***Enterococcus faecium* and Ampicillin Susceptibility Determination: Overestimation of Resistance with Disk Diffusion Method Using 2 Micrograms of Ampicillin?**

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We would like to focus your attention on ampicillin susceptibility testing for *Enterococcus faecium* infections. Ampicillin resistance in *E. faecium* is due to two distinct mechanisms: low-level resistance based on increased quantity of penicillin binding protein 5 (PBP5), which has low affinity for beta-lactams (MIC from 8 to 64 mg/liter), and high-level resistance based on mutation in PBP5 with MICs higher than 16 mg/liter (1). Based on these mechanisms, some French physicians use high doses of amoxicillin to treat *E. faecium* infections, with an ampicillin MIC up to 16 mg/liter. The aim of our study was to find the best method to determine *E. faecium* susceptibility to ampicillin.

Antimicrobial susceptibility testing was performed on a collection of 87 clinical isolates of *E. faecium*. All the isolates had been collected over an 8-year period and retrospectively selected. Five different methods were performed: disk diffusion methods (ampicillin 2 µg according to EUCAST recommendations [2] and ampicillin 10 µg following CLSI recommendations [3]; Bio-Rad, USA), broth microdilution with Sensititre (GN3F plate; Thermo Scientific, USA) as the reference method, Vitek (AST-P606 card; bioMérieux, France), and strips with predefined gradients of antibiotic concentrations with Etest (bioMérieux, France). *E. faecalis* ATCC 29212 and ATCC 51299 were used for quality control.

All the results obtained are summarized in Table 1.

The results of 79 strains are concordant in all 5 methods. The discordances between disk diffusion and Sensititre are as follows. With EUCAST recommendations, 4 resistant or intermediate strains were categorized susceptible with Sensititre (2 major and 2 minor discordances) and 5 resistant strains had MICs between 8 and 16 mg/liter. With CLSI recommendations, three strains categorized as susceptible were intermediate or resistant with Sensititre (two major and one minor discordance), and two resistant strains had an MIC of 16 mg/liter.

In a second study, we investigated whether Etest or Vitek would provide results that were most faithful to those of Sensititre. Neither Etest nor Vitek seemed adequate to replace the reference method, with some relevant differences in categorization and MIC values, notably for MICs from 8 to 32 mg/liter.

It appears that disk diffusion methods can lead to misuse of vancomycin. Nine patients with *E. faecium* strains categorized as intermediate or resistant with ampicillin 2 µg (15%) could have been treated with amoxicillin (considering treatment possible with an MIC of ≤16 mg/liter) if MIC determination had been realized. Two patients infected with *E. faecium* strains categorized as resistant with ampicillin 10 µg (3.8%) could have been treated with amoxicillin.

To conclude, we recommend the use of the microdilution method in severe infections to determine the MIC for *E. faecium* strains that appear resistant by the disk

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TABLE 1 MIC and categorization obtained with the different methods performed on the 87 strains^a

Strain no.	Disk diffusion			Sensititre			Etest		Vitek	
	Ampicillin 2 µg		Inhibition zone (mm)	Ampicillin 10 µg		MIC (µg/ml)	Compared to ampicillin at: ^e		MIC (µg/ml)	Categorization ^d
	Inhibition zone (mm)	Categorization ^b		Inhibition zone (mm)	Categorization ^c		2 µg	10 µg		
1	6	R	20	S	8	I	Concordance	Minor discordance	4 ^f	S ^f
2	6	R	13	R	16	R	Concordance	Concordance	16	R
3	6	R	6	R	16	R	Concordance	Concordance	48	R
4	6	R	17	S	16	R	Concordance	Major discordance	4 ^f	S
5	6	R	17	S	16	R	Concordance	Major discordance	32	R
6	6	R	9	R	32	R	Concordance	Concordance	24	R
7	6	R	12	R	32	R	Concordance	Concordance	32	R
8	6	R	11	R	>32	R	Concordance	Concordance	16	R
9	6	R	15	R	>32	R	Concordance	Concordance	48	R
10	6	R	6	R	>32	R	Concordance	Concordance	48	R
11	6	R	6	R	>32	R	Concordance	Concordance	128	R
12	6	R	6	R	>32	R	Concordance	Concordance	>256	R
13	6	R	6	R	>32	R	Concordance	Concordance	>256	R
14	6	R	9	R	>32	R	Concordance	Concordance	>256	R
15	6	R	10	R	>32	R	Concordance	Concordance	>256	R
16	6	R	9	R	>32	R	Concordance	Concordance	>256	R
17	6	R	9	R	>32	R	Concordance	Concordance	>256	R
18	6	R	6	R	>32	R	Concordance	Concordance	>256	R
19	6	R	6	R	>32	R	Concordance	Concordance	>256	R
20	6	R	6	R	>32	R	Concordance	Concordance	>256	R
21	6	R	6	R	>32	R	Concordance	Concordance	>256	R
22	6	R	6	R	>32	R	Concordance	Concordance	>256	R
23	6	R	12	R	>32	R	Concordance	Concordance	>256	R
24	6	R	16	R	>32	R	Concordance	Concordance	>256	R
25	6	R	6	R	>32	R	Concordance	Concordance	>256	R
26	6	R	10	R	>32	R	Concordance	Concordance	>256	R
27	6	R	6	R	>32	R	Concordance	Concordance	>256	R
28	6	R	12	R	>32	R	Concordance	Concordance	>256	R
29	6	R	11	R	>32	R	Concordance	Concordance	>256	R
30	6	R	6	R	>32	R	Concordance	Concordance	>256	R
31	6	R	10	R	>32	R	Concordance	Concordance	>256	R
32	6	R	6	R	>32	R	Concordance	Concordance	>256	R
33	6	R	6	R	>32	R	Concordance	Concordance	>256	R
34	6	R	9	R	>32	R	Concordance	Concordance	>256	R
35	6	R	6	R	>32	R	Concordance	Concordance	>256	R
36	6	R	6	R	>32	R	Concordance	Concordance	>256	R
37	6	R	6	R	>32	R	Concordance	Concordance	>256	R
38	6	R	6	R	>32	R	Concordance	Concordance	>256	R
39	6	R	6	R	>32	R	Concordance	Concordance	>256	R
40	6	R	6	R	>32	R	Concordance	Concordance	>256	R

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TABLE 1 (Continued)

Strain no.	Disk diffusion			Sensititre			Etest		Vitek			
	Ampicillin 2 µg			Ampicillin 10 µg			Compared to ampicillin at: ^e					
	Inhibition zone (mm)	Categorization ^b	Inhibition zone (mm)	Categorization ^c	MIC (µg/ml)	Categorization ^d	2 µg	10 µg	MIC (µg/ml)	Categorization ^d	MIC (µg/ml)	Categorization ^d
41	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
42	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
43	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
44	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
45	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
46	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
47	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
48	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
49	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
50	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
51	6	R	7	R	>32	R	Concordance	Concordance	>256	R	>16	R
52	6	R	9	R	>32	R	Concordance	Concordance	>256	R	>16	R
53	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
54	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
55	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
56	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
57	6	R	6	R	>32	R	Concordance	Concordance	>256	R	>16	R
58	6	R	21	S	≤4	S	Major discordance	Concordance	4	S	<2	S
59	7	R	22	S	≤4	S	Major discordance	Concordance	2	S	<2	S
60	8	I	23	S	≤4	S	Minor discordance	Concordance	1.5	S	<2	S
61	8	I	25	S	≤4	S	Minor discordance	Concordance	3	S	<2	S
62	12	S	21	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
63	13	S	24	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
64	14	S	24	S	≤4	S	Concordance	Concordance	0.5	S	<2	S
65	14	S	24	S	≤4	S	Concordance	Concordance	1.5	S	<2	S
66	14	S	22	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
67	15	S	26	S	≤4	S	Concordance	Concordance	0.5	S	<2	S
68	15	S	20	S	≤4	S	Concordance	Concordance	1	S	<2	S
69	15	S	24	S	≤4	S	Concordance	Concordance	1	S	<2	S
70	15	S	25	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
71	15	S	25	S	≤4	S	Concordance	Concordance	1	S	<2	S
72	16	S	22	S	≤4	S	Concordance	Concordance	1	S	<2	S
73	16	S	28	S	≤4	S	Concordance	Concordance	0.5	S	<2	S
74	16	S	29	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
75	16	S	28	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
76	16	S	24	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
77	16	S	25	S	≤4	S	Concordance	Concordance	1	S	<2	S
78	17	S	23	S	≤4	S	Concordance	Concordance	0.5	S	<2	S
79	19	S	31	S	≤4	S	Concordance	Concordance	0.75	S	<2	S
80	20	S	28	S	≤4	S	Concordance	Concordance	0.38	S	<2	S
81	20	S	29	S	≤4	S	Concordance	Concordance	0.25	S	<2	S
82	20	S	31	S	≤4	S	Concordance	Concordance	0.19	S	<2	S

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TABLE 1 (Continued)

Strain no.	Disk diffusion		Sensititre		Etest		Vitek	
	Ampicillin 2 µg		Ampicillin 10 µg		Compared to ampicillin at: ^e			
	Inhibition zone (mm)	Categorization ^b	Inhibition zone (mm)	Categorization ^c	MIC (µg/ml)	Categorization ^d	MIC (µg/ml)	Categorization ^d
83	23	S	29	S	≤4	S	0.19	S
84	25	S	29	S	≤4	S	0.19	S
85	25	S	29	S	≤4	S	0.032	S
86	28	S	35	S	≤4	S	0.125	S
87	31	S	36	S	≤4	S	0.19	S

^aBold text and numbers refer to discordance between disk diffusion and Sensititre.^bSusceptibility categorization according to EUCAST recommendations: S, susceptible, ≥10 mm; R, resistant, <8 mm; I, intermediate.^cSusceptibility categorization according to CLSI recommendations: S, susceptible, ≥17 mm; R, resistant, ≤16 mm.^dSusceptibility categorization according to EUCAST recommendations: S, susceptible, ≤4 mg/liter; R, resistant, >8 mg/liter; I, intermediate.^eConcordance, disk diffusion (DD) susceptible and Sensititre susceptible or DD resistant and Sensititre resistant; minor discordance, DD susceptible or resistant and Sensititre intermediate or DD intermediate and Sensititre susceptible; major discordance, DD susceptible and Sensititre resistant or DD resistant and Sensititre susceptible.^fRefers to discordance between Sensititre and Vitek/Etest.

diffusion method. Other *in vitro* studies including more strains are needed to confirm our results. Larger and clinical studies to evaluate the efficacy of high-dose ampicillin treatment of patients infected with *E. faecium* strains with MICs between 8 and 16 mg/liter could be useful.

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