 0.5 or non-confluent growth on the MH agar plate.

The categorical agreements between direct and standard AST are shown in **Table 1**. Overall, error rates were low, both for DST-U and DST-P. Most errors appeared in species other than *Escherichia coli*, with the highest error rates for *Proteus mirabilis* and trimethoprim. Using a score value of  $>1.7$ , 130 samples (66%) were correctly identified to the species level using MALDI-TOF. In four samples, mixed cultures with EB and *Enterococcus faecalis* were reported as EB only, despite the use of the mixed culture algorithm.

**Table 1.** Categorical agreements for direct antimicrobial susceptibility testing of Enterobacteriaceae vs. EUCAST standardised disk diffusion method. Values for *Escherichia coli* only are shown in parentheses.

Antimicrobial agent	Test method	mE	ME	VME	No of isolates
Mecillinam	DST-U	-	2 (2)	1 (1)	177 (123)
	DST-P	-	1 (1)	0 (0)	129 (94)
Cefadroxil	DST-U	-	0 (0)	1 (0)	196 (123)
	DST-P	-	1 (1)	0 (0)	142 (94)
Trimethoprim	DST-U	7 (1)	0 (0)	2 (0)	196 (122)
	DST-P	7 (2)	0 (0)	1 (0)	142 (94)
Nitrofurantoin	DST-U	-	0 (0)	0 (0)	124 (124)
	DST-P	-	0 (0)	0 (0)	94 (94)
Ciprofloxacin	DST-U	0 (0)	2 (1)	0 (0)	196 (123)
	DST-P	0 (0)	0 (0)	0 (0)	140 (93)



**Figure 1.** Comparison of inhibition zones from DST-U and standard AST for Enterobacteriaceae vs. mecillinam, cefadroxil, trimethoprim, nitrofurantoin and ciprofloxacin.

EUCAST breakpoints are shown as lines.

## Conclusions

Both methods for direct antimicrobial susceptibility testing from urine samples correlated well with standardised disk diffusion in samples with  $\geq 10^5$  CFU/mL of one single EB, which implies that DST can be used to reduce the turnover time for antimicrobial susceptibility testing. DST-P was labor intensive and resulted in fewer results compared to DST-U. However the pellet provided from DST-P could be used for rapid species identification in 66% of the samples using MALDI-TOF. The mixed flora algorithm did not detect all mixed cultures and needs further development.