Stichting Werkgroep Antibioticabeleid

Commissie Richtlijnen Gevoeligheidsbepalingen (CRG)

Datum: september 2026

Categorie: signaal vanuit Nederland

Onderwerp: gevoeligheidsbepalingen van temocilline in geautomatiseerde systemen

Signaal:

Vanuit de fabrikant van temocilline werd gemeld dat de gevoeligheidsbepalingen in de Phoenix niet altijd correct zijn.

Achtergrond:

In de tweede helft van 2026 komt temocilline ook op de Nederlandse markt. Het is daarom van belang om de beperkingen van de (geautomatiseerde) systemen die de gevoeligheid van temocilline bepalen te kennen.

Uit de literatuur (zie onder) blijkt dat indien in de Phoenix temocilline resistent gemeten wordt, een deel van de isolaten temocilline gevoelig is. De Vitek geeft minder fouten in de temocilline resistent metingen dan de Phoenix, echter bij de Vitek zijn er meer fouten bij temocilline gevoelig resultaten.

Wanneer een confirmatietest nodig is, kan het beste een gradiënttest gebruikt worden.

Gebruik in België

In België wordt temocilline al vele jaren gebruikt. De laboratoria die de Phoenix gebruiken, vertrouwen op de Phoenix uitslag S en bij een uitslag R, wordt een additionele test gebruik, zoals een gradiënttest. Voor de Vitek is niet bekend hoe de resultaten in België gebruikt worden.

Achtergrond literatuur:

Abstracts 2020

Abstract 1813

Does automated susceptibility testing overcall temocillin resistance?

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Background: Temocillin is a semi-synthetic penicillin with narrow spectrum activity against *Enterobacteriaceae,* including AmpC and extended spectrum beta-lactamase (ESBL) producing organisms. Temocillin has the potential to be an important tool in clinical practice to treat serious infections caused by multi-resistant organisms and for antimicrobial stewardship purposes, including reducing use of critical antibiotics such as carbapenems. Common current clinical microbiology laboratory practice involves use of automated susceptibility testing methods such as the Becton-Dickinson (BD) Phoenix™. However, reported resistance rates to temocillin for *Enterobacteriaceae* isolates at our clinical microbiology laboratory far exceeds that expected by our knowledge of temocillin's stability in the presence of beta-lactamase enzymes, leading to a hypothesis that Phoenix is overcalling temocillin resistance. This potentially poor quality data may lead to greater reluctance to utilise the antibiotic in clinical practice and give incorrect antimicrobial resistance epidemiology data.

Materials/methods: Prospective data was collected on *Enterobacteriaceae* blood culture isolates at our clinical microbiology laboratory from July 2017 to October 2018. Isolates were routinely tested for susceptibility on Phoenix and results recorded. Isolates that were deemed temocillin resistant on Phoenix had the temocillin susceptibility test repeated using an alternative method, for example a temocillin E-test and the concordance of the results was investigated.

Results: 87% (117/134 isolates) of *Enterobacteriaceae* blood culture isolates initially reported to be temocillin resistant, were found to be susceptible when re-tested using an alternative method. This reduced the overall reported temocillin resistance rate from 26% (209/805 isolates) to 2% (17/730 isolates).

Conclusions: Temocillin resistance in *Enterobacteriaceae* blood cultures isolates reported using automated susceptibility testing on BD Phoenix was only confirmed to be true using a second susceptibility testing method in 13% of cases. Phoenix overcalls temocillin resistance. All laboratories relying on BD Phoenix for temocillin susceptibility testing should confirm initial resistant results using a 2nd susceptibility testing method, such as a temocillin Etest. Becton-Dickinson should consider withdrawing temocillin from their Phoenix automated susceptibility testing panels until more reliable resistance results can be obtained.

Trupti A. Patel*, Rachel Dilley, Alan Williams, Gemma L. Vanstone and Indran Balakrishnan. Comparison of the Phoenix automated system, the Etest method and broth microdilution in determining temocillin susceptibility of Enterobacteriaceae. J Antimicrob Chemother 2013. doi:10.1093/jac/dkt049

Vergelijking van 3 methodes: BD PhoenixTM Automated Microbiology System (instrument version 5.15A, software version 6.01A/V5.15A) (Becton Dickinson, Oxford, UK), Etest (AB Biodisk, Solna, Sweden) and broth microdilution (BMD)].

Currently, only the BSAC has defined temocillin breakpoints for Enterobacteriaceae (susceptible if MIC ≤8 mg/L for systemic infections and ≤32 mg/L for UTIs).

Deze breekpunten wijken af van de EUCAST 2026:

Enterobacterales*

Expert Rules and Expected Phenotypes Guidance documents

Penicillins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Temocillin (infections originating from the urinary tract), *E. coli, Klebsiella* spp. (except *K. aerogenes*) and *P. mirabilis*	0.001	16		30	50[F]	17[F]	

Isolates comprised Escherichia coli (194), Klebsiella pneumoniae (19), Proteus mirabilis (16), Enterobacter cloacae (9), Serratia marcescens (7), Citrobacter koseri (6), Enterobacter aerogenes (5), Citrobacter freundii (4), Morganella morganii (4), Klebsiella oxytoca (3), Proteus vulgaris (3), Citrobacter farmeri (2), Pantoea agglomerans (2), Serratia liquefaciens (2), Citrobacter braakii (1), Enterobacter gergoviae (1), Kluyvera sp. (1), Providencia rettgeri (1) and Raoultella ornithinolytica (1).

Conclusie: Our data indicate that, whereas the level of agreement for 'susceptible' results is excellent, the Phoenix system overcalls temocillin non-susceptibility.

Table 1. Summary of temocillin AST results of 264 isolates comparing the BMD, Etest and Phoenix methods (percentages of total shown in brackets for each method)

	Susceptible	Intermediate	Resistant
BMD	246 (93.2%)	17 (6.4%)	1 (0.4%)
Etest	236 (89.4%)	26 (9.8%)	2 (0.8%)
Phoenix	162 (61.4%)	96 (36.4%)	6 (2.3%)

Corentin Deckers et al. Multicenter interlaboratory study of routine systems for the susceptibility testing of temocillin using a challenge panel of multidrug-resistant strains. European Journal of Clinical Microbiology & Infectious Diseases https://doi.org/10.1007/s10096-023-04681-y

Abstract

Accurate susceptibility result of temocillin (TMO) is important for treating infections caused by multidrug-resistant *Enterobacterales*. This multicenter study aimed to investigate the performance of routine temocillin testing assays against *Enterobacterales* challenging strains. Forty-seven selected clinical isolates were blindly analyzed by 12 Belgian laboratories using VITEKR 2 (*n* = 5) and BD Phoenix™ (*n* = 3) automated systems, ETESTR gradient strip (*n* = 3), and disk (3 brands) diffusion method (DD; *n* = 6) for temocillin susceptibility using standardized methodology. Results were interpreted using EUCAST 2023 criteria and compared to the broth microdilution (BMD; Sensititre™ panel) method used as gold standard. Methods' reproducibility was assessed by testing 3 reference strains in triplicate. A total of 702 organism-drug results were obtained against 33 TMO-susceptible and 14 TMO-resistant isolates. Excluding *Proteae* species (*P. mirabilis* and *M. morganii)*, the essential agreement rates were excellent (91.5‑100%) for all MIC-based methods. The highest category agreement was achieved by ETEST (97.5%) followed by VITEK 2 (93.2%), disk diffusion (91.6%), and BD Phoenix™ (88.5%). **BD Phoenix™ and paper disk diffusion overcalled resistance (11.5% and 6.8% of major discrepancies, respectively)**, while ROSCO tablets diffusion and **VITEK 2 generated higher very major discrepancies (7.1% and 4.2% respectively**). Interassay reproducibility was unsatisfactory using recommended *E. coli* ATCC 25922 strain but was excellent with *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 strains. This interlaboratory study suggests that routine testing methods provide accurate and reproducible TMO categorization results except for *Proteae* species.

PS: in deze vergelijking zijn ook ROSCO tabletten gebruikt. Deze kunnen niet gebruikt worden in combinatie met EUCAST breekpunten.

Table 3 Performance of MIC-based temocillin testing methods

TMO MIC-based method (n labs)	Etest (n=3)					BD Phoenix™ (n=3)					VITEK® 2 (n=5)				
Species (total n isolates)	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	AA(%) %[95CI]	EA(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	AA(%) %[95CI]	EA(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	AA(%) %[95CI]	EA(%) %[95CI]
All (n=47)	95,2 [89,8-97,7]	4,0 [1,7-9,0]	0,8 [0,1-4,4]	51,8 [41,2-62,1]	88,2 [78,3-92,6]	85,4 [78,3-90,4]	2,3 [0,8-19,1]	12,3 [7,7-19,1]	54,3 [42,7-65,4]	93,5 [82,5-9,8]	87,3 [82,3-91,1]	7,2 [4,5-11,4]	5,4 [3,1-9,2]	49,3 [38,3-60,4]	90,6 [82,0-95,4]
All except PM/MM (n=43)	97,5 [92,8-99,1]	1,7 [0,4-5,9]	0,8 [0,1-4,6]	53,7 [42,9-64,0]	91,5 [83,3-95,8]	88,5 [81,6-93,0]	0,0 [0,0-3,0]	11,5 [7,6-19,3]	57,6 [45,6-68,7]	100 [91,8-100]	93,2 [88,7-95,6]	4,2 [2,1-11,7]	2,6 [1,1-5,9]	51,6 [39,4-63,6]	96,7 [88,8-99,1]
EC (n=11)	100 [89-100]	0,0 [0,0-11,0]	0,0 [0,0-11,0]	61,9 [40,8-70,2]	100 [84,5-100]	87,5 [71,9-95,0]	0,0 [0-10,7]	12,5 [4,9-28,1]	52,4 [49,7-71,6]	100 [74,1-100]	81,1 [68,6-89,4]	9,4 [4,0-20,2]	9,4 [4,0-20,2]	22,2 [9,0-45,2]	100 [82,4-100]
KP (n=15)	100 [89,0-100]	0,0 [0-10,4]	0,0 [0-10,4]	44,0 [26,6-62,9]	88,0 [70,0-95,8]	100 [91,2-100]	0,0 [0-8,8]	0,0 [0-8,8]	70,0 [48,1-85,4]	100 [83,9-100]	96,9 [89,4-99,1]	3,1 [0,8-10,5]	0,0 [0-5,6]	63,6 [42,9-80,3]	90,9 [72,2-97,4]
Non-EC/KP (n=21)	90 [79,8-95,3]	8,3 [3,0-18,1]	1,7 [0,3-8,9]	55,6 [39,6-70,5]	92,6 [83,0-97,2]	74,1 [61,2-83,6]	5,2 [1,8-14,1]	20,7 [12,2-62,4]	44,8 [28,4-62,4]	82,4 [91,8-100]	84,5 [73,2-90,2]	8,7 [4,6-15,8]	6,8 [3,3-13,3]	54,3 [38,2-69,5]	85,7 [70,6-93,7]
Non-EC/KP/PM/MM (n=17)	94,4 [84,8-98,0]	3,7 [1,0-9,8]	1,9 [0,3-9]	55,5 [39,5-70,4]	92,5 [82,4-97,1]	80 [67,0-88,8]	0 ,0 [0-7,1]	20 [11,2-33,0]	52,0 [33,5-70,0]	100 [78,5-100]	95,2 [88,2-98,1]	4,8 [1,8-11,7]	0,0 [0-4,4]	59,2 [40,7-75,4]	100 [95,6-100]

Caption: EC: *E. coli*; KP: *K. pneumoniae*; MM: *M. morganii*; PM: *P.mirabilis*; %[95CI]: Confidence Interval of 95%; TMO: temocillin; AA: Absolute agreement; CA: Categorical agreement; MD: Major discrepancy; VMD: Very Major Discrepancy;
Interpretation criteria:

VMD/MD (%)	0-3%	VMD/MD (%)	3-5%	VMD/MD (%)	>5%
CA/EA (%)	<80%	CA/EA (%)	80-90%	CA/EA (%)	90-100%

Table 4 Performance of temocillin disk diffusion methods

TMO disk diffusion (n labs)	Total disks (n=6)			BioRad (n=3)			BD (n=2)			Rosco (n=1)		
Species (total n isolates)	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	CA (%) %[95CI]	VMD(%) %[95CI]	MD (%) %[95CI]
All (n=47)	90,9 [86,9-93,7]	2,9 [1,5-5,6]	6,2 [3,9-9,7]	90,1 [84,0-93,4]	2,1 [0,7-6,1]	7,8 [4,4-13,4]	92,0 [84,3-96,0]	1,1 [0,2-6,2]	6,9 [3,2-14,2]	91,3 [79,7-96,6]	8,7 [3,4-20,3]	0,0 [0,0-7,7]
All -PM/MM (n=43)	91,6 [87,5-94,4]	1,6 [0,6-4,0]	6,8 [4,3-10,6]	90,7 [84,4-94,6]	0,8 [0,1-4,3]	8,5 [4,8-14,6]	92,4 [84,4-96,5]	0,0 [0-10,6]	7,6 [3,5-15,6]	92,9 [78,4-96,3]	7,1 [2,4-18,6]	0,0 [0,0-8,2]
EC (n=11)	86,2 [75,7-92,5]	3,1 [0,8-10,6]	10,7 [5,3-20,6]	84,8 [69,1-93,3]	3,0 [0,5-15,3]	12,1 [4,8-27,3]	85,7 [65,3-95,0]	0 ,0 [0-15,4]	14,2 [4,9 -34,6]	90,9 [62,2-98,4]	9,0 [1,6-37,6]	0,0 [0-25,8]
KP (n=15)	92,9 [85,2-96,7]	1,1 [0,2-6,4]	6,0 [2,5-13,1]	93,3 [82,1-97,7]	0,0 [0-7,8]	6,7 [2,9-17,9]	92,0 [75,0-97,7]	0 ,0 [0-13,3]	8,0 [2,2-27,9]	92,8 [68,5-98,7]	7,1 [1,2-31,5]	0,0 [0-21,5]
Non-EC/KP (n=21)	92,0 [85,9-95,6]	4,0 [1,2-7,9]	4,0 [1,2- 7,9]	90,5 [80,7-95,6]	3,2 [0,8-10,8]	6,3 [2,5-15,2]	95,1 [83,9-98,6]	2,4 [0,4-12,6]	2,4 [0,4-12,6]	90,5 [71,1-97,3]	9,5 [2,6-28,9]	0,0 [0,0-15,5]
Non-EC/KP/PM/MM (n=17)	94,0 [87,6-97,2]	1,0 [0,2-5,4]	5,0 [2,1-11,1]	92,2 [81,5-96,7]	0,0 [0-7,0]	7,8 [3,1-,18,5]	97,0 [84,7-99,4]	0,0 [0-10,4]	3,0 [0,5-15,3]	94,1 [73,0-98,9]	5,9 [1,0-26,7]	0,0 [0,18,4]

Caption: EC: *E. coli*; KP: *K. pneumoniae*; MM: *M. morganii*; PM: *P.mirabilis*; %[95CI]: Confidence Interval of 95%; TMO: temocillin;AA: Absolute agreement; CA: Categorical agreement; MD: Major Discrepancy; VMD: Very Major Discrepancy

Interpretation criteria:

VMD/MD (%)	0-3%	VMD/MD (%)	3-5%	VMD/MD (%)	>5%
CA (%)	<80%	CA (%)	80-90%	CA (%)	90-100%

Phoenix is Overcalling the Resistance of Enterobacteriaceae to Temocillin.

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Abstract

Background: Temocillin (TMO) is a 6-α-methoxy-penicillin directed towards Gram negative bacteria. TMO has recently been introduced on Gram negative panels of the Phoenix® (PHX) in Belgium. Since then, increased occurrence of resistance to TMO has been observed with PHX but not with disc diffusion assay or Etest®. In this context, we have compared the susceptibility results generated by PHX with reference methods.

Methods: Enterobacteriaceae isolates declared resistant (R) by PHX were collected for 2 months and their MIC measured by Etest® (E) and broth microdilution (BM). A comparable number of isolates declared susceptible (S) by PHX were then collected for comparison. Categories were determined using the breakpoint by Fuchs et al. (1985 Eur J Clin Microbiol 4:30-33) as applied in the PHX. Quality controls were performed using E. coli ATCC 25922 and 35218.

Results: Isolates Confirmed phenotype by

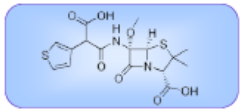
	PHX	E	BM
R (116)		17 (15%)	21 (18%)
S (74)		73 (98%)	72 (97%)

Agreement for S isolates was excellent. Conversely, less than 20% of R isolates were confirmed by either method. Surprisingly the MICs of the non-confirmed isolates were not all close to the breakpoint but distributed over a wide range (5 dilutions).

Conclusions: PHX is clearly overcalling the resistance among Enterobacteriaceae for TMO. This phenomenon seems not to be restricted to strains with borderline MIC. Isolates declared R by PHX should be reconfirmed by another method.

Background

Temocillin is a 6-α-methoxy penicillin directed towards Gram-negative bacteria. It has no useful activity against *Pseudomonas spp.* or *Acinetobacter spp.* but exhibits a remarkable stability against almost all types of β-lactamases including ESBL,[1,2] AmpC,[1] and even carbapenemases.[3]



Temocillin has been recently introduced on the Gram-negative panel of the Phoenix ® (Becton Dickinson & Co) in Belgium. Since then increased occurrences of resistance to temocillin have been reported by Phoenix users, while this did not happen so frequently when using other techniques to assess temocillin susceptibility (discs, E-tests, …)

Objectives

- To reassess Phoenix® validity for testing Enterobacteriaceae susceptibility to temocillin
- To compare MIC distributions of isolates declared resistant and susceptible by Phoenix®

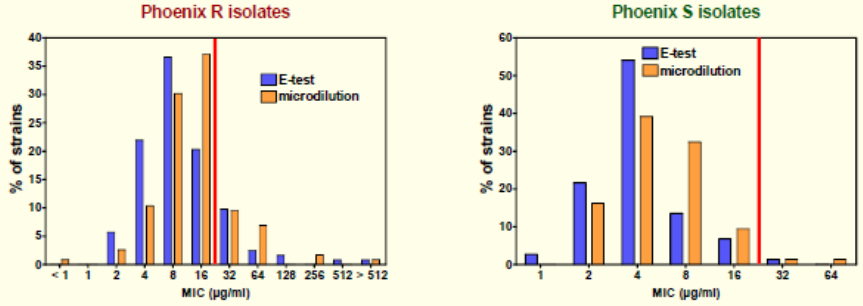
Results

1. Species and Susceptibility to Temocillin

Species	R isolates[a]			S isolates[b]		
	Phoenix	E-test	microdilution	Phoenix	E-test	microdilution
E. coli	39	6 (15%)	6 (15%)	38	38 (100%)	38 (100%)
E. aerogenes	38	1 (2.5%)	3 (8%)	13	13 (100%)	12 (92%)
E. cloacae	18	3 (17%)	6 (33%)	9	9 (100%)	9 (100%)
Serratia spp.	12	5 (42%)	6 (50%)	3	3 (100%)	3 (100%)
others	9	2 (22%)	1 (4%)	11	10 (91%)	10 (91%)
TOTAL	116	17 (15%)	21 (18%)	74	73 (98%)	72 (97%)

[a] number of isolates declared resistant by each method
[b] number of isolates declared susceptible by each method

2. Comparison of MIC distributions for Temocillin



The red line corresponds to the breakpoint used in Belgium[4] and applied by the Phoenix® system (i.e. susceptible ≤ 16 µg/ml)

Materials & Methods

- Isolates declared resistant by the Phoenix system were collected during 2 months in each center
- Isolates declared susceptible were collected in a second phase for sake of comparison
- MIC was determined using Etest and broth microdilution
- Quality controls were performed using *E. coli* ATCC 25922 and ATCC 35218

Main observations and Conclusions

- Agreement for S isolates was excellent (more than 97%).
- Agreement for R isolates was rather poor (less than 20%).
- MICs of non-confirmed isolates were distributed over a 5 dilution range.
- *Serratia spp.* made an exception with a better agreement. *Serratia* has always shown a lower susceptibility rate than the other Enterobacteriaceae towards temocillin which explains the higher number of isolates confirmed resistant.

➢ Phoenix is clearly overcalling the resistance of Enterobacteriaceae to temocillin.
➢ Isolates declared resistant by the Phoenix should be retested using another method.

Acknowledgements

We thank Dr Bart Gordts (AZ St Jan, Brugge, Belgium) for his helpful input and comments on the protocol and the analysis of the data.

References

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