TEMOCILLIN, CEFEPIME AND MEROPENEM SUSCEPTIBILITY OF ESBL-PRODUCING ENTEROBACTER AEROGENES IN BELGIAN HOSPITALS

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ABSTRACT

Background: Treatment options for infections with Extended spectrum β-lactamase (ESBL) producing Enterobacter aerogenes are limited because many β-lactam antibiotica are hydrolysed by ESBLs and because ESBL-producing bacteria are often resistant to other classes of antibiotics. We performed pulsed field gel electrophoresis (PFGE), susceptibility testing for temocillin, cefepime and meropenem and detection and ESBL-characterisation on ESBL-producing E. aerogenes strains.

Methods: On 150 consecutive E. aerogenes strains, collected in 2005 in 5 Belgian hospitals, we performed ESBL-detection by double disk synergy test, ESBL E-test (AB Biodisk.) and PCR for the presence of blaTEM, blaSHV and blaCTX-M genes.

PFGE analysis after XBA1 restriction was performed on the Genepath system (Bio-Rad). For ESBL-producing strains, minimal inhibitory concentrations (MICs) were determined for temocillin with broth microdilution and for cefepime and meropenem with E-test (AB Biodisk).

Results: ESBL-production was detected in 62 (41.3%) strains. Of these, 60 (96.8%) were positive for TEM-24, 1 (1.6%) for TEM-52 and 1 (1.6%) for SHV 4. PFGE analysis revealed two major clones. All TEM-24 positive strains belonged to the same clone.

Of all ESBL-producing strains, 57 (91.9 %) were susceptible to temocillin. All 63 ESBL positive strains (100%) were susceptible to cefepime and meropenem, with MICs < 1 µg/mL for most strains (90.5% for cefepime, 96.7% for meropenem).

Conclusions: ESBL-production was detected in 41.3% of E. aerogenes strains, 96.8%of these strains belonged to the same TEM-24 positive clone. All ESBL producing strains were susceptible to cefepime and meropenem, 91.9% was susceptible to temocillin.

INTRODUCTION

Since the first isolation of ESBLs in Germany from *Klebsella pneumoniae* strains in 1983, several outbreaks caused by ESBL producing *Enterobacteriaceae* have been reported worldwide in hospitals, particularly in intensive care units (1, 2).

At present, more than 200 different ESBLs have been characterized, (website on nomenclature of ESBLs, by G. Jacoby and K. Bush (http://www.lahey.org/studies/webt.htm).

Treatment options of infections with ESBL-producing bacteria are limited because many β–lactam antibiotica are hydrolysed by ESBLs and because ESBL producing bacteria are often resistant to other classes of antibiotics such as aminoglycosides and fluoroquinolones. The role of cefepime in the treatment of infections with ESBL-producing organisms is unclear, mostly because of the limited clinical experience on this issue (3, 4).

Temocillin is a methoxy-derivate of ticarcillin with increased β-lactamase stability, active against Enterobacteriaceae.

In this study we performed susceptibility testing for temocillin, cefepime and meropenem, detection and characterisation of ESBLs and pulsed field gel electrophoresis (PFGE) on 151 consecutive Enterobacter aerogenes strains, collected in the first 3 months of 2005 in 5 Belgian Hospitals.

METHODS (1)

Bacterial strains: origin and identification.

Between January 1st and March 31st 2005, 160 consecutive isolates of *E. aerogenes* were collected in 5 Belgian hospitals (Imelda Hospital, Bonheiden, Hospital Oost-Limburg, Genk, Onze-Lieve Vrouw Hospital, Aalst, Sint-Lucas Hospital, Ghent, Virga Jesse Hospital, Hasselt) from clinical samples of hospitalized and ambulant patients.

After primary bacterial identification by routine procedures of each laboratory, strains were frozen at –70°C till further analysis.

Identification of the strains was confirmed by Phoenix (Becton-Dickinson, Sparks, MD, USA) using the Combo panel (NMIC/ID-51), and by Vitek 2 (bioMérieux, Marcy l'étoile, France) using the Vitek 2 colorimetric GN Card. In case of any discrepancy, strains were identified by API 20E (Biomérieux, Marcy L'Étoile, France) and by sequencing of a 570 base pair long amplicon of the gene coding for the small subunit of 16S ribosomal RNA. The obtained sequences were compared with sequences in Genbank (National Centre for Biotechnology Information) (http://www.ncbi.nih.gov/BLAST). Nine strains were excluded because of false identification or contamination.

Antibiotic susceptibility testing.

The minimal inhibitory concentrations (MICs) for cefepime and meropenem were measured on Mueller-Hinton II agar (Becton Dickinson, Sparks, MD, USA) with E-test (AB Biodisk, Solna, Sweden) and results were categorized according to the Clinical and Laboratory Standards Institute breakpoints (5). MICs for temocillin were determined with broth microdilution and results were interpreted according to breakpoints established by Fuchs et al. (6).

METHODS (2)

Detection of ESBL-production.

All strains were examined for ESBL-production by the double disk synergy test (ceftazidime, cefotaxime and cefepime) (DD) and by the ESBL E-test (AB Biodisk, Solna, Sweden). For the ESBL E-test, a test result was considered as positive in the presence of a "phantom" zone or a distortion of the ellipse or a decrease in MIC of cefepime of more than 3 dilutions in the presence of clavulanic acid.

DNA macrorestriction and PFGE analysis.

DNA macrorestriction with the Genepath reagent kit Group 6 using the XBA1 restriction enzyme (Bio-Rad, Hercules, USA) and PFGE analysis on Genepath system (Bio-Rad, Hercules, USA) were performed according to the manufacturer's instructions. The PFGE patterns were analysed and clustered into dendrograms with the Fingerprinting II software (Bio-Rad, Hercules, USA). Interpretation was done using the criteria of Tenover et al. (7).

Amplification and sequencing of of β-lactamase (BLA) genes:

Strains suspected of ESBL production based on DD or E-test, were examined for the presence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} by polymerase chain reaction (PCR) on a DNA Peltier Thermal Cycler 200 (MJ Research, Nevada, USA) using sequence specific primers (tabel 1). The gene encoding for the 16s rRNA was used as an amplification control. The PCR products were separated on a 1.5 % agarose gel stained with ethidium bromide. Isolates positive for bla_{TEM} were examined for the presence of bla_{TEM-24} with sequence specific primers (table 1). PCR products generated with the consensus primers for the different genes, but negative for bla_{TEM-24} were used for direct sequencing. The PCR products were purified with the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Dye terminator cycle sequencing was performed on a PTC-200 (MJ Research, Nevada, USA) using the CEQ DTCS Quick Start Kit (Beckman Coulter, Fullerton, USA) according to the procedure described by Beckman Coulter.

Sequencing products were purified by ethanol precipitation according to the procedure described by Beckman Coulter, followed by analysis on a CEQ8000 Genetic Analysis System (Beckman Coulter, Fullerton, USA). The obtained sequences were compared with sequences in Genbank (National Centre for Biotechnology Information) (http://www.ncbi.nih.gov/BLAST) *and with sequences on the website on the ESBL nomenclature* (http://www.lahey.org/studies/webt.htm).

Gene	*Primer sequences*
bla_{TEM}	*ATGAGTATTMAACATTTCCG, CCAAWGCTTAATCAGTGAGG*
bla_{TEM}	*ATGGATCCTCAACATTTCCG, CCAAWGCTTAATCAGTGAGG*
bla_{SHV}	*ATGCGTTWTDTTCGCCTGTG, AGCGTTGCCAGTGCTCGATC*
bla_{CTX-M}	*SCVATGTGCAGYACCAGTAA, ACCAGAAYVAGCGGBGC*
bla_{TEM-24}	*GGGCAAGAGCAACTCGGT, AGACCCACGCTTACCGGT*
16S	*AGAGTTTGATCCTGGYTCAG, CTTTACGCCCARTAAWTCCG*

RESULTS

ESBL production was detected in 62 (41.3%) strains. Of these, 60 (96.8%) were positive for TEM-24, 1 (1.6%) for TEM-52 and 1 (1.6%) for SHV 4.

None of the strains was positive for CTX-M.

No strains were positive for more than 1 ESBL-type.

PFGE revealed two major clones. All TEM-24 positive strains belonged to the same clone. The TEM-52 and SHV-4 positive strains belonged to the other major clone.

ESBL negative	ESBL positive		
88 (58.7%)	62 (41.3%)		
	TEM	SHV	CTX-M
	61 (98.4%)	1 (1.6%)	0 (0%)
	TEM-24	TEM-52	SHV-4
	60 (96.8%)	1 (1.6%)	1 (1.6%)
	Clone 1: 60 (100%)	Clone 2: 1 (100%)	Clone 2: 1 (100%)

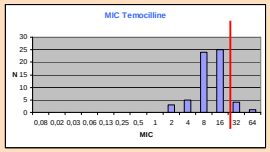
Of all ESBL producing strains, 57 (91.9 %) were susceptible to temocillin.

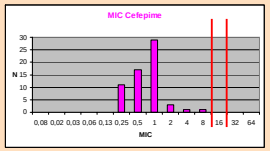
All 5 (8.1%) resistant strains had TEM-24 ESBL-type.

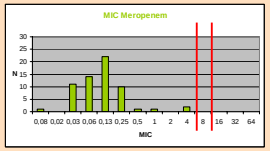
All ESBL producing strains, were susceptible for cefepime, with the following MIC distribution: 11 strains (17.7%) with a MIC of =< 25 µg/mL, 17 strains (27.4%) with a MIC of 1 µg/mL, 29 strains (46.8%) with a MIC of 1 µg/mL, 3 (4.8%) with a MIC of 2 µg/mL, 1 (1.6%) strains with a MIC of 4 µg/mL and 1 (1.6%) strain with a MIC of 8 µg/mL.

All 5 strains (8.1%) with a MIC for cefepime > 1 µg/mL were TEM-24 positive.

All 150 strains were also susceptible for meropenem. For the 62 ESBL-positive strains, the MIC distribution was as follows: 26 strains (41.9%) with a MIC of =< 0.064 µg/mL, 22 strains (35.5%) with a MIC of 0.125 µg/mL, 10 strains (16.1%) with a MIC of 0.25 µg/mL, 1 strain (1.6%) with a MIC of 0.5 µg/mL, 1 strain (1.6%) with a MIC of 2 µg/mL and 2 strains (3.2%) with a MIC of 4 µg/mL.







	TEM-24	TEM-52	SHV-4
Temocillin	S: 55(90.1%) R: 5 (8.9%)	S: 1 (100%) R: 0 (0%)	S: 1 (100%) R: 0 (0%)
Cefepime	S: 60 (100%) R: 0 (0%)	S: 1 (100%) R: 0 (0%)	S: 1 (100%) R: 0 (0%)
Merpenem	S: 60 (100%) R: 0 (0%)	S: 1 (100%) R: 0 (0%)	S: 1 (100%) R: 0 (0%)

DISCUSSION

In 2001 De Gheldre and colleagues published the results of two national surveys of *Enterobacter aerogenes* in Belgian Hospitals, performed in 1996 and 1997. The authors describe the dissemination of two epidemic multiresistant E. aerogenes strains in Belgian hospitals (BE1 and BE2). Almost half of the strains produced an ESBL, 86% was positive for TEM-24 and 16% for TEM-3 (8). In our study, 41.3% of all *E. aerogenes* strains were ESBL-positive, 96.8% of these strains were positive for TEM-24, 1.6% for TEM 52 and 1.6% for SHV 4. None of our strains was positive for TEM-3.

Treatment options of infections with ESBL-producing bacteria are limited because many β–lactam antibiotica are hydrolysed and the plasmids harbouring the genes encoding ESBLs frequently carry genes encoding resistance to aminoglycosides and trimetoprim-sulfamethoxazole. Furthermore, there is a strong association between resistance to fluoroquinolones and ESBL-production (2, 9).

Temocillin is a semisynthetic 6-α-methoxy derivate of ticarcillin active on Enterobacteriaceae and stable against β-lactamases, including AmpC and some extended spectrum β-lactamases (ESBL). Despite this interesting feature, the lack of in vitro and in vivo studies hampers the breakthrough of this antibiotic in clinical use.

Actually, there is agreement that third-generation cephalosporins should not be used in infections with ESBL-producing bacteria (2, 10). The role of cefepime however remains unclear, due to the limited clinical experience on this issue (3, 4). At present, guidelines don't recommend cefepime as a first-line therapy against ESBL-producing organisms. If nevertheless cefepime is used (for example, against organisms with a cefepime MIC of <2 µg/ml), it should be used in high dosage (at least 2 g twice a day) (2).

In a randomized, international multicenter trial, in which cefepime was compared with imipenem-cilastin for the treatment of nosocomial pneumonia, cefepime appeared to be less active against ESBL-producing organisms (11). The susceptibility of ESBL-producing bacteria to cefepime is also dependent on the type of ESBL. CTX-M type ESBLs hydrolyze cefepime with high efficiency, and MICs for cefepime are higher than in bacteria producing other ESBL types (12, 13). A Belgian retrospective study by Goethaert et al. published in 2005, evaluated retrospectively the efficacy of treatment with cefepime versus a carbapenem, in combination with amikacin or ciprofloxacin, in infections of intensive care patients with ESBL-producing *E. aerogenes*. Although there was no significant difference in outcome parameters (clinical improvement, bacteriological eradication) between the cefepime and the carbapenem group, a statistically significant increase in failure to eradicate ESBL-producing *E. aerogenes* was observed as the MIC of cefepime rose (14). In our study all *E. aerogenes* strains were susceptible for cefepime. For 57 of the 62 ESBL-positive strains (91.9%) the MIC was ≤ 1 µg/mL. All 151 *E. aerogenes* strains were also susceptible for meropenem. MIC-values were lower than 1 µg/mL in 95% of the ESBL-producing strains .

CONCLUSIONS

- **ESBL-production was detected in 41.3% of the Belgian E. aerogenes strains.**
- **96.8% of these ESBL-positive strains belonged to the same TEM-24 positive clone.**
- **All ESBL positive strains were susceptible to cefepime and meropenem, 91.9% was susceptible to temocillin.**

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