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## REVISED ABSTRACT

**Objectives** Antibiotic susceptibility tests are the most frequently used tests in clinical microbiology. Due to economic pressure fully or partly automated test systems are developed in order to reduce personal and reagent costs. In this study we compare the MICRONAUT system using a 384 well microtiter plate with a conventional microdilution method according to DIN guidelines.

**Methods** The MICRONAUT system uses vacuum dried antibiotics in a 384-well-plate. The inoculum is applied with an automatic dispenser with a final inoculum of 1x10<sup>6</sup> cells/micro L in 50 microliter cation adjusted Müller Hinton medium with the addition of 0.025 percent phytagel. As the comparator we used the microdilution method according to EUCAST. Evaluation was performed by the comparison of *i.* the performance of quality control strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 29212), and *ii.* the natural sensitivity of clinical isolates of common pathogens (*E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus*). For each species-antibiotic-combination meeting the conditions for comparison (at least five-dilution-series with a mode at the third dilution of the naturally sensitive population) two figures are calculated to determine the degree of similarity of the MIC-distributions: the mode and the percentage of strains within the range of +/- one dilution step of the mode.

**Results** The results for the QC-strains match to 100% for 23 strain-antibiotic pairs. For 16 pairs there is a difference of 1 dilution step for the mode MIC, and only once (*S. aureus* and Penicillin) the mode MIC varied for 2 dilution steps. The results for the MIC distributions of the naturally sensitive population show in 10 out of 15 cases an excellent match with regard to the peak and the width of the MIC distributions. For Imipenem and Gentamicin with *P. aeruginosa*, for vancomycin, moxifloxacin and meropenem with *E. faecalis* differences of the mode are one dilution step only. The percentage of values within the range of +/- one dilution step of the mode for the above mentioned exceptions is nearly identical.

**Conclusions** In general both methods show nearly identical results. Differences in mode MICs rarely exceed one dilution step, although not identical batches of the test medium were used for both methods. This way of evaluation seems more reliable than the use of already evaluated results with S, I and R categories describing major and very major errors.

## INTRODUCTION AND PURPOSE

Antibiotic susceptibility tests are the most frequently used tests in clinical microbiology. In modern medicine it is necessary in many clinical settings to measure MICs of the pathogens. Due to economic pressure fully or partly automated test systems are developed in order to reduce personal and reagent costs. In this study we compare the MICRONAUT system, a semi automated sensitivity test system, using a 384 well microtiter plate with a conventional microdilution method according to EUCAST guidelines. Using the MIC data for quality control strains and for clinical isolates determined with both methods we wanted to evaluate the MICRONAUT microtitration system.

## METHODS

The MICRONAUT<sup>®</sup> system uses vacuum dried antibiotics in a 384-well-plate (Merlin Diagnostika GmbH, Bornheim Germany). The inoculum is applied with an automatic dispenser with a final inoculum of 1x10<sup>6</sup> cells/micro L in 50 microliter cation adjusted Müller Hinton medium with the addition of 0.025 percent phytagel. As the comparator we used the microdilution method according to EUCAST guidelines. Evaluation was performed by the comparison of *i.* the performance of quality control strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 29212), and *ii.* the natural sensitivity of clinical isolates of common pathogens (*E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus*). For each species-antibiotic-combination meeting the conditions for comparison (at least five-dilution-series with a mode at the third dilution of the naturally sensitive population) two figures are calculated to determine the degree of similarity of the MIC-distributions: the mode and the percentage of strains within the range of +/- one dilution step of the mode.

## RESULTS

QC-strain / Antibiotic	EUCAST		MICRONAUT		Species / Antibiotic	EUCAST		MICRONAUT	
	mode	%	mode	%		mode	%	mode	%
<b><i>E. coli</i> ATCC 25922</b>									
Amoxicillin	4	100.0	2	95.9	Amoxicillin	4	94.5	2	80.7
Piperacillin	2	100.0	2	99.2	Piperacillin	2	90.3	2	-
Piperacillin/Tazobactam	2	100.0	2	100.0	Piperacillin/Tazobactam				
Cefuroxime	4	100.0	4	99.0	Cefuroxime	4	93.0	4	89.8
Tobramycin	1	100.0	1	99.5	Tobramycin	1	95.2	1	94.4
<b><i>P. aeruginosa</i> ATCC 27853</b>									
Piperacillin	4	100.0	4	98.3	Piperacillin	4	78.0	4	81.9
Piperacillin/Tazobactam	2	99.0	2	96.8	Piperacillin/Tazobactam	4	74.9	4	80.1
Ciprofloxacin	0.25	97.8	0.25	99.5	Ciprofloxacin	0.12	81.8	0.12	80.9
Levofloxacin	0.5	100.0	1	98.1	Levofloxacin	0.5	86.2	0.5	79.7
Moxifloxacin	2	93.8	2	99.7	Moxifloxacin	1	80.9	1	-
Ceftazidime	8	97.8	16	92.3	Ceftazidime				
Colistin	1	100.0	2	97.8	Colistin	1	75.0	2	73.1
Imipenem	1	100.0	2	78.5	Imipenem	1	86.3	1	82.8
Meropenem	0.25	97.8	0.25	91.1	Meropenem				
Gentamicin	1	100.0	2	96.2	Gentamicin	1	78.2	2	65.6
Tobramycin	0.5	100.0	1	90.6	Tobramycin	0.5	91.1	1	77.2
<b><i>E. faecalis</i> ATCC 29212</b>									
Clindamycin	8	94.3	16	96.5	<i>E. faecalis</i>				
Penicillin	2	97.4	2	95.2	Penicillin				
Vancomycin	4	97.4	2	96.4	Vancomycin	1	90.2	2	87.4
Ciprofloxacin	1	92.3	1	98.1	Ciprofloxacin	2	95.0	1	82.4
Levofloxacin	1	92.3	1	100.0	Levofloxacin	2	97.6	1	87.9
Moxifloxacin	0.25	89.7	0.25	99.4	Moxifloxacin	0.5	96.8	0.25	90.9
Linezolid	1	97.4	1	100.0	Linezolid	1	99.1	1	96.3
Imipenem	0.5	100.0	0.5	82.3	Imipenem	1	86.5	1	84.4
Meropenem	2	100.0	1	98.8	Meropenem	4	86.7	2	86.1
Doxycyclin	8	100.0	4	98.2	Doxycyclin				
<b><i>S. aureus</i> ATCC 29213</b>									
Ampicillin	1	96.2	2	85.0	<i>S. aureus</i>				
Penicillin G	0.5	90.0	2	53.3	Penicillin G				
Linezolid	2	73.8	2	98.5	Linezolid	1	95.9	1	98.6
Vancomycin					Vancomycin	1	99.0	1	99.1

Table 1: mode and +/- one dilution step of the mode results for QC-strains and clinical isolates

## RESULTS continued

All antibiotic-QC-strain combinations, where the MICs +/- one dilution step of the mode were within the range of the tested concentrations with both methods were included in the evaluation. If the lowest concentration tested fell into this range, the number of strains showing their MIC at this concentration should not exceed 10%. The results are depicted in table 1.

The results for the QC-strains match to 100% for 16 strain-antibiotic pairs of 29 pairs evaluated. For 12 pairs there is a difference of 1 dilution step for the mode MIC, and only once (*S. aureus* and Penicillin) the mode MIC varied for 2 dilution steps. The number of tests falling into the +/- one dilution step range was for *E. coli* always about 99% even for the Ampicillin testing, where the mode differed for 1 dilution step. With few exceptions (*P. aeruginosa* – Imipenem 78%, *E. faecalis* – Imipenem 82%, and Ampicillin - *S. aureus* 85%) the number of tests falling into the +/- one dilution step range was above 90%.

The results for the MIC-distributions of the natural sensitive population show in 10 out of 15 cases an excellent match with regard to the peak and the width of the MIC distributions. For Imipenem and Gentamicin with *P. aeruginosa*, for vancomycin, moxifloxacin and meropenem with *E. faecalis* differences of the mode are one dilution step only. The percentage of values within the range of +/- one dilution step of the mode for the above mentioned exceptions is nearly identical.

Antibiotic	Method	MIC (mg/L)															n	Mode	Mod-1 (%)		
		0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>						
Piperacillin	E																		4	100.0	
	M																			4	98.3
Piperacillin/Tazobactam	E																			4	99.0
	M																			4	96.8
Ciprofloxacin	E																			0.25	97.8
	M																			0.25	99.5
Levofloxacin	E																			0.5	98.1
	M																			0.5	98.1
Moxifloxacin	E																			2	99.7
	M																			2	99.7
Colistin	E																			1	97.8
	M																			2	78.5
Imipenem	E																			0.5	78.5
	M																			1	86.3
Gentamicin	E																			1	78.2
	M																			2	96.6
Tobramycin	E																			0.5	90.6
	M																			1	90.6

Table 2: MIC distributions of clinical isolates to demonstrate the strategy for the evaluation

Two examples of the MIC distributions of the naturally sensitive population demonstrate how close the measurements of both methods are.

An example of MIC distributions where the results of the two methods show a mode which differs for one dilution step (Fig 1a), 87.9% of isolates tested with the MICRONAUT system fall into in the +/- one dilution step range for the mode of the reference method.

In a second example, where the mode MICs with both methods are identical, 99% of strains tested with the MICRONAUT system have an MIC within the +/- one dilution step range for the mode of the reference method.

## RESULTS continued

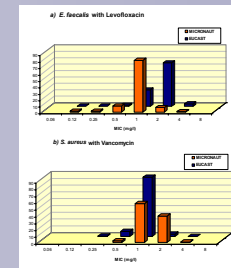


Figure 1: MIC distributions of naturally sensitive populations of a) *E. faecalis* with Levofloxacin b) *S. aureus* with Vancomycin

## CONCLUSIONS

In general the MICRONAUT system, using a 384 well microtiter plate for the determination of MICs generates nearly identical results as compared with the EUCAST reference method. Differences in mode MICs for QC-strains and for clinical isolates rarely exceed one dilution step, although not identical batches of the test medium were used for both methods.

This way of evaluation seems more reliable than the use of already evaluated results with S, I and R categories describing the number of major and very major errors because they can produce identical test results even if the measurements are different, depending on the break-points used. In several clinical settings, especially in intensive care units it is often necessary to know exact MICs to calculate the correct dosing using PK/PD modeling.

## REFERENCES

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