

The Value of Quality Control Strains in Susceptibility Tests

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ABSTRACT

Objectives The goals of a quality control program are to assist in monitoring the precision and accuracy of the susceptibility test procedure, the performance of reagents used in the test and the performance of persons who carry out the tests and read the results. They are best accomplished by the testing of quality control (QC) strains with known susceptibility to the antimicrobial agents to be tested (NCCLS). Therefore, QC strain measurements done by laboratories taking part in the GENARS-project (German Network for Antimicrobial Resistance Surveillance) were used for a comparison of the performance of three different methods for MIC determination.

Methods In the GENARS-project two commercial MIC test systems and one manual microdilution system according to NCCLS are used for the determination of antimicrobial susceptibility. The commercial systems are the Vitek 2[®] (bioMérieux) and the Micronaut[®] system (Merlin Diagnostics) with 384-well microtiter-plates. QC strains measured by all test systems were evaluated for those antibiotics where a range of \pm one dilution step of the modal value of the respective QC strain is included in the range of concentrations tested. For reliable assessment of the test quality the distance of the modal value from the lowest and highest concentration tested has to be two or more dilution steps.

Results From a multitude of antibiotics tested only few drugs are tested with a range of concentrations which meets the above mentioned requirements. Table 1 indicates the number of test combinations available for evaluation. The Vitek 2[®] system offers the shortest ranges of concentrations. However, from the range of concentrations only few are tested, while the others are calculated, e.g. for Gentamicin the range includes six concentrations while only three are measured (AST-P526).

Conclusions An evaluation of QC-strain measurements should be possible for all antibiotics tested. However, due to the concentrations chosen and the short ranges of concentrations available in the different test-systems only few antimicrobial agents can be used for a comparison of the performance of the test methods. Therefore, either the range of concentrations has to be extended, or more suitable QC strains have to be implemented in a way that their MIC's fall into the range of concentrations which are sufficient in clinical terms.

INTRODUCTION AND PURPOSE

For an assessment of quality in a network of antimicrobial resistance surveillance where data from different laboratories are merged, quality control strains (QC strains) should be measured by each laboratory. As realized in the GENARS-project (German Network for Antimicrobial Resistance Surveillance) all laboratories involved agreed in testing the same QC strains with a minimal requirement on frequency. This is the basis for a comparison of the three different systems for MIC determination used in the GENARS project. By comparing MIC distributions for QC strains measured, we want to demonstrate whether the three different methods give identical or at least comparable results.

METHODS

Six laboratories of medical microbiology of university hospitals presently participate in the GENARS-project in Germany. These laboratories collect MIC-data for bacterial susceptibility to antibiotics for all species identified in the laboratory routine from clinical material. For evaluation these data are sent online to the central office of GENARS in Bonn.

For susceptibility testing four laboratories use the Micronaut[®]-system (Merlin GmbH, Bornheim), a microdilution system. In an automatic process 384-well microtiter-plates containing vacuum-dried antibiotics are filled with freshly inoculated Mueller-Hinton broth (Becton Dickinson/Difco; Heidelberg). The plates are read photometrically. Another center uses a self prepared manual microdilution system (MMS). All these centres are using the same batch of kation adjusted Mueller-Hinton broth, which is reserved by the manufacturer for the study. According to DIN and NCCLS the inoculum contains approximately 5×10^5 CFU/ml. A further center uses the Vitek 2[®] system (BioMérieux GmbH, Nürtingen).

In order to compare these three systems for susceptibility testing four QC strains were chosen for evaluation. Because the spectrum of antibiotics tested and the concentrations tested are different with each system, for evaluation of QC-strains only those antibiotics came into account which are tested by all test systems and where the range of \pm one dilution step of the modal value for the respective QC strain is within the range of concentrations tested.

RESULTS

Due to the requirements mentioned in Material and Methods, for each test system only a few drugs still remain for evaluation. Table 1 indicates the number of antibiotics which are tested in the respective test system with at least one concentration above and below the modal value of the MIC distributions for four QC strains.

MIC test system	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>E. faecalis</i> ATCC 29212	<i>S. aureus</i> ATCC 29213
Micronaut [®]	2 / 32	10 / 32	11 / 32	11 / 32
Vitek 2 [®]	2 / 20	2 / 20	3 / 20	3 / 20
MMS	10 / 23	12 / 23	9 / 23	9 / 23

Table 1 Number of antibiotics (bold) which are tested in the respective test system with at least one concentration above and under the modal value of the MIC distribution for the respective QC strain in relation to the total number of antibiotics tested. MMS = manual microdilution system

As exemplified in Table 2 for *E. coli* ATCC 25922 and three different antibiotics, for all three tests systems the range of concentrations available for cefotaxime does not allow any quality assessment. Because the range of \pm one dilution step of the modal value of the MIC distribution for gentamicin is located completely within the concentrations tested, the MMS (manual micro dilution system) can be assessed. However, only for cefuroxime the ranges of concentrations measured are sufficient for a comparison of all three test systems.

		MIC (mg/l)											
		0.12	0.25	0.5	1	2	4	8	16	32	64	128	n
CTX	VITEK 2 [®]	-	-	-	100.0	0.0	0.0	0.0	0.0	0.0	0.0	-	51
	MMS	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	12	
	MICRONAUT [®]	-	99.5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	-	198
GEN	VITEK 2 [®]	-	-	-	100.0	0.0	0.0	0.0	0.0	-	-	-	51
	MMS	0.0	0.0	58.3	25.0	16.7	0.0	0.0	0.0	-	-	-	12
	MICRONAUT [®]	-	4.6	87.7	31.1	6.1	0.5	0.0	0.0	0.0	0.0	-	198
CXM	VITEK 2 [®]	-	-	-	0.0	0.0	100.0	0.0	0.0	0.0	0.0	-	51
	MMS	0.0	0.0	0.0	0.0	0.0	66.7	33.3	0.0	0.0	-	12	
	MICRONAUT [®]	-	-	0.0	0.0	63.9	15.1	0.5	0.0	0.0	0.5	199	

Table 2 MIC distributions (in %) of *E. coli* ATCC 25922 for cefotaxime (CTX), cefuroxime (CXM) and gentamicin (GEN) determined by 3 test systems (MMS = manual microdilution system). Bold red letters indicate the modal value. Blue areas indicate the range of \pm one dilution step of the modal value when completely within the range of concentrations measured. n = number of tests - = not done

In the Tables 3a-d for four QC strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213) those drugs are indicated, which can be used for a comparison of all test systems used in the GENARS project. With the exception of *E. coli* ATCC 25922 where two antibiotics can be used, for all other QC strains only one antibiotic meets the above mentioned requirements. Due to the extremely short ranges of concentrations available by the Vitek2[®] system only few drugs can be used for a quality control by QC strains.

<i>S. aureus</i> (ATCC 29213)				<i>E. faecalis</i> (ATCC 29212)			
3a	MICRONAUT	VITEK 2	MMS	3b	MICRONAUT	VITEK 2	MMS
Ampicillin	X			Ampicillin	X		
Oxacillin	X			Oxacillin	X		
Penicillin	X			Penicillin	X		X
Ciprofloxacin		X		Ciprofloxacin	X		X
Levofloxacin		X		Levofloxacin	X		X
Moxifloxacin	X	X		Moxifloxacin	X	X	X
Imipenem	X	X		Imipenem	X	X	X
Meropenem	X	X		Meropenem	X	X	X
Vancomycin	X	X	X	Vancomycin	X	X	X
Doxycycline	X			Doxycycline	X		X
Erythromycin	X	X		Erythromycin	X	X	X
Linezolid	X	X		Linezolid	X		X
Quinsuprastin/Dalfoipristin	X	X		Quinsuprastin/Dalfoipristin	X	X	X
				Riftampicin			X

<i>P. aeruginosa</i> (ATCC 27853)				<i>E. coli</i> (ATCC 25922)			
3c	MICRONAUT	VITEK 2	MMS	3d	MICRONAUT	VITEK 2	MMS
Cefotaxime	X	X		Ampicillin / Subactam	X	X	X
Ceftazidime	X	X		Ampicillin	X		X
Ciprofloxacin	X	X		Cefuroxime	X	X	X
Gatifloxacin	X	X		Ciprofloxacin	X		X
Levofloxacin	X	X		Piperacillin	X		X
Moxifloxacin	X	X		Piperacillin / Subactam	X		X
Imipenem	X	X	X	Piperacillin / Tazobactam	X		X
Piperacillin	X	X	X	Amikacin	X		X
Piperacillin / Subactam	X	X	X	Gentamicin	X		X
Piperacillin / Tazobactam	X	X	X	Tobramycin	X		X
Amikacin	X	X		Doxycycline	X		X
Gentamicin	X	X					
Tobramycin	X	X					

Tables 3a-3d Antibiotics suitable for an assessment of the respective test-system and the respective QC strain. Coloured rows indicate antibiotics suitable for all test systems used in the GENARS project. MMS = manual microdilution system

The MIC distributions measured by the three test systems (Fig. 1 - 5) showed a narrow range of only 2 - 3 concentrations. For *E. coli* ATCC 25922 and cefuroxime all modal values were at the same concentration of 4 mg/l (Fig. 1). Depending on the test system and the antibiotic tested, for all other QC strains the modal values varied within two dilution steps (Fig. 2 - 5).

The number of tests lying within a range of \pm one dilution step of the modal value are: *E. coli* / ampicillin = 97,4 %; *E. coli* / cefuroxime = 99,6 %; *P. aeruginosa* / imipenem = 96,0 %; *S. aureus* / vancomycin = 98,5 %; *E. faecalis* / levofloxacin = 99,6 %. The clear sharp columns, often without any variations, detected by the Vitek2[®] system (Fig. 1 and Fig. 5) may be due to values rather calculated than really measured.

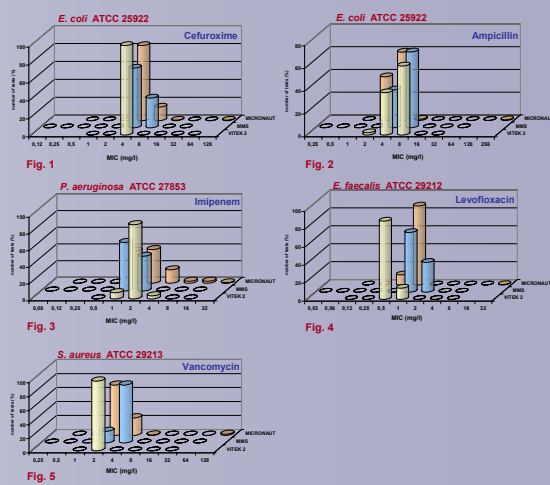


Fig. 1 - 5 Comparison of MIC distributions measured by different test-systems. MMS = manual micro dilution system. Uncoloured circles indicate concentrations measured but no strains were detected.

CONCLUSIONS

Due to the concentrations chosen and the short ranges of concentrations available in the different test-systems only few antimicrobial agents can be used for a comparison of the performance of the test methods. Using those drugs which meet the aforementioned requirements for a comparison of QC strains measured by the different test systems, an assessment of quality is possible. For the antibiotics selected for this evaluation the good conformity of test results detected by the different systems points out the high qualitative performance of the test systems used by GENARS. However, an evaluation of QC-strain measurements should be possible for all antibiotics tested. Therefore, either the range of concentrations has to be extended, or more suitable QC strains have to be implemented in a way that their MIC's fall into the range of concentrations which are sufficient in clinical terms.