

Establishing quality control ranges for temocillin following CLSI-M23-A3 guideline

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Objectives: This study aimed to establish acceptable quality control ranges for temocillin disk diffusion tests and Etest[®] minimal inhibitory concentrations.

Methods: According to Clinical and Laboratory Standards Institute (CLSI) guideline, a Tier 2 quality control study was performed and involves seven laboratories. Each of them tested 10 replicates of two quality control strains (*Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218) on three different media lots and, for disk diffusion, two disk lots.

Results: Proposed zone diameter quality control ranges were 12–25 mm for *E. coli* ATCC 25922 and 19–28 mm for *E. coli* ATCC 35218. Proposed Etest quality control ranges were 3–24 mg/l for *E. coli* ATCC 25922 and 2–6 mg/l *E. coli* ATCC 35218.

Conclusion: Based on our results, we would advise the use of *E. coli* ATCC 35218 as QC strain for temocillin susceptibility testing and Etest because ranges obtained are narrower than with *E. coli* ATCC 25922 and do not overlap temocillin breakpoint.

Keywords: Temocillin, Quality control, Susceptibility testing, Etest

Introduction

Temocillin is a narrow-spectrum penicillin mainly active against Enterobacteriaceae and stable towards beta-lactamases, including most AmpC and extended-spectrum β -lactamases (ESBL).¹ The rise of ESBL-producing Enterobacteriaceae in the mid-2000 and the recent increase of resistance to carbapenems, have participated to the regain of interest in this old antibiotic.^{1,2}

Routine susceptibility testing for temocillin has been described using several methods as automates, strips, and disk diffusion susceptibility testing and, for the latter, has been recently validated according to EUCAST methodology.^{3–5} According to Clinical and Laboratory Standards Institute (CLSI), testing of standard quality control (QC) strains on a regular basis is needed to ensure test performance but to date, very little has been described about the QC ranges for temocillin. Laboratories currently rely on (1) Etest[®] temocillin leaflet⁶ which suggests quality control limits for *Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218 of 4–16 and 2–8 mg/l, respectively; and (2) a

newsletter⁷ from the former company SmithKline Beecham suggesting limits for paper disk diffusion (30 μ g temocillin) of 20–24 mm for *E. coli* ATCC 25922 and 24–26 mm for *E. coli* ATCC 35218. It is however not clear how these limits were defined and, in our experience those described for disk diffusion susceptibility testing are not realistic. The aim of the present study is to determine QC ranges for temocillin against *E. coli* ATCC 25922 and *E. coli* ATCC 35218 QC strains for disk diffusion susceptibility testing and Etest according to CLSI guideline.

Materials and Methods

Etest and disk diffusion QC ranges were determined using a Tier 2 study following the CLSI-M23-A3 guideline.⁸ Data from seven laboratories in Belgium (AZ Sint-Lucas & Volkskliniek [Gent], Jan Yperman ziekenhuis [Ieper], Jessa ziekenhuis [Hasselt], O. L. V. ziekenhuis [Aalst], Imelda ziekenhuis [Bonheiden], H. Hartziekenhuis [Lier], Ziekenhuis Oost-Limburg [Genk]) were collected and temocillin minimum inhibitory concentrations (MICs) and inhibitory zones against two QC strains, *E. coli* ATCC 25922 and *E. coli* ATCC 35218, were analysed. Three separate lots of Mueller-Hinton (MH) agars from three different

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manufacturers (Becton Dickinson [BD]; Oxoid-ThermoFischer Scientific [TF], and BioMerieux [BM]) were used and each of the seven laboratories tested each QC strain on each of the three media brands during ten working days. For disk diffusion susceptibility testing, the evaluation also includes two lots of 30 µg temocillin disks from two different manufacturers (BD and TF). Disk diffusion susceptibility testing was performed according to CLSI performance standards⁹ and, Etest susceptibility tests according to manufacturer leaflet.⁶ Aztreonam (Etest and 30 µg disks) was chosen as internal QC for *E. coli* ATCC 25922 strain and piperacillin/tazobactam (Etest and 100/10 µg disks) as internal QC for *E. coli* ATCC 35218 strain, the latter lacking QC limits for aztreonam. Whenever a control value was out of the QC range, test results of that day were discarded and tests repeated. In each laboratory, only one technician or microbiologist was designated for the reading of the results.

From the data generated in the Tier 2 study means, standard deviations, medians and zone diameter ranges were calculated for each medium lot, disk lot and laboratory. For each QC strains, comparisons between groups (disk lots, MH lots, centres) were performed by non-parametrical approaches (difference between two groups was explored using the Wilcoxon test and, for three or more groups using

Kruskal–Wallis test. *Post-hoc* analysis was performed using the Steel–Dwass test).

From the pooled data set, zone diameter ranges were calculated to encompass 95%, that is, from the lower 2.5% of the distribution to the upper 97.5%. These values were adjusted downwards and upwards, respectively, to the nearest whole millimetre, this ensuring that at least 95%, and mostly more, of the predicted zone diameter distribution were included in the QC range.

Data from Etest were first transformed in mid log₂ MIC allowing for the calculation of the mean, standard deviation and ranges.

Results

Of the control agents, aztreonam and piperacillin/tazobactam and for each of the 10 days in each centre, 100% of zone diameters and MIC were within the CLSI QC ranges.

Zone diameter ranges

Four hundred and twenty independent measures for each QC strains were examined. Overall distributions of zone diameters (column 'Total') and sub-analysis by MH manufacturer, disk manufacturer, and laboratory are shown in Table 1 for *E. coli* ATCC 25922 and in Table 2 for *E. coli* ATCC 35218. There was no obvious outlier laboratories but as shown in Table 3, significant differences ($P < 0.05$) were obser-

Table 1 Temocillin zone diameter test results for *E. coli* ATCC 25922 QC strain according to disk lots, medium lots, and centres

Zone diam. (mm)	Number of occurrences at each zone diameter for <i>E. coli</i> ATCC 25922												Total*	
	Disk lot		Medium lot			Centres								
	BD	TF	BD	TF	BM	A	B	C	D	E	F	G		
	<i>n</i> =210	<i>n</i> =210	<i>n</i> =140	<i>n</i> =140	<i>n</i> =140	<i>n</i> =60	<i>n</i> =60	<i>n</i> =60	<i>n</i> =60	<i>n</i> =60	<i>n</i> =60	<i>n</i> =60	<i>n</i> =60	<i>n</i> =420
9	2	1	0	1	2	1	0	0	0	2	0	0	0	3
10	3	1	0	3	1	3	0	0	0	1	0	0	0	4
11	6	0	1	5	0	0	0	0	0	5	0	1	6	6
12	4	5	0	9	0	2	1	1	1	3	0	1	9	9
13	4	1	1	3	1	3	0	0	0	2	0	0	5	5
14	7	9	2	12	2	8	2		0	5	0	1	16	16
15	4	3	4	3	0	2	1	1	0	3	0	0	7	7
16	23	18	9	19	13	13	2	10	2	8	4	2	41	41
17	18	13	6	11	14	2	6	3	5	9	5	1	31	31
18	41	29	17	30	23	5	8	5	15	9	21	7	70	70
19	25	19	6	23	15	6	12	6	4	4	6	6	[44]	44
20	33	34	24	16	27	6	8	8	14	5	14	12	67	67
21	18	28	21	3	22	6	11	10	5	3	4	7	46	46
22	13	24	23	2	12	1	6	7	8	0	5	10	37	37
23	6	15	14	0	7	2	3	6	2	1	0	7	21	21
24	2	6	8	0	0	0	0	3	2	0	1	2	8	8
25	1	2	2	0	1	0	0	0	2	0	0	1	3	3
26	0	1	1	0	0	0	0	0	0	0	0	1	1	1
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	1	1	0	0	0	0	0	0	0	0	1	1	1
Mean	18.1	19.3	20.2	16.8	19.0	16.7	19.2	19.6	19.7	16.1	19.0	20.4	18.7	18.7
Median	18	20	21	18	19	16	19	20	20	17	19	20	19	19

Note: Horizontal lines designate the 2.5 and 97.5% QC limits for *E. coli* ATCC 25922, and the brackets indicate the overall mean zone diameter.

Table 2 Temocillin zone diameter test results for *E. coli* ATCC 35218 QC strain according to disk lots, medium lots, and centres

Zone diam. (mm)	Number of occurrences at each zone diameter for <i>E. coli</i> ATCC 35218												Ttotal*
	Disk lots		Medium lots			Centres							
	BD	TF	BD	TF	BM	A	B	C	D	E	F	G	
	n=210	n=210	n=140	n=140	n=140	n=60	n=60	n=60	n=60	n=60	n=60	n=60	
10	0	1	0	1	0	1	0	0	0	0	0	0	1
11	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	1	1	0	0	0	0	0	1	0	0	0	1
13	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	1	0	1	0	1	0	0	0	0	0	0	1
17	2	0	1	0	1	1	0	0	1	0	0	0	2
18	4	3	1	4	2	5	1	0	0	1	0	0	7
19	4	1	2	0	3	2	0	0	0	3	0	0	5
20	10	6	3	8	5	7	0	2	2	5	0	0	16
21	16	8	7	10	7	1	7	5	0	10	1	0	24
22	29	25	7	29	18	15	2	4	6	13	6	8	54
23	37	26	8	34	21	12	12	10	4	11	8	6	63
24	59	48	29	37	41	4	22	17	15	9	24	16	[107]
25	32	43	37	12	26	7	11	6	16	7	18	10	75
26	10	27	23	1	13	2	1	9	9	1	2	13	37
27	7	14	17	2	2	2	3	7	4	0	1	4	21
28	0	4	3	1	0	0	1	0	2	0	0	1	4
29	0	1	1	0	0	0	0	0	0	0	0	1	1
30	0	1	0	0	1	0	0	0	0	0	0	1	1
Mean	23.2	23.9	24.4	22.7	23.5	21.9	23.7	24.1	24.2	22.4	24.0	24.7	23.6
Median	24	24	25	23	24	22	24	24	25	22	24	25	24

Note: Horizontal lines designate the 2.5 and 97.5% QC limits for *E. coli* ATCC 35218, and the brackets indicate the overall mean zone diameter.

ved between some of them. Significant differences were also observed between all MH manufacturers and disk manufacturers.

For *E. coli* ATCC 25922, the overall calculated mean±standard deviation of zone diameters was 18.7±3.1 mm. The 95% confidence interval of the zone diameter was 12.6–24.7 mm, which when rounded down and up respectively to the nearest whole mm became the range 12–25 mm. In all laboratories, frequent appearance (69% of the observations) of one or several ‘inner colonies’ in the inhibition zone was observed.

For *E. coli* ATCC 35218, the overall calculated mean±standard deviation of zone diameters was 23.6±2.1 mm. The 95% confidence interval of the zone diameter was 19.2–27.9 mm, which when rounded became the range 19–28 mm. Inner colonies were also reported in all laboratories but were less frequent than with *E. coli* ATCC 25922 (51% of the observations).

MIC ranges (Etest)

A total of 210 independent measures per QC strains were examined. MIC distributions are shown in Table 4. No obvious outlier laboratories were detected. The presence of inner colonies was also frequent and reported in 53% of the observations for *E. coli* ATCC 25922 and for *E. coli* ATCC 35218, 56% of the observation.

For *E. coli* ATCC 25922, the calculated mean±standard deviation of mid log₂ MIC converted observations was 2.9±0.7. The 95% confidence interval of mid log₂ converted observations around the mean was 1.4–4.3. Converting these values back to MIC values, they became 2.7–19.7 mg/l, which when rounded up to the standard Etest dilution series became the range 3–24 mg/l.

Table 3 Comparison of rank of mean inhibitory zones obtained by each laboratory

	Rank ¹	Laboratory	Mean ²
<i>E. coli</i> 25922	1	E	16.1
	2	A	16.7
	3	F	19.0
	4	[B C D G]	19.2
	5		19.6
	6		19.7
	7		20.4
<i>E. coli</i> 35218	1	A	21.9
	2	E	22.4
	3	B	23.7
	4	F	24.0
	5	C	24.1
	6	D	24.2
	7	G	24.7

Note: ¹From the smallest to the largest mean zone.

²Mean inhibitory zone diameter (mm).

³Braces group mean inhibitory diameter that do not differ significantly by Steel–Dwass multiple range test ($P>0.05$).

For *E. coli* ATCC 35218, the calculated mean \pm standard deviation of mid \log_2 MIC converted observations was 1.4 ± 0.5 . The 95% confidence interval of mid \log_2 converted observations around the mean was 0.4–2.4. Converting these values back to MIC values, they became 1.3–5.3 mg/l, which when rounded up to the standard Etest dilution series became the range 2–6 mg/l.

Discussion

Quality control ranges for antimicrobial agents against QC strains for both dilution and disk diffusion testing are currently set by the Clinical and Laboratory Standard Institute using data gathered in predefined structured multi-laboratory studies, so-called Tier two studies.⁹ In the present study, we used this protocol to define zone diameter QC ranges for temocillin against two QC strains and, this method was also applied to define MIC QC ranges from Etest as no separate protocol for this technique has been published so far.

Based on our results, the proposed zone diameter QC ranges for temocillin disk diffusion susceptibility testing are 12–25 mm for *E. coli* ATCC 25922 and 19–28 mm for *E. coli* ATCC 35218. As suspected, those ranges are far from those previously reported.⁷ The proposed MIC QC ranges for Etest is 3–24 mg/l for *E. coli* ATCC 25922 and 2–6 mg/l for *E. coli* ATCC 35218. Those ranges are in line with those proposed by the manufacturer (4–16 and 2–8 mg/l, respectively).⁶

Although it has never been reported before, frequent appearance of inner colonies were reported by all laboratories. Interestingly, when cultured and retested,

those inner colonies remain resistant to temocillin. The underlying mechanism remains unclear and worth further investigation. Nevertheless, the use of *E. coli* ATCC 35218 as QC strain allows for less inner colonies and when reported, were more situated at the exterior border of the inhibition zone than those reported using *E. coli* ATCC 25922, thus influencing less the final results. This could explain why *E. coli* ATCC 35218 returns narrower QC ranges than *E. coli* ATCC 25922.

Based on our results, we would advise the use of *E. coli* ATCC 35218 as QC strain for temocillin because it allows for lower variability and ranges do not overlap the epidemiological breakpoint (≤ 16 mg/l, ≥ 19 mm) and in the case of Etest range, do not overlap the pharmacokinetic/pharmacodynamics breakpoint (8 mg/l). Although *E. coli* ATCC 35218 is currently recommended for beta-lactam/beta-lactamase inhibitor combination only, the stability of temocillin to beta-lactamases makes it a suitable candidate as QC strain.

Disclaimer Statements

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Conflicts of interest The authors have no conflict of interest.

Ethics approval None.

Table 4 MIC data of replicate tests in seven laboratories using Etest strips on MH agar

MIC (mg/l)	Mid \log_2 MIC	Number of occurrences at each zone diameter										Total
		Medium lot			Centres							
		A	B	C	A	B	C	D	E	F	G	
<i>E. coli</i> ATCC 25922 (n=210)												
3	1	1	0	0	0	0	0	0	0	0	1	1
4	1.5	16	0	0	1	4	0	4	3	1	3	16
6	2	24	0	23	3	2	5	6	9	8	14	47
8	2.5	17	4	24	9	7	11	7	3	3	5	45
12	3	7	42	14	7	10	12	11	7	10	6	63
16	3.5	4	13	5	4	5	2	2	4	4	1	22
24	4	0	8	2	4	1	0	0	2	3	0	10
32	4.5	0	3	2	1	1	0	0	2	1	0	5
48	5	1	0	0	1	0	0	0	0	0	0	1
Total (n)		70	70	70	30	30	30	30	30	30	30	210
<i>E. coli</i> ATCC 35218 (n=210)												
1.5	0	1	0	0	0	0	0	0	0	0	1	1
2	0.5	41	0	3	4	6	10	6	6	4	8	44
3	1	19	22	26	3	8	18	9	9	6	14	67
4	1.5	8	26	30	17	14	2	13	7	8	3	64
6	2	1	13	10	4	2	0	0	7	10	1	24
8	2.5	0	8	1	2	0	0	1	1	2	3	9
12	3	0	1	0	0	0	0	1	0	0	0	1
Total (n)		70	70	70	30	30	30	30	30	30	30	210

Note: Horizontal lines designate the 2.5 and 97.5% QC limits for each strain.

References

- 1 Livermore DM, Tulkens PM. Temocillin revived. *J Antimicrob Chemother.* 2009;63(2):243–5.
- 2 Pulcini C, Bush K, Craig WA, Frimodt-Møller N, Grayson ML, Mouton JW, et al. Forgotten Antibiotics: An Inventory in Europe, the United States, Canada, and Australia. *Clin Infect Dis.* 2012;54(2):268–74.
- 3 Rodriguez-Villalobos H, Cardentey-Reyes A, Thiroux C, Nonhoff C, Struelens MJ. Comparison of four commercial methods for determining temocillin susceptibility of *Escherichia coli*. *J Antimicrob Chemother.* 2009;63(4):832–4.
- 4 Patel TA, Dille R, Williams A, Vanstone GL, Balakrishnan I. Comparison of the Phoenix automated system, the Etest method and broth microdilution in determining temocillin susceptibility of Enterobacteriaceae. *J Antimicrob Chemother.* 2013;68(7):1685–6.
- 5 Vanstone GL, Dille R, Schwenk S, Williams A, Balakrishnan I. Temocillin disc diffusion susceptibility testing by EUCAST methodology. *J Antimicrob Chemother.* 2013;68(11):2688–9.
- 6 BioMérieux A. AB bioMérieux Etest® temocillin. Antimicrobial susceptibility testing for in vitro diagnostic use. Marcy l’Etoile: BioMérieux A; 2011.
- 7 Paeme G. Bepaling van de *in-vitro* gevoeligheid voor temocilline. *Bacteriology Newsletter.* Philadelphia, PA: Smithkline Beecham Pharmaceuticals; 1998.
- 8 Clinical and Laboratory Standards Institute. Development of *in vitro* susceptibility testing criteria and control parameters. Approved guideline — 3rd ed. CLSI document M23-A3. Wayne, PA: CLSI; 2008.
- 9 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility test. 10th ed. CLSI approved standard M02-A10. Wayne, PA: CLSI; 2009.