Journal of Global Antimicrobial Resistance 41 (2025) 253–257

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Minimum inhibitory concentration distribution of Mecillinam in clinical *Staphylococcus saprophyticus* isolates from Europe

Minna Rud Andreasen[a,b,†], Filip Jansåker[c,d,†,*], Jesper Iversen[b], Opeyemi U. Lawal[e,f,g], Maria Miragaia[e], Luisa Gonçalves[h], Paulo Paixão[i], Elsa Gonçalves[j], Cristina Toscano[j], Maria D. Luzon[k], Małgorzata Urbaś[l], Lotte Jelsbak[a], Henrik Westh[b,m,†], Jenny Dahl Knudsen[c,†]

[a] *Department of Science and Environment, Roskilde University, Roskilde, Denmark*
[b] *Department of Clinical Microbiology, Hvidovre University Hospital, Hvidovre, Denmark*
[c] *Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark*
[d] *Center for Primary Health Care Research, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden*
[e] *Laboratory of Bacterial Evolution and Molecular Epidemiology, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB-NOVA), Oeiras, Portugal*
[f] *Laboratory of Molecular Genetics, ITQB-NOVA, Oeiras, Portugal*
[g] *Canadian Research Centre for Food Safety, University of Guelph, Ontario, Canada*
[h] *SAMS Hospital, Lisbon, Portugal*
[i] *Hospital da Luz, Lisbon, Portugal*
[j] *Hospital Egas Moniz, Lisbon, Portugal*
[k] *Department of Epidemiology and Clinical Microbiology, Narodowy Instytut Leków, Warszawa, Poland*
[l] *Hospital Universitari de Bellvitge, Barcelona, Spain*
[m] *Department of Clinical Medicine, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark*

ARTICLE INFO

Article history:
Received 30 May 2024
Revised 31 December 2024
Accepted 18 January 2025
Available online 28 January 2025

Editor: Stefania Stefani

Keywords:
Cystitis
Mecillinam
Minimal inhibitory concentration (MIC)
Pivmecillinam
Staphylococcus saprophyticus
Urinary tract infection (UTI)

ABSTRACT

Objectives: *Staphylococcus saprophyticus* (*S. saprophyticus*) is the second most common bacteria causing uncomplicated urinary tract infections (UTIs). It is considered non-susceptible to mecillinam, with no defined breakpoint and only few available minimal inhibitory concentration (MIC) observations. However, this consideration does not correlate with clinical outcome. With this study, we aimed to provide a comprehensive MIC distribution analysis of mecillinam for *S. saprophyticus*, which could be useful for determining potential breakpoints.

Methods: We studied 112 isolates of *S. saprophyticus* from human urine samples from 4 European countries. The broth microdilution and MIC test strip methods were used to determine mecillinam MIC.

Results: Broth microdilution MICs ranged from 4 to ≥ 256 mg/L, with a binary clustering at 32 to 64 and ≥ 256 mg/L. The MICs were duplicated for each isolate with similar results. The MIC distribution from the test strip method aligned well with the results from the broth microdilution method. Disc diffusion test yielded an 8 mm inhibitory zone in three isolates with MIC of 32 mg/L.

Conclusions: Considering mecillinam concentration in the urine usually reach 200 mg/L in conventional treatment, the clinical success frequently seen with pivmecillinam treatment for UTIs caused by *S. saprophyticus* may be explained by the MIC cluster of 32 to 64 mg/L. This cluster might be identified by an 8 mm inhibitory zone in disc diffusion tests. Clinical studies with MIC data are needed to examine potential breakpoints. As of now, clinicians should not switch empirical pivmecillinam treatment to other antibiotics based solely on the presence of *S. saprophyticus*. © 2025 The Author(s). Published by Elsevier Ltd on behalf of International Society for Antimicrobial Chemotherapy.

* Corresponding author. Mailing address: Center for Primary Health Care Research, Department of Clinical Sciences Malmö, Lund University, Jan Waldenströms gata 35, SE-205 02, Malmö, Sweden.
E-mail address: Filip.Jansaker@med.lu.se (F. Jansåker).
† These authors contributed equally to this work.

https://doi.org/10.1016/j.jgar.2025.01.015

Introduction

Staphylococcus saprophyticus (*S. saprophyticus*) is a Gram-positive coccus that predominantly is isolated from uncomplicated lower urinary tract infections (UTIs) [1]. It is considered the second most common pathogen (after *Escherichia coli*) in UTIs, causing about 5% to 10% of all cases [1]. However, there are currently no defined minimum inhibitory concentration (MIC) breakpoint or epidemiological cutoff values (ECOFFs) in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for mecillinam for *S. saprophyticus*. As of April 2024, only 25 MIC observations exist for mecillinam in the EUCAST database of MIC distribution for *S. saprophyticus*. Considering that a UTI is very common and that pivmecillinam is one of the major recommended and widely used empirical treatment option for UTIs in Scandinavia [2–6], there is a need to clarify the clinical breakpoints and mecillinam MIC distribution for *S. saprophyticus*. The absence of such knowledge may cause unnecessary overtreatment in an UTI caused by *S. saprophyticus* (e.g. clinicians switch to other oral antibiotics from empirical pivmecillinam treatment due to growth of *S. saprophyticus* in the urine sample) or potentially under-treatment, that is, if pivmecillinam treatments cannot achieve sufficient mecillinam concentrations (time over MIC) for *S. saprophyticus*.

Clinical data of the effect of pivmecillinam on UTI caused by *S. saprophyticus* is very sparse [7–10]. However, recent clinical studies on pivmecillinam empirical efficacy for UTIs have shown the drug to be effective [9] and also superior to placebo [8] in the subpopulations with *S. saprophyticus* as the causative agent. This could be explained by the pharmacological properties of pivmecillinam: high bioavailability and rapid accumulation of mecillinam in the urine causing very high concentrations (about 200 mg/L) with conventional pivmecillinam therapies [11,12].

Considering this, we aimed to investigate the mecillinam MIC distribution in *S. saprophyticus* isolated in human urine samples. We hypothesized that there could be a population with MICs below mecillinam urine concentration, as suggested by the few available MIC observations [13], which could be an explanation to the high cure rates seen in clinical settings.

Materials and methods

The study included 112 isolates of *S. saprophyticus*. The isolates had been obtained from human urine samples collected in four different European countries, as previously described [14]. These isolates had been preserved in glycerol stock and stored at −80 °C. The isolates were thawed and revived for the purpose of this study.

Antimicrobial Susceptibility Testing: Media preparation and antimicrobial susceptibility testing against mecillinam was performed using standardized methods according to EUCAST [15,16]. The MIC of mecillinam was determined using these 2 different approaches on the 112 clinical *S. saprophyticus* isolates:

Broth Microdilution Method: The MIC was determined using the broth microdilution technique following the guidelines outlined by EUCAST [16]. Mecillinam concentrations ranging from 0.125 mg/L to 256 mg/L were tested in duplicates.

MIC Test Strip Method: The test strip method, as recommended by the manufacturer (Liofilchem™, Roseto degli Abruzzi, Italy), was also used to determine the MIC values of mecillinam. This method (which is the same as the Epsilometer test/Etest method) provides a gradient of antibiotic concentration on a solid medium for MIC determination [17].

Disc diffusion test: To further assess mecillinam susceptibility, a disc diffusion test was conducted using 10 µg mecillinam Thermo Scientific™ Oxoid™ discs on 6 routine urine sample isolates from the Department of Clinical Microbiology, Hvidovre University Hospital, Hvidovre, Denmark. Disc diffusion tests were conducted on three isolates from each MIC cluster (i.e. 3 isolates with MIC of 32 mg/L and 3 isolates with MIC ≥ 256 mg/L) to explore whether disc diffusion test could differentiate isolates from each cluster. Mecillinam MIC test strips/Epsilometer tests were performed to determine MICs on these isolates.

Statistics: Statistical analysis using the paired *t* test was conducted to compare the MIC values obtained from the test strip and broth microdilution methods. $P < 0.05$ was defined as statistical significance.

Results

The study included 112 isolates from human UTI sources in Europe (Denmark = 91; Portugal = 12; Spain = 8; and Poland = 1). Fig. 1 and Table 1 show the MIC distribution of mecillinam in these isolates determined by broth microdilution and test strips. The broth microdilution tests yielded MICs for mecillinam that ranged from 4 to ≥ 256 mg/L, with a binary clustering around 32 to 64 (57 isolates, 51%) and ≥ 256 mg/L (42 isolates, 38%), respectively. All isolates were tested in duplicates with similar results.

We found no statistical difference between the results from the test strips and the broth microdilution methods ($P = 0.099$). Only one isolate in the broth microdilution test and one isolate in the test strip were below the currently considered breakpoint (8 mg/L) of mecillinam for *Enterobacterales* [18]. Fig. 2 shows the disc diffusion test with mecillinam, which yielded small but sharp inhibitory zones (8–9 mm) for isolates with MICs of 32 mg/L ($n = 3$) but no bacterial growth inhibition at ≥ 256 mg/L ($n = 3$).

Discussion

The study found a binary clustering of mecillinam MIC distributions, including one likely mecillinam susceptible population clustering around an MIC of 32 to 64 mg/L and one likely resistant population with an MIC of ≥ 256 mg/L. The duplications with broth microdilution method yielded similar MIC distribution, and the results from the MIC test strip method correlated well with those of the broth microdilution method, showing no significant difference between the two methods ($P = 0.099$).

The finding of binary clustering of mecillinam MIC distribution in *S. saprophyticus* represents new knowledge that could have useful clinical implications. Pivmecillinam treatment for UTIs are given as 400 mg or 200 mg tablets [9]. Following the intake of a 400 mg pivmecillinam tablet, the urine concentration of mecillinam reaches ca. 200 mg/L during the first 6 hours [12], surpassing the MIC in the 32 to 64 mg/L cluster but not the ≥ 256 mg/L cluster. Therefore, it is possible that the clinical success frequently observed following pivmecillinam treatment for UTI caused by *S. saprophyticus* [7–9] could be explained by the binary clusters of mecillinam MICs observed in our study. However, clinical studies are needed to examine this hypothesis, because the current knowledge on treatment outcome with MIC data is sparse and inconclusive regarding pivmecillinam treatment for UTI caused by *S. saprophyticus* [7–10]. For example, in a recent study, which included 12 cases of UTIs caused by *S. saprophyticus* treated with pivmecillinam, we found no correlation between MIC and treatment outcome [9]. Moreover, only one isolate in the broth microdilution test and one isolate in the test strip method were below the currently considered breakpoint (8 mg/L) of mecillinam for *Enterobacterales* [18]. Therefore, before clinical studies have determined whether clinical outcomes correlate with MIC, a breakpoint of mecillinam for *S. saprophyticus* cannot be suggested at this point; however, it may potentially lie within the observed 32 to 64 mg/L cluster.

A study by Zykov et al. [19] demonstrated significant reduction in bacterial load in a murine UTI model comparing mecillinam (mimicking human doses of 200 mg or 400 mg) and vehicle-

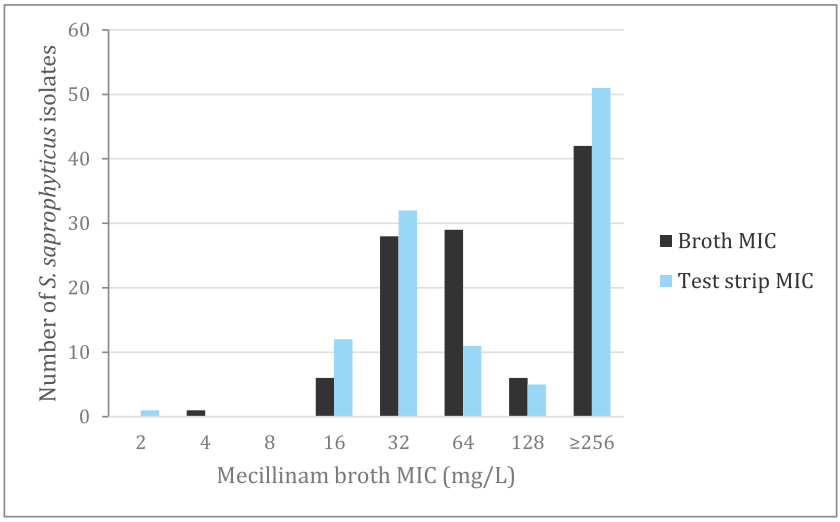

Fig. 1. Distribution of mecillinam minimum inhibitory concentration (MIC) in 112 clinical *Staphylococcus saprophyticus* urine isolates determined by broth microdilution and test strip.

Table 1
Relation between minimum inhibitory concentration (MIC, mg/L) values determined by broth microdilution and test strip for mecillinam in 112 clinical Staphylococcus saprophyticus urine isolates.

	Broth microdilution									
	MIC, mg/L	2	4	8	16	32	64	128	≥ 256	
Test strip	2				1					
	4									
	8									
	16				3	5	4			
	32				1	18	13			
	64					1	8	2		
	128					1		3	1	
	≥ 256		1		1	3	4	1	41	
	Sum		**1**	**0**	**6**	**28**	**29**	**6**	**42**	**112**

treated mice, which had been infected with multidrug resistant *Enterobacterales* with mecillinam MIC of up to 64 mg/L. This provides further support for the hypothesis generated in this study, that is, that the clinical success observed in humans treated with pivmecillinam for UTI caused by *S. saprophyticus* could be explained by the MIC clustering around 32 to 64 mg/L in 51 % of the clinical isolates tested by broth dilution. Interestingly, the bacterial reducing effect in mice (for multidrug resistant *Enterobacterales*) [19] was observed irrespective of whether the administered doses correlated with human doses of 200 mg or 400 mg pivmecillinam, 3 times daily. A similar study with mecillinam on *S. saprophyticus* has not been conducted. However, both a 200 mg and a 400 mg pivmecillinam regimen appear clinically effective for UTI caused by *S. saprophyticus* [9]. Although no study has compared these two regimens for UTI caused by *S. saprophyticus*, a comparative study of different pivmecillinam regimens may therefore be more clinically relevant than murine studies. As of now, the observed MIC distribution of mecillinam together with pharmacokinetic data of pivmecillinam [12], suggest that 400 mg tablets and three times daily dosing of pivmecillinam may be the most optimal regimen for UTI caused *S. saprophyticus,* to achieve sufficient time over MIC.

A limitation with our study is that it only encompasses 112 *S. saprophyticus* isolates from 4 European countries (91 from Denmark), potentially limiting the generalizable beyond these specific regions Studies examine the MIC distributions of mecillinam in clinical *S. saprophyticus* isolates from other countries are warranted. Further studies could also examine mechanisms, potentially differences in penicillin-binding proteins, that could explain the findings of two MIC clusters for mecillinam in *S. saprophyticus*. The main strength of our findings is the duplication of MIC distributions determined using two methods.

The disc diffusion test is generally used as a surrogate measure to MIC in routine screening of resistance [20]. An inhibition zone diameter < 15 mm around the disc is regarded as equivalent to a clinical break-point in *Enterobacterales* and thus considered to signify mecillinam resistance in the bacteria [18]. Based on the explorative observations in our study, we suggest that, for *S. saprophyticus* from UTI sources, the interpretation may be a “high level of resistance” (no clear inhibition zone) and “likely susceptible” (i.e. a clear inhibitory zone: e.g. > 7 mm). However, our observations need to be confirmed in larger samples and clinical studies also have to be done to investigate whether clinical outcomes correlate with this suggestion.

In conclusion, this study revealed a binary clustering pattern of mecillinam MICs among clinical *S. saprophyticus* isolates collected from four European countries. Notably, one of these clusters may explain the clinical success often observed in pivmecillinam treatment for UTIs caused by this bacterium. Whereas the establishment of a definite breakpoint for *S. saprophyticus* in a UTI requires further clinical studies with MIC data, a tentative breakpoint for mecillinam could be considered at this stage. As of now, clinicians should not switch empirical pivmecillinam treatment to other oral alternative based solely on the presence of *S. saprophyticus* in urine cultures from patients with uncomplicated UTIs, such decision should instead be based on the clinical response of the treatment.

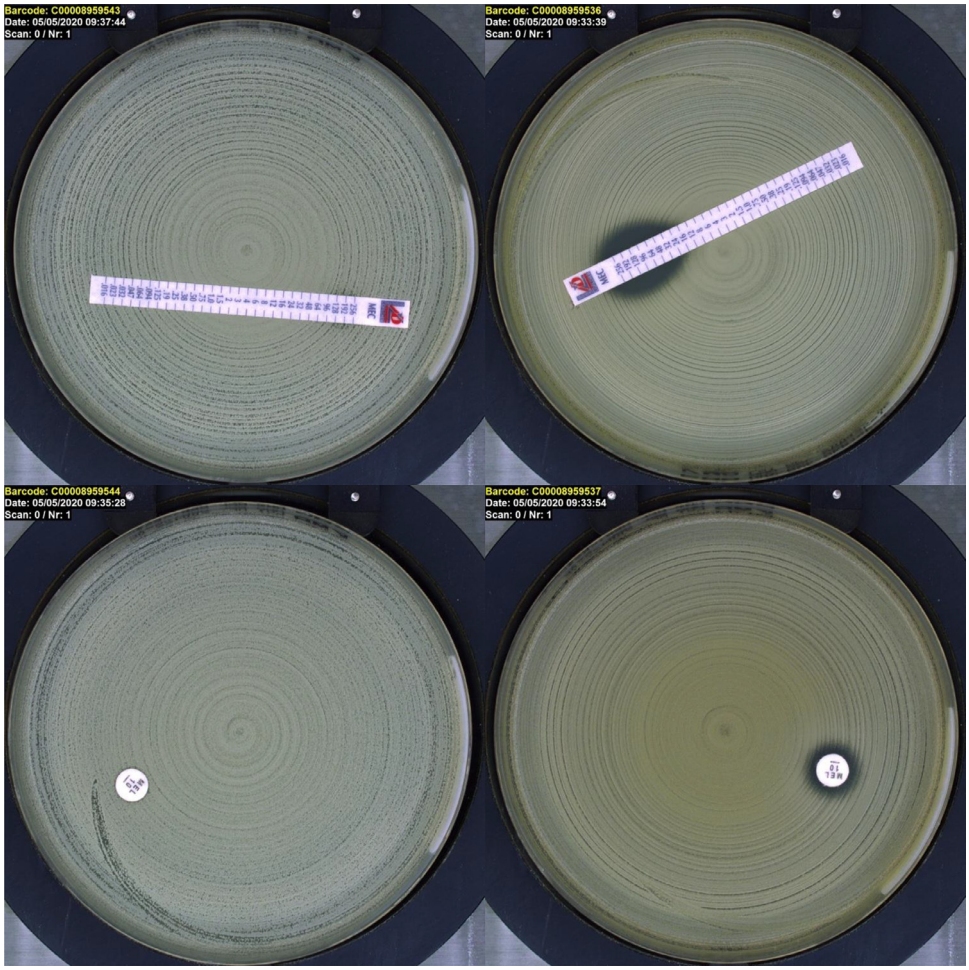

Fig. 2. Disc diffusion of mecillinam (10 µg) in Staphylococcus saprophyticus isolates with minimum inhibitory concentrations (MIC) of 32 mg/L and ≥ 256 mg/L. Legend: The left isolate had an MIC of ≥ 256 mg/L with no inhibition zone, whereas the right isolate had an MIC of 32 mg/L with a disc diffusion zone of 8 mm (replicated with three isolates each). The MICs were determined by MIC test strips according to the recommendations by the manufacturer (Liofilchem™, Roseto degli Abruzzi, Italy).

Declaration of competing interest: None.

Author contributions: Conception: F.J., H.W., and J.D.K. Design of the study: M.R.A., F.J., H.W., and J.D.K. Experiment: M.R.A. and J.I. Supervision: L.J., H.W., and J.D.K. Acquisition, analysis, or interpretation of data: All authors. Drafting the manuscript: M.R.A. and F.J. Revising the manuscript critically for important intellectual content: All authors. Final approval of the version to be published: All authors. Agreement to be accountable for all aspects of the work: All authors.

Funding: No specific funding was acquired for this study.

References

[1] Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urol Clin North Am Feb 2008;35(1):1–12 v. doi:10.1016/j.ucl.2007.09.004.
[2] Frimodt-Moller N, Simonsen GS, Larsen AR, Kahlmeter G. Pivmecillinam, the paradigm of an antibiotic with low resistance rates in Escherichia coli urine isolates despite high consumption. J Antimicrob Chemother Dec 23 2022;78(1):289–95. doi:10.1093/jac/dkac396.
[3] Kornfalt Isberg H, Melander E, Hedin K, Molstad S, Beckman A. Uncomplicated urinary tract infections in Swedish primary care; etiology, resistance and treatment. BMC Infect Dis Feb 13 2019;19(1):155. doi:10.1186/s12879-019-3785-x.
[4] Holm A, Cordoba G, Aabenhus R. Prescription of antibiotics for urinary tract infection in general practice in Denmark. Scand J Prim Health Care Mar 2019;37(1):83–9. doi:10.1080/02813432.2019.1569425.
[5] Haugom LEA, Ruths S, Emberland KE, Eliassen KER, Rortveit G, Wensaas KA. Consultations and antibiotic treatment for urinary tract infections in Norwegian primary care 2006-2015, a registry-based study. BMC Fam Pract Jun 24 2021;22(1):127. doi:10.1186/s12875-021-01470-4.
[6] Li X, Sundquist K, Jansaker F. Fluoroquinolones and other antibiotics redeemed for cystitis-A Swedish nationwide cohort follow-up study (2006-2018). Antibiotics (Basel) Jan 28 2022;11(2). doi:10.3390/antibiotics11020172.
[7] Granlund M, Landgren E, Henning C. Pivmecillinam in treatment of Staphylococcus saprophyticus urinary tract infections. Scand J Infect Dis 1983;15(1):65–9. doi:10.3109/inf.1983.15.issue-1.10.
[8] T. J. Monsen, S. E. Holm, B. M. Ferry, and S. A. Ferry, "Mecillinam resistance and outcome of pivmecillinam treatment in uncomplicated lower urinary tract infection in women," *APMIS: acta pathologica, microbiologica, et immunologica scandinavica,* vol. 122, no. 4, pp. 317–23, Apr 2014, doi: 10.1111/apm.12147.
[9] Jansaker F, et al. Pivmecillinam for uncomplicated lower urinary tract infections caused by Staphylococcus saprophyticus-cumulative observational data from four recent clinical studies. Antibiotics (Basel) May 7 2019;8(2). doi:10.3390/antibiotics8020057.
[10] Hovelius B, Mardh PA, Nygaard-Pedersen L, Wathne B. Nalidixic acid and pivmecillinam for treatment of acute lower urinary tract infections. Scand J Prim Health Care Nov 1985;3(4):227–32. doi:10.3109/02813438509013954.
[11] Anderson JD, Adams MA. Urinary excretion of mecillinam by volunteers receiving film-coated tablets of pivmecillinam hydrochloride. Chemotherapy 1979;25(1):1–4. doi:10.1159/000237814.
[12] Kerrn MB, Frimodt-Moller N, Espersen F. Urinary concentrations and urine ex-vivo effect of mecillinam and sulphamethizole. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases Jan 2004;10(1):54–61. [Online]. Available http://www.ncbi.nlm.nih.gov/pubmed/14706087 .
[13] "The European Committee on Antimicrobial Susceptibility Testing (EUCAST). MIC distributions for *Staphylococcus saprophyticus* at August 05, 2023. Available on https://mic.eucast.org/search/. Method: MIC. Antimicrobial: Species: *Staphylococcus saprophyticus*. Last accessed April, 2024."

[14] Lawal OU, et al. Staphylococcus saprophyticus from clinical and environmental origins have distinct biofilm composition. Front Microbiol 2021;12:663768. doi:10.3389/fmicb.2021.663768.
[15] The European Committee on Antimicrobial Susceptibility Testing (EUCAST)Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version January 1, 2022;7. Available at www.eucast.org Last accessed April, 2024..
[16] The European Committee on Antimicrobial Susceptibility Testing (EUCAST) reading guide for broth microdilution. Version 4.0 January 2022. Available at: www.eucast.org. Last accessed August 05, 2023.
[17] "MIC Test Strip, International Patent Liofilchem® and the Liofilchem company logo are registered trademarks of LIOFILCHEM s.r.l. © copyright LIOFILCHEM 2017. F00023 rev.32 /30.11.2017. http://www.liofilchem.net/. ."
[18] "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. Available at: www.eucast.org. Last accessed August 09, 2024."
[19] Zykov IN, Frimodt-Moller N, Smabrekke L, Sundsfjord A, Samuelsen O. Efficacy of mecillinam against clinical multidrug-resistant Escherichia coli in a murine urinary tract infection model. Int J Antimicrob Agents Feb 2020;55(2):105851. doi:10.1016/j.ijantimicag.2019.11.008.
[20] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal Apr 2016;6(2):71–9. doi:10.1016/j.jpha.2015.11.005.