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Temocillin susceptibility among Enterobacterales strains recovered from blood culture in France

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Running title: change in temocillin susceptibility among Enterobacterales

Keywords: Temocillin; Extended-spectrum beta-lactamases (ESBL); third-generation cephalosporin (3GC); Antibiotic resistance; cephalosporinase-overproducing strain (COPE)

Highlights

- Cephalosporinase-overproducer Enterobacterales are significantly more resistant to temocillin than ESBL-producing Enterobacterales (37.7% vs 23.5%; $P < 0.01$);
- The rate of temocillin resistance is correlated to the number of inactive beta-lactams, ranging from 3.7% to 60.0% in strain susceptible to all beta-lactams and those resistant to 3 beta-lactams;
- Among third-generation cephalosporin-resistant Enterobacterales, temocillin was active against 64.9% of the strains non-susceptible to piperacillin-tazobactam, ofloxacin, and cotrimoxazole.

- In ESBL-producing strains, the rate of resistance to temocillin trend to increase from 13.9% in 2014 to 23.9% in 2017 (*P < 0.01*).

Abstract

Temocillin is used for several years in some European countries but, only since 2015 in France. We assessed the susceptibility of Enterobacterales strains isolated from blood culture one year before (2014) and two years after (2017) its use in France. 1,387 strains were included by 17 clinical laboratories located throughout France: 363 in 2014 and 1,024 in 2017. The rate of resistance to temocillin was 4.6% and 26.7% in 3rd generation cephalosporin (3GC) susceptible and resistant strains respectively. Cephalosporinase-overproducer (COPE) strains were significantly more resistant to temocillin (37.7%) than ESBL-producer (ESBL-PE) (23.5%) (*P<0.01*). The rate of temocillin resistance was correlated to the number of inactive beta-lactams. The rate of resistance to temocillin trend to increase from 13.9% in 2014 to 23.9% in 2017 (*P<0.01*). Temocillin remains highly active against Enterobacterales but the trend in resistance should be assessed over time.

Introduction

Temocillin is a 6-α-methoxy derivative of ticarcillin which has been synthesized in the early 1980's (Labia et al. 1984). The 6-α-methoxy group confers to the molecule intrinsic stability against most beta-lactamases such as penicillinase, extended-spectrum beta-lactamase (ESBL), AmpC, and KPC carbapenemase (Woodford et al. 2014). Temocillin has been introduced in Belgium and Luxembourg in the 1980s, and in the United Kingdom in 2006. In the context of the 1980s, characterized by the development and marketing of several new antibiotics and the low prevalence of antibiotic resistance, temocillin was not considered by the health authority and the medical community useful enough in France as in many other countries. However, the worldwide pandemic of ESBL-producing Enterobacterales (ESBL-PE) and then the emergence of carbapenemase-producing Enterobacterales have led to a renewed interest in this molecule. In European countries, the average rate of resistance to a third-generation cephalosporin (3GC) in 2018 was 15.1% and 31.7% in invasive strains of *E. coli* and *K. pneumoniae* respectively (European Centre for Disease Prevention and Control 2018).

In France, the registration of temocillin follows a request from several professional organizations and associations (Haute Autorité de Santé 2015). The molecule has obtained a marketing authorization in December 2015 for the treatment of intra-abdominal and complicated urinary tract infections (UTI) due to ESBL-PE.

Temocillin breakpoints lack in EUCAST and CLSI guidelines for antibiotics susceptibility testing. Fuchs *et al.* first proposed to categorize as susceptible a strain displaying a MIC ≤16 mg/L and resistant if MIC ≥ 32 mg/L (Fuchs et al. 1985). The BSAC guidelines latter defined temocillin MIC breakpoints for Enterobacterales as susceptible if ≤ 8 mg/L for systemic infections and ≤32 mg/L for non-complicated UTI (British Society for antimicrobiol Chemotherapy 2013). In contrast, French guidelines proposed a unique breakpoint of 8 mg/L (CA-SFM 2020). In clinical laboratories, the routine assessment of temocillin susceptibility could be performed by discs diffusion methods, gradient strip test, or microdilution MIC. In comparison to microdilution or agar dilution, gradient strip tests appear a reliable method while major and very major error are more frequent using the discs diffusion method (Patel et al. 2013; Alexandre et al. 2018; Winckert et al. 2018).

We aim to assess the trend of temocillin resistance among invasive Enterobacterales strains isolated in France before and two years after the obtention of the marketing authorization. The GMC-11 project

was carried out by the collaborative GMC study group, an association of 30 French clinical laboratories involved in clinical microbiology research.

2. Methods

2.1. Clinical strains

Seventeen French clinical laboratories spread over the country were enrolled in the study: 7 in the Paris area, 3 in the east, 4 in the north-west, 1 in the south-east, and 2 in the center of France (Table 1). Each center included 100 non-duplicate consecutive Enterobacterales strains (50 strains collected in 2014 and 50 strains collected in 2017) recovered from blood culture. The laboratories that did not keep the strains collected in 2014 were invited to include 100 strains collected in 2017. As temocillin is recommended for the treatment of Enterobacterales resistant to 3GC, each center has to include 60% of strains resistant to third-generation cephalosporins (3GC-R). Bacterial identification was performed using conventional biochemical methods (e.g. VITEK 2) or MALDI-TOF mass spectrometry as recommended by the manufacturers.

2.2. Susceptibility testing

The minimum inhibitory concentration (MIC) of temocillin was assessed by Etest (bioMérieux, Lyon, France) as recommended by the manufacturer. Nowadays, there are no consensual guidelines for temocillin susceptibility testing interpretation. Temocillin susceptibility was interpreted using breakpoints of 8 mg/L according to CA-SFM EUCAST and BCSA systemic breakpoints (British Society for antimicrobiol Chemotherapy 2013; Comité de l'antibiogramme de la Société Française de Microbiologie 2020), 16 mg/L as first suggested by Fuchs *et al.* (Fuchs et al. 1985), and 32 mg/L according to BSAC urinary breakpoints (British Society for antimicrobiol Chemotherapy 2013). The susceptibility to eight others routinely tested antibiotics including piperacillin-tazobactam, cefotaxime, pivmecillinam, nalidixic acid, ofloxacin, cotrimoxazole, amikacin, and ertapenem, was assessed by the disk diffusion method. For these antibiotics, susceptibility testing was assessed using EUCAST v7.1 guidelines (European Committee on Antimicrobial Susceptibility Testing 2019). A phenotypic-based

approach was used to distinguish ESBL-PE and cephalosporinase-overproducing (COPE) strains as previously described (Comité de l'antibiogramme de la Société Française de Microbiologie 2020).

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 20.0.0 (IBM Corp., Armonk, NY, USA). Categorical and continuous variables were compared using the Chi-square and Student test, respectively. As the clinical strains were included according to their susceptibility to 3GC, all analyses were performed within 3GC-susceptible (3GC-S) and 3GC-resistant (3GC-R) strains. The rate of temocillin resistance among the three main species and overall strains was analyzed after grouping strains collected in 2014 and 2017.

3. Results

3.1. Clinical strains

A total of 1,387 strains were included ranging in 363 strains collected in 2014 and 1,024 strains collected in 2017 (Table 2). Eight laboratories included clinical strains collected both in 2014 and 2017 while the others included strains collected only in 2017. Enterobacterales species collected are listed in Table S1: *E. coli* accounted for 56.4%, *K. pneumoniae* 15.3%, *E. cloacae* complex 12.0%, and other Enterobacterales species 16.3%. The species distribution was not significantly different between 2014 and 2017 for *E. coli* and *E. cloacae* complex. However, a significantly higher proportion of *K. pneumoniae* (10.5% vs 17.0%, P = 0.003) were included in 2017. 3GC-R strains proportion was significantly higher in 2014 than in 2017 (54.5% *vs* 46.9%, P = 0.012). Among 3GC-R strains, ESBL and cephalosporinase-overproduction mechanisms accounted for about two-thirds (n=460) and one third (n=198), respectively, with no significant difference between 2014 and 2017 (P = 0.22).

3.2. Overall temocillin resistance

At breakpoints of 8 mg/L, 16 mg/L and 32 mg/L, the overall rate of temocillin resistance was under 5% (4.6%, 0.4% and 0.0%, respectively) for 3GC-S strains. The rate of resistance was significantly higher

(P < 0.001) for 3GC-R strains, ranging from 2.2% at the breakpoint of 32 mg/L to 7.6% and 26.7% at the breakpoints of 16 mg/L and 8 mg/L. At the breakpoint of 8mg/L, COPE displayed a significantly higher rate of temocillin resistance than ESBL-PE (36.0% *vs* 21.1%; P < 0.001). Considering the breakpoint of 16 mg/L, 3.5% of ESBL-PE were resistant to temocillin versus 13% of COPE (P <0.001).

MIC_{50} and MIC_{90} for 3GC-S strains were 4 mg/L and 8 mg/L, respectively. MIC distribution was higher for 3GC-R strains and much more for COPE strains. Overall, COPE displayed MIC_{50} and MIC_{90} of 8 mg/L and 24 mg/L, respectively, while ESBL-PE MIC_{50} and MIC_{90} were 6 mg/L and 12 mg/L respectively. At the breakpoint of 32 mg/L, almost all strains (3GC-S and 3GC-R) were susceptible to temocillin.

3.3. Temocillin resistance by species

At the breakpoint of 8 mg/L, the rate of temocillin resistance was low among 3GC-S strains, ranging from 3.7% in *E. coli* to 4.9% in *K. pneumoniae* and 5.7% in *E. cloacae complex* (P =0.32) (Table 4). In contrast, among 3GC-R strains at the same breakpoint, *E. cloacae complex* displayed a significantly higher rate of resistance to temocillin (40%) in comparison to *K. pneumoniae* (24.0%), and *E. coli* (19.6%) (P = 0.001). Among ESBL-PE, 18.6%, 24.6%, and 27.1% of *E. coli*, *K. pneumoniae*, and *E. cloacae* complex were resistant to temocillin at the breakpoint of 8 mg/L, respectively, while among COPE 27.0% and 48.0% of. *E. coli* and *E. cloacae* complex were resistant to temocillin respectively.

3.4. Trend in temocillin resistance between 2014 and 2017

The rates of temocillin resistance were not significantly different between 2014 and 2017 for both 3GC-S and 3GC-R strains except for ESBL-PE. Despite the small number of ESBL-PE included in 2014 (130 strains), strains collected in 2017 were significantly more resistant to temocillin than those included in 2014 (23.9% vs 13.9%; P = 0.02) at the breakpoint of 8 mg/L (Table 3). This finding was not noticed using higher breakpoints of 16 and 32 mg/L. If considering solely the eight laboratories that include strains collected in 2014 and 2017, the same trend in increased resistance to temocillin was noticed for ESBL-PE at the breakpoint of 8 mg/L. In contrast, temocillin resistance rate in COPE strains was similar in 2014 and 2017 (34.9% vs 36.6%, P = *0.8*).

3.5. Associated resistance

Associated resistances determined by the disc diffusion method were higher in 3GC-R strains than 3GC-S strains (Table 5). Regarding 3GC-S strains, temocillin was the most active antibiotic with amikacin, and ertapenem (Table S2) while mecillinam, amikacin, and ertapenem were more active than temocillin against 3GC-R strains. Among 3GC-S strains non-susceptible to at least one other antibiotic tested, resistance to temocillin ranged from 6.6% to 15.1% among cotrimoxazole, and mecillinam resistant strains respectively (Table 5). On the other hand, the rate of temocillin resistance in 3GC-R isolates ranged from 22.1% to 48.6% in cotrimoxazole, and ertapenem resistant isolates respectively.

Temocillin remained active against 64.9% of 3GC-R strains also resistant to piperacillin-tazobactam, ofloxacin and cotrimoxazole.

Of 1,270 strains tested for cefotaxime, piperacillin-tazobactam, and ertapenem, temocillin resistance was strongly correlated ($P < 0.01$) to the number of inactive beta-lactams. At the breakpoint of 8 mg/L, the rate of temocillin resistance ranged from 3.7% (no resistance to other beta-lactams) to 60% (resistance to all three beta-lactams) (Table 6, Fig. 1).

4. Discussion

Temocillin remains highly active against Enterobacterales strains recovered from blood culture. It, therefore, represents a useful alternative to broad-spectrum antibiotics, such as carbapenem or piperacillin-tazobactam, to treat infections due to 3GC-R strains. However, our results highlight 4 significant changes and trends: i) a higher rate of temocillin resistance among COPE than ESBL-PE; ii) the rate of temocillin resistance is correlated to the number of inactive beta-lactams; iii) a trend in increase resistance in ESBL-PE between 2014 and 2017; iv) two-third of the isolates resistant to 3GC, piperacillin/tazobactam, ofloxacin, and cotrimoxazole were susceptible to temocillin, confirming this molecule as a mainstay for the treatment of complicated UTI due to multi-drug resistant Enterobacterales.

The mechanisms of resistance to temocillin in Enterobacterales are largely unknown. Interestingly, we found here that among 3GC-R strains, temocillin resistance was significantly higher in COPE than in ESBL-PE. To our knowledge, this finding has never been reported yet, probably because our study is based on a larger collection of strains than the previous reports. Moreover, these latter mainly focused on ESBL-PE. However, natural cephalosporinase-producer susceptible to 3GC trend to display higher MIC_{90} than *E. coli* or natural penicillinase producer (Jules and Neu 1982; Livermore et al. 2006). In our work, this trend was significant for 3GC-R strains and especially for the species *E. cloacae complex* probably reflecting the existence of genetic determinants. Furthermore, the rate of temocillin resistance is strongly correlated to the number of inactive beta-lactams suggesting the accumulation of mechanisms of resistance to other beta-lactams might affect its susceptibility. It could be hypothesized that the high production of ESBL or AmpC might also hydrolyze temocillin at a low-level. Otherwise, it was suggested the resistance to temocillin could be due to a failure of the molecule entry via outer membranes porins (Verbist 1982). Indeed, the *in vitro* addition of EDTA allows a decrease of temocillin MICs for these strains (Jules and Neu 1982). As the modal distribution of MIC is close to the breakpoint of 8 mg/L, a slight decrease of susceptibility would impact the temocillin susceptibility rate at this breakpoint (Verbist 1982; Rodriguez-Villalobos et al. 2006).

At the breakpoint of 8 mg/L, the rate of 3CG-S strains resistant to temocillin was low and similar to previous studies including those published during the developing steps of the molecule (Verbist 1982; Yang and Livermore 1988). In contrast, 74.5% of the 3GC-R strains are resistant to temocillin. The rate of temocillin resistance among 3GC-R Enterobacterales was previously reported ranging from 63% to 94.9% (Livermore et al. 2006; Glupczynski et al. 2007; Tärnberg et al. 2011; Titelman et al. 2011; Duployez et al. 2016; Zykov et al. 2016; Ip et al. 2017; Mischnik et al. 2017; Alexandre et al. 2018; Kresken et al. 2018). These differences might be due to variability in epidemiology such as i) the rate of COPE and ESBL-PE among 3GC-R strains, ii) ESBL type as previously suggested (Livermore et al. 2006; Tärnberg et al. 2011), iii) the type of infection, *E. coli* strains collected from community-acquired UTI being less resistant to temocillin than those recovered from healthcare-associated infections (Alexandre et al. 2018).

The rate of temocillin resistance remained stable over the study period except for ESBL-PE for which a significant increase was noticed. It is noteworthy that we could rule out any bias regarding MICs achievement methodology between the two periods of study since all the laboratories used collections

of frozen strains for the first period, and all MICs were assessed in 2017 using the same methodology. Two other facts might affect the rate of temocillin resistance in ESBL-PE. First, a significantly higher proportion of *K. pneumoniae* was included in 2017 than in 2014. But the same observation is made within *K. pneumoniae* strains. Then, a fewer number of laboratories included clinical strains collected in 2014 than in 2017. However, the same trend was also noticed for these laboratories. We can also hypothesize a change in ESBL enzyme type, but it is unlikely as it would have occurred at the same time and in all the centers located all over the country. It is possible the patients infected with ESBL-PE were more frequently treated by antibiotics including temocillin which could explain this increase is only noticed for these strains.

In countries where temocillin is available and used for many years, the rate of temocillin resistance seems to be higher among ESBL-PE strains. Indeed, up to 37% and 33.8% of ESBL-PE strains recovered in United-Kingdom and Belgium during the 2000s were resistant to temocillin, respectively (Titelman et al. 2011; Mischnik et al. 2017). In Belgium, this rate is stable since the early 2000s (Vanhoof et al. 2001; Rodriguez-Villalobos et al. 2006; Livermore and Tulkens 2008). In France, Duployez *et al.* reported a rate of temocillin resistance of 28.7% among Enterobacterales strains collected in 3 hospitals located next to the Belgium border in 2015 (Duployez et al. 2019), which is higher than in the present study (13.9%) but slightly less to that in Belgium (Rodriguez-Villalobos et al. 2006; Glupczynski et al. 2007). As no participant laboratories of the present study are located near the Belgium border, these differences could suggest a circulation of Enterobacterales strains across the French-Belgian border. The molecule was introduced in Belgium for clinical use in 1988 at the same time of the emergence of ESBL-PE, thus, no studies prior to this date including ESBL-PE were performed. Furthermore, in Hong-Kong, where the molecule is not available, the rate of temocillin resistance among ESBL-PE was assessed as 16.1% (Ip et al. 2017), which is similar to the rate recovered before the marketing authorization in France. The trend in increased resistance to temocillin among ESBL-PE needs to be confirmed by further epidemiological studies.

5. Conclusion

Temocillin represents a useful alternative to broad-spectrum antibiotics for the treatment of severe infections due to 3GC-R strains. However, as a high proportion of these strains are resistant to

temocillin, empiric treatment using temocillin should be avoided. To the best of our knowledge, this work is the first that include such a large collection of Enterobacterales species and strains in order to determine temocillin-resistant strains prevalence. Indeed, most of the previous studies focused only on *E. coli* and *K. pneumoniae*. This finding allowed us to notice a higher level of resistance among COPE and increasing resistance among ESBL-PE over time. Also, the resistance to temocillin is strongly correlated to the number of inactive other beta-lactams suggesting a multifactorial mechanism of resistance. Further epidemiological surveys are needed to assess trends in temocillin resistance among Enterobacterales.

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Competing interest

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Tables.

Table 1 Location of participating center in France

Hospital name	Hospital Type	City	Location
Hôpital Foch	PPE*	Suresnes	Paris area
GH Paris - Saint-Joseph	PPE*	Paris	
HIA Bégin	Military hospital	Saint Mandé	
CHU Avicenne	Teaching hospital	Bobigny	
CHU Henri Mondor	Teaching hospital	Créteil	
CHU Necker Enfants-malades	Teaching hospital	Paris	
Institut Curie	PPE*	Villejuif	
CHU Caen Normandie	Teaching hospital	Caen	North-west
CHU de Nantes	Teaching hospital	Nantes	
CHU Pontchaillou	Teaching hospital	Rennes	
Hôpital Pasteur	General hospital	Dieppe	
CHU de Nice	Teaching hospital	Nice	South-east
CHU Jean Bernard	Teaching hospital	Poitiers	Center
CHU Bretonneau	Teaching hospital	Tours	
CHU de Reims	Teaching hospital	Reims	East
Hôpital Civil de Strasbourg	Teaching hospital	Strasbourg	
CHU Jean Mimoz	Teaching hospital	Besançon	

* Private hospital with public engagement

Table 2 Characteristics of Enterobacteriales included in 2014 and 2017

	2014	2017	*P*
Total number of strains	363	1024	/
Species distribution			
E. coli	210 (57.9%)	572 (55.9%)	0.5
E. cloacae complex	43 (11.8%)	124 (12.1%)	0.9
K. pneumoniae	38 (10.5%)	174 (17.0%)	**0.003**
Other species	72 (19.8%)	154 (15.0%)	**0.03**
3GC resistance			
3GC-S strains	165 (45.5%)	544 (53.1%)	**0.012**
3GC-R strains	198 (54.5%)	480 (46.9%)	
COPE	66 (33.3%)	134 (28.0%)	0.22
ESBL-PE	130 (65.7%)	330 (68.7%)	
Other mechanisms	2 (1%)	16 (3.3%)	

Table 3 Temocillin susceptibility of Enterobacterales included in 2014 and 2017

	8mg/L			16 mg/L			32 mg/L		
	2014	2017	p	2014	2017	p	2014	2017	p
3GC-S strains	11 (6.7%)	21 (3.9%)	0.133	0 (0.0%)	3 (0.6%)	/	0 (0.0%)	0 (0.0%)	/
3GC-R strains	43 (21.7%)	138 (28.8%)	0.061	14 (7.1%)	38 (7.7%)	/	3 (1.5%)	12 (2.5%)	0,6
ESBL-PE	18 (13.9%)	79 (23.9%)	**0.02**	3 (2.3%)	13 (3.9%)	0.6	0 (0.0%)	1 (0.3%)	1
COPE	23 (34.9%)	49 (36.6%)	0.8	9 (13.6%)	17 (12.7%)	0,8	1 (1.5%)	4 (3.0%)	1
Other mechanisms	2 (100%)	10 (62.5%)	0.53	2 (100%)	8 (50%)	0.6	2 (100%)	6 (37.5%)	1

Table 4 Temocillin susceptibility of 3GC-S and 3GC-R Enterobacterales strains collected in 2014 and 2017 at breakpoint of 8, 16, and 32 mg/L.

	Nb	MIC_{50}	MIC_{90}	% of resistant strains at breakpoint		
				8 mg/L	16 mg/L	32 mg/L
Overall species						
3GC-S	709	4	8	4.6	0.4	0.1
3GC-R	678	6	16	25.5	6.2	0.9
- COPE	198	8	24	35.9	12.6	2.5
- ESBL-PE	460	6	12	20.9	3.5	0.2
E. coli						
3GC-S	459	4	6	3.7	0.2	0.0
3GC-R	316	6	12	19.6	3.2	0.6
- COPE	37	6	16	27.0	8.1	2.7
- ESBL-PE	279	6	12	18.6	2.6	0.4
K. pneumoniae						
3GC-S	81	3	8	4.9	0.0	0.0
3GC-R	129	4	16	24.0	3.9	0.0
- COPE	11	/	/	/	/	/
- ESBL-PE	118	4	16	24.6	3.4	0.0
E. cloacae complex						
3GC-S	35	3	6	5.7	5.7	2.9
3GC-R	125	8	24	40.0	12.8	0.8
- COPE	77	8	24	48.0	16.9	1.3
- ESBL-PE	48	6	16	27.1	6.3	0.0

Table 5 Rate of temocillin resistance among non-susceptible Enterobacterales to other antibiotics according to 3CG susceptibility

Non-susceptible antibiotics	3CG-R	3CG-S
Pivmecillinam	26 (32.5%)	11 (15.1%)
Piperacillin/tazobactam	124 (36.8%)	6 (9.8%)
Ertapenem	17 (48.6%)	2 (66.7%)
Nalidixic acid	94 (26.0%)	12 (10.5%)
Ofloxacin	94 (27.0%)	12 (13.9%)
Cotrimoxazole	89 (22.1%)	9 (6.6%)
Amikacin	17 (25.4%)	2 (18.2%)
Piperacillin/tazobactam + Ofloxacin + Cotrimoxazole	49 (35.1%)	2 (20%)
Piperacillin/tazobactam + Ofloxacin + Cotrimoxazole + Pivmecillinam	6 (33.3%)	1 (20%)
Ofloxacin + Cotrimoxazol + Pivmecillinam	10 (32.3%)	2 (20%)

Table 6 Correlation between temocillin-resistant Enterobacterales and inactive beta-lactams (cefotaxime, piperacillin-tazobactam, ertapenem)

Number of inactive beta-lactam	**Total number of strains**	**Number (%) of strains non-susceptible to temocillin according to breakpoint**		
		8 mg/L	**16 mg/L**	**32 mg/L**
0	596	22 (3.7%)	1 (0.2%)	0 (0.0%)
1	362	50 (13.8%)	5 (1.4%)	1 (0.3%)
2	272	100 (36.8%)	29 (10.7%)	6 (2.2%)
≥3	40	24 (60.0%)	16 (40.0%)	8 (20.0%)

Figures.

Fig. 1 Correlation between temocillin-resistant Enterobacterales and inactive beta-lactams (cefotaxime, piperacillin-tazobactam, ertapenem)