

# Antibiotic Resistance Surveillance on the Basis of High Quality Routine Data: German Network for Antimicrobial Resistance Surveillance

Huppertz K., Noll I., Wiedemann B. and the GENARS-group

Central Office of GENARS; Pharmaceutical Microbiology; University of Bonn; Meckenheimer Allee 168; 53115 Bonn; Germany

# GENARS

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## ABSTRACT

**Objectives** Worldwide every day billions of susceptibility tests for human pathogens are performed for diagnostic reasons. Unfortunately these data are not sufficient for surveillance studies because of the lack of comparability, due to methodological variability. The 6 laboratories of the GENARS-project measure MIC's of about 30 antibiotics. Methods are standardized and quality controlled. This paper describes the reproducibility of the results of the QC strains and in addition the quality control by the comparison of MIC-distributions of clinical isolates.

**Methods** In each laboratory involved in the GENARS-project, all clinical isolates are identified to species level. Generally, all isolates are tested against 32 different antibiotics. In contrast to usual QC strain evaluation we look for those data, where the MIC's of the strains are within the range of the concentrations tested, which increases the sensitivity of the method. The second quality control measure is the comparison of MIC-distributions of clinical isolates, where the modal value of the sensitive population of one species should be identical for laboratories involved.

**Results** Evaluation of QC strain measurements gives a good impression of the quality of laboratory work. For the first half of 2002 more than 130 tests for each QC strain and antibiotic were evaluated. Some examples are shown in table 1, demonstrating that the reproducibility of the test  $\pm$  one dilution step is close to 100 % with few exceptions. The second tool to demonstrate reproducibility of test results is shown in fig. 3. The MIC-distribution for cefuroxime with *E. coli* demonstrates, that the naturally sensitive population peaks at 4 mg/l.

**Conclusions** The high methodological standard of sensitivity testing of the GENARS-project will not only improve the surveillance including an early warning system, a demonstration of resistance trends and finding of new resistance mechanisms, but will also be advantageous for the patients who benefit from more reliable test results and better hospital epidemiology.

## INTRODUCTION AND PURPOSE

Worldwide every day billions of susceptibility tests for human pathogens are performed for diagnostic reasons. Unfortunately, almost all of these test-results are lost for any antimicrobial resistance surveillance because of the lack of comparability due to methodological variability. Therefore surveillance studies need a specific protocol which has to be followed by all participating laboratories (1, 2, 3). This is the reason why surveillance data are mostly based on random data and not on continuous clinical samples. If the every day data for susceptibility testing shall enter a surveillance program which recognizes trends and new mechanisms, the every day testing has to be improved to a standard of quality including inter- and intra laboratory quality control that is not achieved by most routine laboratories. The GENARS-project (German Network for Antimicrobial Resistance Surveillance) which is funded by the German Federal Ministry of Health tries to reach this goal.

The 6 laboratories of the project agreed to use a unique high methodical standard for routine diagnostics by measuring MIC's for each clinical isolate. This method allows the recognition of a development of antimicrobial resistance and is used for each isolate identified in the daily routine. Methods are standardized and quality controlled.

Evaluation of QC strain measurements and the quality controls by comparison of MIC-distributions of clinical isolates provide the good reproducibility of the GENARS results

## METHODS

In Germany, presently six medical microbiology laboratories of university hospitals are taking part in the GENARS-project. These laboratories collect MIC-data on bacterial susceptibility against antibiotics for all species of clinical material identified in the laboratory routine.

In fig. 1 the geographical positions for the centres involved are indicated. For central evaluation, each lab transmits its data to the central office to Bonn once a week.

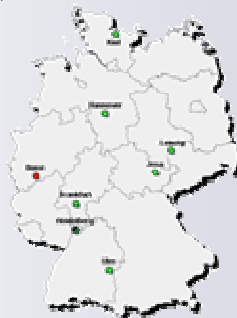


fig. 1 geographical positions of GENARS centres

- central office
- centres already integrated
- centres not yet integrated

For susceptibility testing four laboratories use the Micronaut®-system (Merlin GmbH; Bornheim), a microdilution procedure. In an automatic process 384-well microtiter-plates containing vacuum-dried antibiotics are filled with freshly inoculated Mueller-Hinton broth (Becton Dickinson/Difco; Heidelberg). All 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  $5 \times 10^5$  CFU/ml (4, 6). The plates are read photometrically and results are transferred online for evaluation. One center uses a self prepared microdilution, another Vitek 2® (Biomérieux).

At present 49 antibiotics are tested in the GENARS-project. According to the particular needs, resistance outbreaks and the market situation, the antibiotics can be exchanged. For quality assurance each lab tests QC strains regularly. MIC data from all isolates from clinical material enter the data collection. For early recognition of a development of antimicrobial resistance only non-validated MIC's are accepted (see frame "Project-Characteristics"). Due to the weekly data-transmissions to the central office the project may function as an early warning system.

## RESULTS

In the first half of 2002 each QC strain was tested about 130 times by all GENARS centres. The number of tests within a range of  $\pm$  one dilution step of the modal value is close to 100 % (see table 1). For *E. faecalis* (ATCC 29212) and the antibiotics Cefazolin, Ciprofloxacin, Levofloxacin and Meropenem all tests are within this range of  $\pm$  one dilution step of the modal value. In the case of *S. aureus* (ATCC 29213) and the antibiotics Ampicillin and Erythromycin low values were detected.

	Ampicillin	Ampicillin AmSul	Piperacillin	Ceftazidim	Cefotaxime	Cefuroxime	Tobramycin	Ciprofloxacin	Levofloxacin	Meropenem	Erythromycin	Tobramycin
<i>E. coli</i> (ATCC 25922)	94,8	-	97,7	-	-	99,4	-	-	-	-	-	90,2
<i>P. aeruginosa</i> (ATCC 27853)	-	-	92,4	-	91,7	-	-	93,9	99,3	100	-	95,6
<i>E. faecalis</i> (ATCC 29212)	97,7	97,5	-	100	-	-	99,2	100	100	100	97,7	-
<i>S. aureus</i> (ATCC 29213)	71,8	-	-	-	-	-	-	95,5	-	-	-	72,7
<i>S. aureus</i> (ATCC 43300)	-	100	-	-	-	-	98,1	-	-	-	-	98,1

Table 1 Percent of tests which are in the range of  $\pm$  one dilution step of the modal value

Comparing the MIC distributions of QC strains from four centres of the first half 2002 (fig. 2 and fig. 3), with few exceptions a good uniformity of test-quality can be detected. For *P. aeruginosa* (ATCC 27853) and Ciprofloxacin (fig. 2) the modal value peaks clear at a concentration of 0.25 mg/l in all centres. In centre 3, from the 26 measurements two are due to a methodological error. In *E. coli* (ATCC 25922) and Ampicillin (fig. 3) the shape of the distributions of test results is much wider. With the exception of centre 3, the modal values of all other centres are peaking at 4 mg/l. However, using the acceptable limits for QC strains according to DIN (1-8 mg/l) (7) or NCCLS (2-8 mg/l) (5) for this strain and antibiotic nearly all tests of all centres are within these ranges.

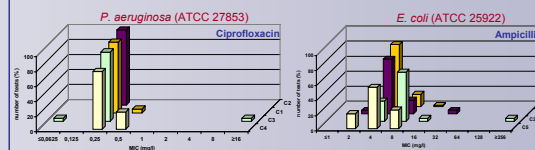


fig. 2 MIC distributions of *P. aeruginosa* (ATCC 27853) and Ciprofloxacin for four centres. Period under observation: first half of 2002. C1 – C4 = centre 1 – centre 4.

fig. 3 MIC distributions of *E. coli* (ATCC 25922) and Ampicillin for four centres. Period under observation: first half of 2002. C1 – C4 = centre 1 – centre 4.

A second tool for quality control is the comparison of MIC-distributions of clinical isolates, an example is shown in fig. 3 for *E. coli* and Cefuroxime for the first half of 2002. For all centres involved, the naturally sensitive population peaks at 4 mg/l. In this way, abnormalities from the typical shape of a MIC-distribution can be easily detected. Quality is checked by matching of the peak values and by the width of distribution.

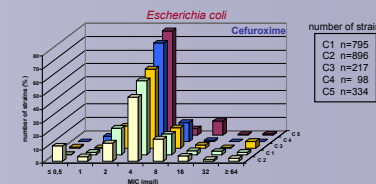


fig. 3 Distribution of MIC-values for *E. coli* and Cefuroxime for five GENARS centres.

## CONCLUSIONS

In the GENARS-project antimicrobial susceptibility against antibiotics is determined by measuring MIC-values in the every day laboratory routine. These non-validated data are the basis for antimicrobial resistance surveillance. For all centres involved, both evaluations of QC strain measurements and comparisons of MIC-distributions of clinical isolates demonstrate the high comparability and reproducibility. The high methodological standard of sensitivity testing of the GENARS-project will not only improve the surveillance including an early warning system, a demonstration of resistance trends and detection of new resistance mechanisms, but will also be advantageous for the patients who benefit from more reliable test results and better hospital epidemiology.

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