Multi-resistance in *P. aeruginosa*: Epidemiological Results from the GENARS-Project

Noll I.[1], Beer J.[2], Huppertz K.[1], Pfister W.[3], Pietzcker T.[4], Schubert S.[5], Wichelhaus T.[6], Ziesing S.[7] and Wiedemann B.[1]

GENARS-Project; Pharmaceutical Microbiology; University of Bonn; Meckenheimer Allee 168; 53115 Bonn; Germany; www.genars.de

GENARS

funded by
Federal Ministry of Health and Social Security

P 1831

REVISED ABSTRACT

Objectives Natural resistance of *P. aeruginosa* to many antibiotics reduces the usefulness of many drugs for treatment of infections with *P. aeruginosa*. Most statistics on resistance report only resistance to specific drugs. However, more important is the knowledge of the epidemiology of multi-resistant strains, because these usually cause difficulties in antibiotic treatment. The GENARS-project (German Network for Antimicrobial Resistance Surveillance) is designed to provide epidemiological data for German university hospitals. Since 2002 resistance data are collected for all clinical relevant pathogens.

Methods Analysis was based on first isolates of *P. aeruginosa* from six laboratories, collected from January 2002 to June 2004. Minimal inhibitory concentrations (MICs) were determined by broth microdilution method (DIN) for ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), meropenem (MER) and piperacillin (PIP). Resistance patterns were evaluated by using break-points according to DIN, grouping susceptible and intermediate as non-resistant; multi-drug resistance was defined as resistance to at least four of the five agents.

Results A total of 6,150 isolates was analysed. 26% of these isolates were resistant to at least one agent: The most common pattern was a mono-drug resistance to GEN (7.4%) followed by CIP (5.1%) and co-resistance to GEN and CIP (4.0%). 1.9% of the isolates were classified as multi-resistant, 0.4% were resistant to all five class representatives. Comparing isolates from cystic fibrosis (CF) patients with those from other patients revealed significant differences with respect to resistance patterns: While the rate of non-resistant isolates among non-CF-patients is 71% it decreases to 47% among CF patients. This corresponds with higher proportions of strains with mono-resistances (33% to 15%) as well as triple-resistances (8.2% to 3.2%) among CF patients.

Conclusions The relevance of multi-resistance in *P. aeruginosa* as a major clinical problem is proven by an overall rate of almost two percent for German university hospitals and an even higher proportion for cystic fibrosis patients. Among the agents tested gentamicin plays an eminent role in regard to its mono-resistant rate as well as a component of the most frequent resistance patterns.

INTRODUCTION AND PURPOSE

Only a limited number of antibiotics is available for the treatment of infectious diseases caused by *P.aeruginosa*, as this species is intrinsically resistant to many drugs and thus multi-resistance causing infectious diseases untreatable with antibiotics are easily selected. Here we present data collected by the GENARS project to elucidate, how common multi-resistant *P.aeruginosa* strains are in german hospitals. As multi-resistance is specially common in patients with cystic fibrosis we compared strains from these patients with data from all other patients.

METHODS

GENARS – funded by the German Federal Ministry of Health and Social Security – is a national network for antimicrobial resistance surveillance. At present, six laboratories affiliated to university hospitals are collecting data continuously for all clinical relevant pathogens in a widely standardized and quality controlled way (1).

Susceptibility tests are performed by determination of minimal inhibitory concentrations (MICs) by broth microdilution method according to DIN guidelines (2), one center provides data achieved by the automated system VITEK 2.

This analysis was based on first isolates of *P. aeruginosa* from six centers, collected from January 2002 to June 2004. MICs were determined for the following five class representatives: ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), meropenem (MER) and piperacillin (PIP). Resistance patterns were evaluated by using breakpoints according to DIN (3); multi-drug resistance was defined as resistance to at least four of the five agents. Data analysis was executed by WHONET software (4), significance tests were computed by Epi Info™(5).

RESULTS

A total of 6,150 isolates was analysed. The number of isolates collected per center varied from 224 to 1,329 due to differences in size and structure of the hospitals. With regard to patient type the sample is composed as follows: 26.2% isolates from patients of intensive care units, 44.2% from non-icu inpatients and 22.9% from outpatients, for the remaining 6.9% information was missing. Of the isolates, 37.2% were from respiratory specimens, 14.0% from urine, 3.4% from blood cultures, and 45.3% from other sites or unknown origin.

Basic information about susceptibility of the *P. aeruginosa* isolates tested is given in terms of SIR proportions (table 1) and more detailed as distributions of MICs (figure 1):

Antimicrobial	MIC breakpoints				
	S (<=)	R (>)	S%	I%	R%
Gentamicin	4	16	27.3	56.4	16.3
Ciprofloxacin	1	2	81.8	5.3	12.9
Piperacillin	1	4	58.9	33.4	7.7
Ceftazidime	4	16	85.8	8.7	5.6
Meropenem	4	32	88.9	8.6	2.5

Table 1: MIC breakpoints according to DIN and susceptibility rates of *P. aeruginosa* (N=6,150)

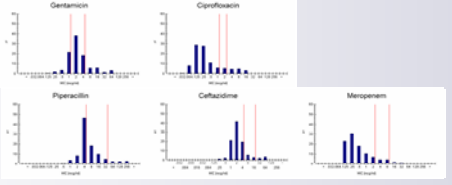

Figure 1: Distributions of MICs of selected antibiotics for *P. aeruginosa* (red lines indicate DIN breakpoints)

Rates of susceptibility were highest with meropenem (88.9%) followed by ceftazidime (85.8%) and ciprofloxacin (81.8%). In contrast, gentamicin showed the highest rate of resistance (16.3%) as well as a very high proportion of intermediate strains (56.4%, see table 1).

Table 2a shows the results of the analysis of resistance patterns. 4,538 isolates (73.8%) were not resistant to any of the selected antibiotics, for the remaining 1.612 isolates 30 different resistance patterns were detected: The most common pattern was a mono-drug resistance to GEN (7.4%) followed by CIP (5.1%) and co-resistance to GEN and CIP (4.0%). 116 isolates (1.9%) were classified as multi-resistant according to our definition - resistance to at least four of the five agents - including 22 strains (0.4%) that were resistant to all five antibiotics (table 2b).

	complete sample		by patient type	
			CF	others
a) resistance pattern	No.	%	%	%
none	4538	73.8	47.0	71.1
GEN	453	7.4	29.6	7.8
CIP	311	5.1	2.7	5.7
PIP	53	0.9	0.9	1.2
CAZ	24	0.4		0.1
MER	20	0.3		0.1
GEN - CIP	243	4.0	4.9	3.2
PIP - CAZ	122	2.0	0.9	2.9
GEN - PIP	41	0.7	1.8	1.8
CIP - MER	35	0.6		0.2
PIP - CIP	18	0.3		0.2
GEN - CAZ	15	0.2	0.6	0.1
GEN - MER	6	0.1		
PIP - MER	2	< 0.1		
CIP - CAZ	2	< 0.1		
CAZ - MER	1	< 0.1		
GEN - PIP - CIP	57	0.9	1.2	1.5
GEN - PIP - CAZ	47	0.8	5.8	0.8
GEN - CIP - CAZ	12	0.2	0.6	0.3
PIP - CIP - CAZ	11	0.2		0.2
GEN - CIP - MER	10	0.2	0.6	0.1
GEN - PIP - MER	7	0.1		0.2
GEN - CAZ - MER	3	< 0.1		
PIP - CAZ - MER	2	< 0.1		0.1
PIP - CIP - MER	1	< 0.1		
GEN - PIP - CIP - CAZ	49	0.8	1.5	1.0
GEN - PIP - CAZ - MER	25	0.4	1.5	0.7
GEN - PIP - CIP - MER	11	0.2		0.1
PIP - CIP - CAZ - MER	7	0.1		0.1
GEN - CIP - CAZ - MER	2	< 0.1		0.1
GEN - PIP - CIP - CAZ - MER	22	0.4	0.3	0.4
b) number of resistances				
none	4538	73.8	47.0	71.1
one	861	14.0	33.2	14.9
two	485	7.9	8.2	8.4
three	150	2.4	8.2	3.2
four or five = multi-resistant	116	1.9	3.4	2.4
total no. of isolates	6150		328	1001

Table 2: resistance patterns and number of resistances in *P. aeruginosa*
center columns: complete sample (N=6,150)
right columns: by patient type (CF- cystic fibrosis; data from center 2; N=1,329)

Multi-resistance in *P. aeruginosa* is known as a major problem in the treatment of cystic fibrosis patients. To assess differences in resistance patterns between cystic fibrosis versus other patients data of one center specialized in the treatment of these patients were analyzed; the results are displayed in the right half of table 2. The two patient samples differ significantly with regard to number of resistances of the strains ($Chi^2 = 79.97$; df = 4; p<= 0.0001):

Among cystic fibrosis patients strains with mono-drug resistances as well as triple-resistances are more than twice as frequent as in other patients (see figure 2).

Figure 2: Comparison of resistance in *P. aeruginosa* for CF versus other patients

CONCLUSIONS

Especially with *P. aeruginosa* it is often difficult to put the MIC measurements into the categories S, I,and R. MIC distributions show that the breakpoints used usually do not clearly separate resistant strains from the rest of the population. Many so called intermediate strains might already have genetic alterations. The naturally susceptible gentamicin distribution e.g. falls into the intermediate category.

The data demonstrate that most *P. aeruginosa* isolates are still treatable with antibiotics. However, 22 strains were completely resistant, not treatable with any antibiotic. These strains were not only from patients with cystic fibrosis but also from other severely ill patients, in different hospitals. Studies to elucidate possible clonal spread are on the way.

The most striking difference between CF isolates and the rest is with the number of sensitive isolates being low in the CF isolates and the high number of strains with 3 resistance markers in these patients.

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GENARS project-group:

1) GENARS-Office, c/o Institute of Pharmaceutical Microbiology, University of Bonn
2) Institute of Medical Microbiology and Epidemiology of Infection, University Hospital Leipzig
3) Institute of Medical Microbiology, University of Jena
4) Institute of Microbiology and Immunology, University of Ulm
5) Institute of Medical Microbiology and Virology, University Hospital of Schleswig-Holstein, Campus Kiel
6) Institute of Medical Microbiology, University of Frankfurt a.M.
7) Institute of Medical Microbiology and Hospital Epidemiology, Medical School Hannover