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funded by
Federal Ministry of Health and Social Security

REVISED ABSTRACT

Objectives The increasing resistance of *Enterobacteriaceae* to mono-bactams and oxyimino-cephalosporines like cefotaxime, ceftazidime, ceftriaxone and cefpodoxime is due to an increasing spread of strains expressing extended spectrum β -lactamases (ESBL). ESBL rates of more than 50 % have already been reported for southern European countries. For Germany, Austria and Switzerland a multi-center study of the Paul Ehrlich Society (PEG) in 2001 detected ESBL-rates of 0.8 % for *E. coli* and 8.2 % for *K. pneumoniae* (1). More recent and detailed information about the occurrence of ESBL phenotypes in Germany is provided by the GENARS project funded by the German Federal Ministry of Health and Social Security.

Methods All laboratories involved determine antimicrobial susceptibility by testing the Minimal Inhibitory Concentration (MIC) in the laboratory routine for about 25 different antibiotics. According to NCCLS guidelines, some of these antibiotics tested, could be used as indicators for an ESBL screening (cefotaxime, ceftazidime, cefpodoxime). The ratio of cefpodoxime and cefpodoxime/clavulanic acid was used for phenotypical confirmation.

Results In the 1st half of 2004, the percentage of strains with a positive ESBL-screening amounts to 4.3 % and to 10.5 % for *E. coli* (n=1944) and *K. pneumoniae* (n=382), respectively. Phenotypically confirmed as ESBL are 1.7 % of all *E. coli* strains and 7.1 % of all *K. pneumoniae* strains. In both species strains were detected in which ESBLs are phenotypically confirmed, but where NCCLS parameters for initial screening failed. Strains with a MIC for cefpodoxime of ≥ 8 mg/L which can not be reduced by clavulanic acid are identified as AmpC expressing strains (*E. coli* 1.1 %; *K. pneumoniae* 1.8 %). In GENARS hospitals ESBL rates differ considerably (*E. coli* : 1.1 % - 4.7 %; *K. pneumoniae*: 1.4 % - 13.4 %).

Conclusions To prevent spread in hospitals, cities and countries, a detailed surveillance of ESBL-strains is necessary. To reach this goal an improvement of our epidemiological knowledge about the occurrence and spread of ESBL-strains is needed. In Germany the MIC-determination as demonstrated for GENARS hospitals allows a correct detection of ESBL producing strains.

INTRODUCTION AND PURPOSE

The increasing resistance of *Enterobacteriaceae* to mono-bactams and oxyimino-cephalosporines like cefotaxime, ceftazidime, ceftriaxone and cefpodoxime is due to an increasing spread of strains expressing extended spectrum β -lactamases (ESBL). ESBL rates of more than 50 % have already been reported for southern European countries. For Germany, Austria and Switzerland a multi-center study of the Paul Ehrlich Society (PEG) in 2001 detected ESBL-rates of 0.8 % and 8.2 % for *E. coli* and *K. pneumoniae*, respectively. In this study we want to provide more recent and de-tailed information about the occurrence of ESBL phenotypes in Germany by analysis of the GENARS database. In addition we want to find evidence for the occurrence of plasmid mediated AmpC β -lactamases.

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 (2). Susceptibility tests are performed by determination of minimal inhibitory concentrations (MICs) by broth microdilution method according to DIN guidelines (3).

Analysis was based on first isolates of *E. coli* and *K. pneumoniae* from three centers, collected from January 2004 to June 2004. Analysis was restricted to these three centers, because only these used the relevant antibiotics in sufficient concentrations for the evaluation.

All laboratories involved determine antimicrobial susceptibility by testing the Minimal Inhibitory Concentration (MIC) in the laboratory routine for about 25 different antibiotics, including ceftazidime (CAZ), cefotaxime (CTX), cefpodoxime (CPP) and cefpodoxime/clavulanic acid (CPC). According to NCCLS guidelines (4), these antibiotics can be used as indicators for an

> ESBL screening: CAZ ≥ 2 mg/L or CTX ≥ 2 mg/L or CPP ≥ 8 mg/L

> ESBL phenotypical confirmation: ratio of CPP and CPC acid ≥ 8

> AmpC β -lactamase: ratio of CPP and CPC acid ≤ 1

Data analysis was executed by WHONET software (5).

RESULTS

Evaluation of data is shown in table 1 for *E. coli* and table 2 for *K. pneumoniae*. The corresponding summaries are depicted in table 3.

While ESBLs can be detected by the inhibition with clavulanic acid, AmpC β -lactamases are not or nearly not inhibited with clavulanic acid. As *K. pneumoniae* strains naturally only produce class A β -lactamases, which can be inhibited by clavulanic acid, resistance to cefpodoxime, which cannot be reduced by the enzyme inhibitor is an indicator of the presence of a plasmid coded AmpC β -lactamase. As *E. coli* naturally produces a small amount of AmpC β -lactamase, in this species the presence of a resistance to cefpodoxime, which cannot be reduced by clavulanic acid indicates the presence of a mutation in the chromosome which leads to an increased production of the enzyme or the presence of a plasmid coded AmpC β -lactamase.

Strains which showed an MIC ratio of cefpodoxime to cefpodoxime/clavulanic acid of 2 – 4 were categorised as indifferent. These strains have an elevated MIC towards at least one of the cephalosporins in question but the ratio did not confirm them as ESBLs. These strains might have a mixture of AmpC and ESBLs or may be hyperproducers of broad spectrum plasmid encoded β -lactamases.

RESULTS continued

	Ratio CPP/CPC				No.	% of total
	<=1	2 or 4	>=8	nd		
Screening negative		1859	1		1860	95.7
positive	21	20	32	11	84	4.3
No.	21	1879	33	11	1944	
% of total	1.1	96.7	1.7	0.6		100.0

Amp C indifferent	CPP/CPC<=1
ESBL	CPP/CPC=2 or 4
undefined	CPP/CPC>=8
	CPP and/or CPC >32

Tab.1: ESBL in *E. coli* strains, screening and confirmational test

	Ratio CPP/CPC				No.	% of total
	<=1	2 or 4	>=8	nd		
Screening negative		340	2		342	89.5
positive	7	4	25	4	40	10.5
No.	7	344	27	4	382	
% of total	1.8	90.1	7.1	1.0		100.0

Amp C indifferent	CPP/CPC<=1
ESBL	CPP/CPC=2 or 4
undefined	CPP/CPC>=8
	CPP and/or CPC >32

Tab. 2: ESBL in *K. pneumoniae* strains, screening and confirmational test

Result of ESBL screening and confirmation test	<i>E. coli</i>		<i>K. pneumoniae</i>	
	No.	%	No.	%
ESBL	33	1.7	27	7.1
AmpC indifferent	21	1.1	7	1.8
	20	1.0	4	1.0
Total no. of isolates	1944	100.0	382	100.0

Tab. 3: ESBLs and AmpC β -lactamases in *E. coli* and *K. pneumoniae*

The high proportion of AmpC β -lactamases in *K.pneumoniae* demonstrates the wide spread of plasmid encoded AmpC β -lactamases in these pathogens.

RESULTS continued

ESBL rates in the three centers are not evenly distributed. While center A has a high ESBL rate in *E. coli*, center B has a high frequency of ESBLs in *Klebsiella*.

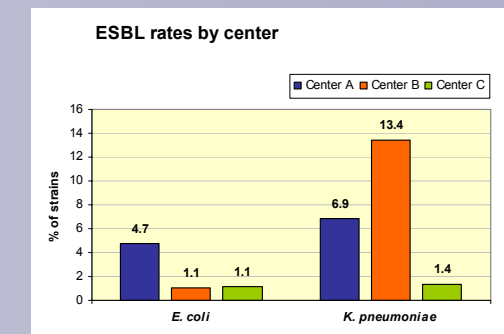


Fig 1: ESBL rates in *E. coli* and *K. pneumoniae* in three centers

CONCLUSIONS

1. With routine MIC testing it is possible to detect ESBL producing pathogens quickly, if the range of tested concentrations is sufficient.
2. In general, ESBL producing *E. coli* and *K. pneumoniae* are still rare in German hospitals.
3. ESBLs are not evenly distributed but seem to spread in specific centers.
4. Plasmid encoded AmpC β -lactamases become more and more common.

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GENARS centers involved:

- 1) GENARS-Office, c/o Institute of Pharmaceutical Microbiology, University of Bonn
- 2) Institute of Medical Microbiology, University of Jena
- 3) Institute of Microbiology and Immunology, University of Ulm
- 4) Institute of Medical Microbiology and Hospital Epidemiology, Medical School Hannover