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ARTICLE

In vitro activity of temocillin against prevalent extended-spectrum beta-lactamases producing *Enterobacteriaceae* from Belgian intensive care units

Y. Glupczynski · T.-D. Huang · C. Berhin · G. Claeys · M. Delmée · L. Ide · G. Ieven · D. Pierard · H. Rodriguez-Villalobos · M. Struelens · J. Vaneldere

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Abstract Temocillin is a narrow spectrum penicillin with high stability to most β-lactamases including AmpC types and extended-spectrum types (ESBLs). We have analysed its in vitro activity against 652 clinical isolates of *Enterobacteriaceae* prospectively collected from patients hospitalised in intensive care units at seven different university hospitals in Belgium in 2005. Strains were screened for ESBL production using cefotaxime and ceftazidime screen agar plates and by double ESBL E-tests. The MIC of temocillin and of five comparators was determined using the E-test method. ESBLs were characterized at one central laboratory by isoelectric focusing, PCR for *bla* genes of the SHV, TEM, and CTX-M families, and by DNA sequencing. The prevalence of ESBL-producing *Enterobacteriaceae* averaged 11.8% and ranged between 3.0 and 29% in the different hospitals. Meropenem exhibited the highest in vitro activity overall (mode MIC 0.064 μg; MIC_{90}; 0.19 μg/ml), whereas ceftazidime (MIC_{90}>256 μg/ml) and ciprofloxacin (MIC_{90}>32 μg/ml) scored the worst. Temocillin was active against more than 90% of the isolates including most AmpC- and ESBL-producing isolates. These data indicate the well preserved activity of temocillin over the years against *Enterobacteriaceae* and show the wide variation in prevalence of ESBL-producing *Enterobacteriaceae* isolates in Belgian intensive care units. Prospective clinical studies are, however, needed to validate the usefulness of temocillin in the treatment of microbiologically documented infections caused by ESBL- and/or AmpC- overproducing nosocomial *Enterobacteriaceae* pathogens.

Y. Glupczynski (✉) · T.-D. Huang · C. Berhin
Cliniques universitaires UCL de Mont-Godinne,
Laboratoire de Microbiologie,
5530 Yvoir, Belgium
e-mail: youri.glupczynski@skynet.be

G. Claeys
Laboratorium Microbiologie, Universitair Ziekenhuis Gent,
9000 Gent, Belgium

M. Delmée
Cliniques universitaires UCL Saint-Luc, Unité de Microbiologie,
1200 Brussels, Belgium

L. Ide · J. Vaneldere
UZ KU Leuven, Laboratorium Microbiologie,
3000 Leuven, Belgium

G. Ieven
UZ Antwerpen, Laboratorium Microbiologie,
2650 Edegem, Belgium

D. Pierard
AZ VUB Jette, Laboratorium Microbiologie,
1090 Brussels, Belgium

H. Rodriguez-Villalobos · M. Struelens
Hôpital universitaire Erasme ULB, Laboratoire de Microbiologie,
1070 Brussels, Belgium

Introduction

Temocillin is the 6-α-methoxy derivative of ticarcillin. This structural modification increases stability to AmpC β-lactamases as well as to extended-spectrum beta-lactamase (ESBLs) enzymes, but reduces its activity against *Pseudomonas aeruginosa*, *Acinetobacter* spp. and almost completely removes its affinity for the essential penicillin-binding proteins of Gram-positive bacteria and anaerobes (*Bacteroides fragilis* and others) [1]. Overall temocillin appears as a narrow spectrum antimicrobial agent with very consistent activity against *Enterobacteriaceae* [2, 3] and selectively against some glucose non-fermenting Gram-negative bacilli (e.g. *Burkhol-*

deria cepacia, *Delftia acidovorans* and *Comamonas* spp.) [4, 5] that may be involved in lung infection among patients with cystic fibrosis.

Temocillin marketed in Belgium and in the UK in the late eighties by Beecham Pharmaceuticals and presently licensed to Eumedica has been used on a limited scale in these two countries in microbiologically documented therapy of infections known to be caused by pathogens with potent β-lactamases. In Belgium, temocillin is currently recommended for parenteral treatment of acute pyelonephritis (Belgium Antibiotic Politic Coordination Committee guidelines: www.health.fgov.be/vesalius) and it has been approved recently in the US for treatment of *Burkholderia cepacia* lung infection in cystic fibrosis [5]. Recent studies on the in vitro activity of temocillin are limited [6–8]. One multicentric survey in Belgium showed susceptibility rates of 79–100% among clinical isolates of *Enterobacteriaceae* collected from hospitalized patients [6]. Another local survey indicated good activity of temocillin against multiresistant ESBL-producing *Escherichia coli* isolates (92% of the strains were found susceptible to temocillin) [7], while a recent study from the UK showed that nearly 90% of the AmpC- and ESBL-producing isolates *Enterobacteriaceae* isolates were susceptible to temocillin [8].

Owing to the increasing incidence of infections caused by multi-drug resistant Gram-negative bacteria and also because of the limited number of clinically active drugs available against such organisms, we aimed to determine the prevalence of ESBL-producing *Enterobacteriaceae* in several Belgian intensive care units (ICUs) and to assess the in vitro activity of temocillin against these isolates.

Materials and methods

Bacterial strains

Non-duplicate, consecutive clinical isolates of various *Enterobacteriaceae* were prospectively obtained from patients hospitalized in ICUs for more than 72 h in order to enrich the collection of organisms as much as possible on nosocomial pathogens. The survey took place at seven different university hospitals distributed throughout the country during a 6-month period in 2005. Each laboratory collected up to 100 clinical isolates. Origins of the specimens included lower respiratory tract (sputum, bronchial or endotracheal aspirates, broncho-alveolar lavage), pus, wounds and blood samples. Routine screening cultures (i.e. rectal swabs and stools) as well as screening samples from environmental sources were excluded. Urine specimens were not retained because nosocomial urinary tract infections seldom occur in patients hospitalized in ICUs and the clinical significance of positive urine cultures in patients with bladder catheters is most often uncertain.

Identification of the isolates was performed at each participating laboratory following its routine methodology. All strains were stored at −80°C and were centralized at one laboratory (UCL Mont-Godinne University Hospital) for susceptibility testing, confirmation of ESBL production and characterization of the enzymes involved in resistance.

Susceptibility testing

MICs were determined by E-test method (AB BIODISK, Solna, Sweden) for amikacin, ciprofloxacin, ceftazidime, meropenem, temocillin and piperacillin/tazobactam (only for ESBL-producing isolates) according to the manufacturer's instructions and results were categorized using breakpoints and interpretive criteria provided by the CLSI [9]. Susceptibility to temocillin was determined according to the breakpoint values provided by Fuchs et al. [10]: susceptible if MIC≤16 μg/ml and resistant if MIC≥32 μg/ml. The *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 (SHV-18 ESBL-producing) strains were used at least twice as controls to validate the MIC results and ESBL detection procedures.

Detection of ESBL production

Screening of ESBL production was carried out using a species-adapted algorithm. All *Escherichia coli* and *K. pneumoniae* isolates were subcultured on two different selective Mueller-Hinton agar plates (Oxoid, Basingstoke, UK) containing cefotaxime (0.5 μg/ml) and ceftazidime (0.5 μg/ml). Colonies growing on one or both ESBL screening plates were further tested by ESBL E-tests (ceftazidime/ceftazidime-clavulanate and cefotaxime/cefotaxime-clavulanate) (AB BIODISK, Solna, Sweden) for confirmation of ESBL production.

Isolates of *K. oxytoca* were assessed for ESBL production by double ESBL E-test using ceftazidime/ceftazidime-clavulanate when ceftazidime MIC was≥4 μg/ml.

For AmpC inducible *Enterobacteriaceae* isolates (*Citrobacter freundii, Enterobacter* spp., *Serratia* spp., *Providencia* spp. and *Morganella morganii*), strains displaying ceftazidime MICs≥4 μg/ml were also evaluated for ESBL production by cefepime/cefepime-clavulanate ESBL E-test strips as recommended by Stürenburg et al. [11].

An ESBL MIC ratio≥8 of any of the tested cephalosporin substrates alone versus its combination with clavulanic acid was used as an indicator of ESBL production. Isolates with ceftazidime MIC>2 μg/ml and for which no ESBL production could be detected by phenotypic tests were considered as probable AmpC hyperproducers.

Characterization of ESBLs

Isoelectric focusing was performed on crude bacterial extracts frozen/thawed several times from bacterial growth in exponential phase in brain heart infusion medium using the Phastsystem apparatus (Pharmacia AB, Uppsala, Sweden). Isoelectric points (PIs) were determined by comparing PI values of selected β-lactamases with known PIs (5.4, 6.3, 6.5, 7.6, 8.1) after staining of gels with nitrocefin (500 μg/ml).

ESBLs were further characterized by PCR for *bla* genes of TEM, SHV, and CTX-M families for all isolates. Detection of bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ gene families was performed with PCR using specific primers designed in house as described previously [12]. PCR products were analysed on a 1% agarose gel. Identification of TEM, SHV and CTX-M types and subgroups was performed on selected strains using DNA sequencing of full-length ESBL gene PCR products (ABI 3100, Perkin Elmer). Sequence homology was determined using BLAST for comparison in gene Bank databases. Selected strains of the same species showing homology in the resistance profiles and resistance mechanisms were analysed.

Results

In total, 652 isolates (mean number of isolates per hospital: 93; range: 65–100) were tested. Fifty percent (330/652) of the isolates originated from the lower respiratory tract, 28% (182/652) from pus or wounds, 7% (45/652) from blood and 15% (95/652) from other body sites.

Escherichia coli was the most prevalent species (186 isolates [29%]), followed by *Enterobacter cloacae* (115 isolates; 18%), *Klebsiella pneumoniae* (76 isolates; 12%) *Enterobacter aerogenes* (75 isolates; 11.5%) and *Klebsiella oxytoca* (9.5%). Concerning groups of organisms, the non-inducible *Enterobacteriaceae* spp (*Escherichia coli*, *Klebsiella* spp. and *Proteus* spp.) were more prevalent (57%) than the inducible AmpC-producing species (*Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *M. morganii* and *Providencia* spp.) which represented 43% of all isolates. Overall, meropenem displayed the highest activity (MIC_{50} of 0.064 μg/ml and less than 1% of the strains being non-susceptible) followed by amikacin (MIC_{50} of 2 μg/ml; 5% non-susceptible) and temocillin (MIC_{50} of 4 μg/ml; 8% non-susceptible isolates). Ceftazidime and ciprofloxacin were found to be less active (20% non-susceptible strains). The proportion of non-susceptible strains by species is shown in Table 1. *Enterobacter aerogenes* accounted as the most resistant species (66.7 and 57.0% of isolates non-susceptible to ceftazidime and to ciprofloxacin, respectively) while high resistance rates (around 30%) to ceftazidime were observed in *Enterobacter cloacae*, *C. freundii* and in *K. pneumoniae* isolates. Among *Escherichia coli*, the highest level of resistance was found for ciprofloxacin (17 %). Globally, temocillin had good activity against all *Enterobacteriaceae* species except against *Enterobacter aerogenes* and *Serratia marcescens* which were found less susceptible (25% non susceptibility for each of these two species).

Table 2 shows the MIC distribution of temocillin against a subset of *Enterobacteriaceae* isolates with defined resistance mechanisms. In all, 86% of the *Enterobacteriaceae* isolates with hyperproduced or acquired AmpC were susceptible to temocillin at ≤16 μg/ml (modal MIC=16 μg/ml). Likewise, ESBL-producing isolates had a temocillin modal MIC of 16 μg/ml with the single exception of ESBL and AmpC co-

Table 1 Number and percentage of non-susceptible isolates to the tested antimicrobials, classified by species

	Number of non-susceptible isolates (%)				
Species[a] (No. isolates)	Ceftazidime	Meropenem	Temocillin	Amikacin	Ciprofloxacin
Escherichia coli (186)	10 (5.4)	0	4 (2.2)	2 (1.2)	32 (17.2)
Enterobacter cloacae (115)	38 (33)	0	7 (6.1)	3 (2.6)	5 (4.3)
K. pneumoniae (75)	24 (32.0)	0	9 (12)	11 (14.7)	16 (21.3)
Enterobacter aerogenes (72)	48 (66.7)	2 (2.8)	18 (25)	11 (15.3)	41 (57)
K. oxytoca (62)	3 (4.8)	0	3 (4.8)	1 (1.6)	18 (29)
S. marcescens (39)	1 (2.6)	0	10 (25.6)	2 (5.1)	8 (20.5)
Proteus mirabilis (35)	0	0	1 (2.9)	0	5 (14.3)
M. morganii (27)	5 (18.5)	0	0	0	2 (7.4)
Citrobacter freundii (15)	4 (26.7)	0	0	1 (6.7)	2 (13.3)
Total[a] (652)	134 (20.5)	2 (0.4)	53 (8.1)	31 (4.8)	131 (20.1)

[a] In addition to the species groups detailed, this total also includes: *Proteus vulgaris* (9); *Hafnia* spp. (6), *Citrobacter diversus* (5); *Providencia* spp (3); *Serratia liquefaciens* (2), *Proteus penneri* (1)

These isolates were all susceptible to the tested agents with the single exception of one ESBL-producing *Providencia rettgeri* (resistant to ceftazidime (MIC≥32 μg/ml) and to ciprofloxacin (MIC≥256 μg/ml)

Table 2 MIC distribution of temocillin for *Enterobacteriaceae* isolates with characterized resistance mechanisms

	MIC (μg/ml)[b]							
	1	2	4	8	16	32	64	≥128
All species combined[c]								
Hyperproduced AmpC (80)			12	22	35[a]	8	2	1
Non-CTX-M ESBL (64)		1	8	4	26[a]	23	2	
CTX-M (13)			2	6[a]	4	1		
Hyperproduced K1 enzyme (16)				10[a]	5	1		
Escherichia coli								
Hyperproduced AmpC (8)			3	3[a]	2			
Non-CTX-M ESBL (6)			1		4[a]		1	
CTX-M (6)				5[a]	1			
Klebsiella[d]								
Non-CTX-M ESBL (23)			3	1	11[a]	8		
CTX-M (4)					3[a]	1		
Hyperproduced K1 enzyme (16)				10[a]	5	1		
Enterobacter aerogenes								
Hyperproduced AmpC (24)			3	6	12[a]	3		
Non-CTX-M ESBL (26)			2	2	7	14[a]	1	
Enterobacter cloacae								
Hyperproduced AmpC (31)			1	6	18[a]	5	1	
Non CTX-M ESBL (6)			1	1	4[a]			
CTX-M (3)			1	2[a]				
Morganella morganii								
Hyperproduced AmpC (6)			1	1	2[a]	1		1
Citrobacter freundii								
Hyperproduced AmpC (5)			2		1[a]	1	1	
S. marcescens								
Hyperproduced AmpC (4)				1	1[a]	1	1	

[a] Modal values
[b] E-test MICs falling within two dilutions in a log2 base scale were rounded up to the highest value
[c] In addition to the species groups detailed, this total also includes three ESBL-positive isolates (two *Proteus mirabilis* and one *Providencia stuartii*)
[d] Includes 23 *K. pneumoniae* isolates all ESBL producing and 20 *K. oxytoca* isolates (16 K1 enzyme hyperproducers and 4 ESBL producers)

producing *Enterobacter aerogenes* which were less susceptible (MIC_{50} of 32 μg/ml).

Overall, 77 (11.8%) of the tested isolates were found to be ESBL-producers. The prevalence of ESBL-producing strains ranged between 3 and 29% in the different hospitals. Regarding the species distribution, ESBLs were predominantly found in *Enterobacter aerogenes* (26/72 [36%]) and in *K. pneumoniae* (23/75 [31%]) and less commonly among *Enterobacter cloacae* (9/115 [8%], *Escherichia coli* (12/186 [6%]), *K. oxytoca* (4/62 [6%] and *Proteus mirabilis* (2/35 [6%]).

Table 3 shows the distribution of ESBL enzymes determined by the consistent results of primers specific PCR followed by DNA sequencing for most of the isolates and the β-lactamases isoelectric points as determined by isoelectric focusing. The ESBL enzymes were distributed differently between health care centres and among the different *Enterobacteriaceae* species which may either reflect a clonal dissemination of outbreak-associated nosocomial isolates within and between hospitals (for instance in *Enterobacter aerogenes* and *K. pneumoniae*) or alternatively a limitation of horizontal plasmid or gene transfer between bacterial hosts. For instance, among the TEM-related ESBLs, the TEM-24 enzyme largely predominated in *Enterobacter aerogenes* and it was also occasionally found among other species (*K. pneumoniae*, *Enterobacter cloacae*) while TEM-17 and TEM-52 β-lactamases were exclusively found in *Escherichia coli.* On the other hand, the SHV-derived ESBLs were essentially found in *K. pneumoniae* isolates (SHV-4 and SHV-5 like) and in *Enterobacter cloacae* (SHV-12) as well as in one single *Providencia* spp. isolate (SHV-4); CTX-M enzymes were predominantly found in *Escherichia coli* (mainly CTX-M-1 group enzymes) and to a lesser extent in other *Enterobacteriaceae* species. In *Enterobacter cloacae*, CTX-M-9 group enzymes were often found in association with SHV-12. Two *Proteus mirabilis* isolates (one with TEM-24 and one with a TEM-131) were susceptible or displayed low resistance to ceftazidime (MIC of 0.38 μg/ml and 2 μg/ml) which may reflect the low level expression of ESBL enzymes in some *Enterobacteriaceae* groups such as *Proteus*

Table 3 Characterization of extended-spectrum beta-lactamases in ESBL-positive *Enterobacteriaceae* isolates

	Classes of ESBL enzymes[a]				
Species (No. isolates)	TEM	SHV	CTX-M	SHV + CTX-M	Unidentified[b]
Enterobacter aerogenes (26)	TEM-24 (24)	SHV-4 (2)			
K. pneumoniae (23)	TEM-3 (1)	SHV-5 like[c] (11)	CTX-M-1 (2)		2
	TEM-24 (1)	SHV-4 like[c] (6)			
Escherichia coli (12)	TEM-52 (3)	SHV-4 (1)	CTX-M-1 (4)		
	TEM-17 (1)		CTX-M-2 (1)		
	TEM-24 (1)		CTX-M-9 (1)		
Enterobacter cloacae (9)	TEM-24 (2)	SHV-12 (2)		SHV-12 + CTX-M-9 (3)	1
	TEM-3 (1)				
K. oxytoca (4)	TEM-3 (1)		CTX-M-9 (2)		
	TEM-24 (1)				
Proteus mirabilis (2)	TEM-24 (1)				
	TEM-131 (1)				
Providencia rettgeri (1)		SHV-4 (1)			

[a] Additional non-ESBL β-lactamases are not included in this table.
[b] No bla_{TEM}, bla_{SHV} or bla_{CTX-M} gene could be detected by PCR assays targeting bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes in these isolates which were all confirmed as ESBL-producers by phenotypic tests
[c] SHV ESBL types are based on the detection of bla_{SHV} by PCR as well as by determination of PIs—SHV-4 like: PI: 7.8; SHV-5 like: PI of 8.2 No DNA sequencing of bla_{SHV} amplicons was performed on these isolates which also co-produced an SHV-1 enzyme (PI of 7.6)

species. Interestingly, no ESBL could be characterized for three isolates (two *K. pneumoniae* and one *Enterobacter cloacae*; Table 3) which may possibly reflect the presence in these strains of uncommon ESBL enzymes (e.g., VEB, PER, GES, BES, TLA) that were not targeted by our PCR assays. Additionally, other non-ESBL narrow-spectrum β-lactamases were also often detected; mainly TEM-1/ TEM-2 in *Escherichia coli* and in *Proteus mirabilis* and SHV-1 in *K. pneumoniae* (data not shown).

Concerning the activity of the different drugs, according to the mechanisms of resistances it was very striking that all agents except meropenem displayed a lower activity against ESBL-producing isolates (Table 4). In particular, the activity of ceftazidime and of ciprofloxacin was highly influenced by the presence of ESBLs. Non-susceptibility to ciprofloxacin or to ceftazidime was observed in respectively 58 and 66% of the ESBL-producing *Escherichia coli* isolates. In *K. pneumoniae* and in *Enterobacter aerogenes*, resistance rates were even higher and reached almost 100% for ceftazidime and more than 80% for ciprofloxacin. The activity of piperacillin-tazobactam was also markedly reduced and less than 50% of the ESBL-producing isolates were susceptible to this agent. On the other hand, temocillin displayed a very similar activity against ESBL-producing or non-ESBL producing *Escherichia coli* (92 vs. 98% temocillin susceptible isolates). However, a lower proportion of temocillin-susceptible isolates was found among ESBL-producing AmpC-derepressed *Enterobacter aerogenes* isolates (24% resistant and 40% intermediately susceptible isolates) and to a lesser extent in ESBL-producing *K. pneumoniae* (4% resistant and 30% intermediately susceptible isolates).

Discussion

In the present survey, we were able to document a mean prevalence of ESBL of roughly 12% among *Enterobacderiaceae* isolates collected from patients hospitalised in ICUs from seven Belgian university hospitals. Wide variations

Table 4 Comparative activity of the tested antibiotics against ESBL-positive and ESBL-negative *Enterobacteriaceae* isolates

	Number of resistant isolates (%)					
No. isolates	Piperacillin-tazobactam	Ceftazidime	Meropenem	Temocillin	Amikacin	Ciprofloxacin
ESBL producing isolates (77)	34 (44.2%)	67 (87.0%)	1 (1.3%)	26 (33.8%)	14 (18.2%)	54 (70.1%)
ESBL non-producing isolates (575)	Not tested	62 (10.8%)	1 (0.2%)	27 (4.7%)	17 (3.0%)	77 (13.4%)
Fisher exact test	–	$P<0.00001$	$P=0.22$	$P<0.00001$	$P<0.00001$	$P<0.00001$

were, however, observed between centres both in the prevalence rates (from 3 to 29%) as well as in the species distribution among which these enzymes were found. *Enterobacter aerogenes* still accounted as the most prevalent ESBL-producing species (35% of the isolates belonging to this species were found to be ESBL-producers) but it was closely followed by *K. pneumoniae* (31% ESBL-producers) and to a lesser extent *Enterobacter cloacae* and *Escherichia coli*. Two previous national surveys have shown higher figures of ESBL prevalence in *Enterobacter aerogenes* (65% in 2001; 58% in 2003) [13] and the lower figures observed here could possibly reflect a decrease in clonal spread of the epidemic strains which affected most Belgian hospitals over more than 10 years since the early nineties [14, 15]. Alternatively, our data may be biased by the small sample size of this survey and its restriction only to ICU patients while several of the former studies screened isolates originating from all hospitalisation units and wards.

Also, the high rate of ESBL-producing *K. pneumoniae* observed in this study (31% of the isolates) is at variance with other longitudinal Belgian multicentric surveys carried out between 1998 and 2005 in which a lower prevalence of ESBL (ranging between 5 and 15%) was observed in *Klebsiella* species [16]. Of note, the ESBL-producing *K. pneumoniae* isolates (SHV-4 like and SHV 5 like) found in the present study almost exclusively originated from two hospitals and would thus probably reflect the occurrence of local outbreaks in these institutions although the clonality of the collection of strains was not investigated in the present survey.

By contrast the high frequency of ESBL-producing *Escherichia coli* isolates which were found in all participating centres is consistent with the global trend towards increase in the incidence of these enzymes in *Escherichia coli*, and clearly reflects the recent emergence of the CTX-M ESBL enzymes which rose by a three-fold factor in Belgium between 2000 and 2004 [17].

The other major aim of this survey was to evaluate the activity of temocillin as well as selected comparators against resistant nosocomial *Enterobacteriaceae* isolates with defined acquired resistance mechanisms including ESBL and/or AmpC β-lactamases. We found that temocillin still retained good in vitro activity against the majority of *Enterobacteriaceae* isolates with a modal MIC of 4 μg/ml and a MIC_{90} of 16 μg/ml. Among the different comparators, meropenem appeared as the most active agent showing activity against more than 99% of the isolates regardless of the species and/or resistance mechanisms. On the other hand, ceftazidime and ciprofloxacin (89 and 72% non-susceptible isolates, respectively) were poorly active not only against ESBL-producing isolates but also against a significant proportion of AmpC overproducing species among which *Enterobacter aerogenes* again proved as the most resistant. Overall, amikacin retained good in vitro activity and it displayed an activity profile rather comparable to temocillin against the different *Enterobacteriaceae* species.

Although all mechanisms of resistance of the different isolates could not be fully determined in this survey, temocillin most notably retained activity against ESBL-producing *Escherichia coli* isolates. This observation agrees with the data of Rodriguez et al. [7] who found that 92% of the ESBL-producing *Escherichia coli* isolates identified at their hospital were susceptible to temocillin at a breakpoint value of 16 μg/ml. On the other hand, ESBL-producing *Enterobacter aerogenes* and *K. pneumoniae* isolates were found less susceptible to temocillin than ESBL-negative isolates belonging to these species.

In this study, we were not able to find a relationship between the level of resistance to temocillin and the presence of specific types of ESBLs. The highest rate of resistance was however observed in ESBL and AmpC co-producing *Enterobacter aerogenes* isolates as well as in *S. marcescens*, a species notoriously known to be intrinsically less susceptible to temocillin [1, 2, 6]. Contrary to Rodriguez-Villalobos et al. [7], we did not found higher rates of temocillin-resistant isolates among CTX-M-1 group isolates in comparison to isolates producing other types of ESBL enzymes. Acquired resistance to temocillin usually arises via combination of several mechanisms including the presence of ESBLs and/or AmpC hyperproduction with impermeability and/or upregulated efflux but these aspects were not investigated in the present study. Although the efficacy of carbapenems against ESBL-producing *Enterobacteriaceae* isolates has been widely documented, carbapenem resistance has emerged over the last years either by the acquisition of plasmidic metallo-β-lactamases or through the loss of porins. Hence, temocillin could be considered as a second line alternative therapeutic option to carbapenems for the treatment of infections caused by ESBL-producing strains which are causing an increasing number of infections in critically ill patients. However its use in this setting must be conditioned by microbiological investigations which should demonstrate the susceptibility to temocillin of these ESBL-positive strains and which at the same time rule out the presence of *Pseudomonas aeruginosa* or other temocillin naturally resistant non-fermenting organisms. Prospective clinical studies are therefore warranted in order to confirm its therapeutic efficacy.

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References

1. Slocombe B, Cooper CE, Griffin KE, White AR (1985) Temocillin: in vitro antibacterial activity. Drugs 29(Suppl 5):49–56
2. Van Landuyt H, Boelaert J, Piot P, Verbist L (1985) In vitro activity of temocillin against clinical isolates. Drugs 29(Suppl 5):1–8
3. Spencer RC (1990) Temocillin. J Antimicrob Chemother 26:735–737
4. Taylor RF, Gaya H, Hodson ME (1992) Temocillin and cystic fibrosis: outcome of intravenous administration in patients infected with *Pseudomonas cepacia*. J Antimicrob Chemother 29:341–344
5. Lekkas A, Gyi KM, Hodson ME (2006) Temocillin in the treatment of *Burkholderia cepacia* infections in cystic fibrosis. J Cyst Fibros 5:121–124
6. Vanhoof R, Berhin C, Carpentier M, et al (2001) Comparative in vitro activity of temocillin and other antimicrobial agents against *Enterobacteriaceae* isolated from patients admitted to five Belgian hospitals. Acta Clin Belg 56:354–359
7. Rodriguez-Villalobos H, Malaviolle V, Frankard J, de Mendonça R, Nonhoff C, Struelens MJ (2006) In vitro activity of temocillin against extended spectrum β-lactamase-producing *Escherichia coli*. J Antimicrob Chemother 57:771–774
8. Livermore DM, Hope R, Fagan EJ, Warner M, Woodford N, Potz N (2006) Activity of temocillin against prevalent ESBL- and AmpC-producing *Enterobacteriaceae* from south-east England. J Antimicrob Chemother 57:1012–1014
9. Clinical and Laboratory Standards Institute (2006) Performance standards for antimicrobial susceptibility testing. Approved standard MS100-S16. CLSI, Wayne, PA, USA
10. Fuchs PC, Barry AL, Thornsberry C, Jones RN (1985) Interpretive criteria for temocillin disk diffusion susceptibility testing. Eur J Clin Microbiol 4:30–33
11. Stürenburg E, Sobottka I, Noor D, Laufs R, Mack D (2004) Evaluation of a new cefepime-clavulanate ESBL Etest to detect extended-spectrum β-lactamases in an *Enterobacteriaceae* strain collection. J Antimicrob Chemother 54:134–138
12. De Gheldre Y, Avesani V, Berhin C, Delmee M, Glupczynski Y (2003) Evaluation of Oxoid combination discs for detection of extended-spectrum β-lactamases. J Antimicrob Chemother 52:591–597
13. De Gheldre Y, Glupczynski Y, Berhin C et al (2004) Epidemiology of *Enterobacter aerogenes* in Belgium: results of a multicentre survey. In: Abstracts of the 14th European Congress of Clinical Microbiology and Infectious Diseases. Abstract no. P-1122. Clin Microbiol Infect 10(Suppl 3):305 (abstract)
14. De Gheldre Y, Struelens MJ, Glupczynski Y, et al (2001) National epidemic surveys of *Enterobacter aerogenes* in Belgian hospitals from 1996 to 1998. J Clin Microbiol 39:889–896
15. Glupczynski Y, Delmée M, Goossens H, Struelens MJ (2001) Distribution and prevalence of antimicrobial resistance among gram-negative isolates in intensive care units in Belgian hospitals between 1996 and 1999. Acta Clin Belg 56:297–306
16. Ieven M, Delmee M, De Mol P et al (2006) Eight years of Belgian MYSTIC surveillance: 1998–2005. In: Abstracts of the 16th European Congress of Clinical Microbiology and Infectious Diseases, Nice, France, April 2006, Abstract no. P-1387
17. Rodriguez-Villalobos H, Malaviolle V, Frankard J et al (2005) Emergence of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* in Belgium. Euro-surveillance Weekly 10:63. Available online: (http://www.eurosurveillance.org/ew/2005/050224.asp#3)