VITEK 2 and PHOENIX fail to detect high-level gentamicin-resistant *Enterococcus faecium* isolates with aac-aph gene

Dear Editor,

Detection of high-level gentamicin resistance is important to evaluate the use of β-lactam and aminoglycoside combination for treatment. The most common mechanism of high-level resistance to gentamicin is due to the presence of bifunctional inactivation enzyme AAC-APH. Twenty-seven vancomycin-resistant strains were tested for gentamicin susceptibility by using automated methods VITEK and PHOENIX systems as well as agar dilution MICs, E test, and disk diffusion using 120 µg disks. The presence of resistance genes was tested by PCR using specific primers. All isolates tested carried *vanA* and *aac-aph* genes. No inhibition zone was obtained with highly charged gentamicin disks as well as E test. Agar dilution MICs showed that 5, 3, and 19 strains had MIC 256, 512, and 1024 mg/L, respectively. Four of five isolates with gentamicin MICs 256 mg/L were susceptible by both VITEK and PHOENIX systems, and the remaining one was susceptible by PHOENIX and resistant by VITEK. Three isolates with MICs 520 mg/L were reported resistant by both systems. One isolate with MIC >1024 mg/L was reported susceptible by both automates which may be due to a growth problem. Gentamicin breakpoints for *Enterococcus faecium* of British, French, European, and American institutions have some differences [Table 1]. EUCAST accepts that there is no synergy between β-lactams and aminoglycosides

Table 1: Critical values accepted by British, American, French, and European organizations for high-level gentamicin resistance in enterococci

	MICs (mg/L)							
	≤8	16	32	64	128	256	512	≥1024
[1]Susceptible strains	*	*	*	*				
[2]BSAC breakpoint						*	*	*
[2]CLSI breakpoint							*	*
[2]CA-SFM breakpoint						*	*	*
EUCAST no synergie point						*	*	*
[3]PHOENIX/VITEK 2 test								*

[1]Distribution of gentamicin MICs among wild-type *E. faecium* shows the highest MIC as 64 µg/mL (EUCAST). [2]Breakpoints accepted by BSAC, CLSI, and CA-SFM for gentamicin resistance are shown in gray. No synergy point is >128 mg/L for EUCAST. [3]VITEK and PHOENIX use one well with 500 mg/L gentamicin to detect high-level resistance. Automated systems detect only strains with MICs ≥1024 so they fail to detect *E. faecium* with MICs 256 and 512 mg/L on which use of β-lactams and gentamycin has no synergic effect.

Additional information. MIC values obtained by agar dilution, E test, disk diffusion methods, and automated systems for 27 *E. faecium* isolates and presence of *vanA* and *aac-aph* genes

Organism	Genotype	Gentamicin susceptibilities				
		Agar dilution MICs	E test	Disk diffusion (120 µg)	PHOENIX	VITEK2
E. faecium SMH1	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH2	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH3	vanA, aac-aph	256	>256	6 mm	S	S
E. faecium SMH4	vanA, aac-aph	512	>256	6 mm	R	R

Additional information (contd..)

Organism	Genotype	Gentamicin susceptibilities				
		Agar dilution MICs	E test	Disk diffusion (120 µg)	PHOENIX	VITEK2
E. faecium SMH5	vanA, aac-aph	256	>256	6 mm	S	S
E. faecium SMH6	vanA, aac-aph	256	>256	6 mm	S	S
E. faecium SMH7	vanA, aac-aph	512	>256	6 mm	R	R
E. faecium SMH8	vanA, aac-aph	512	>256	6 mm	R	R
E. faecium SMH9	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH10	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH11	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH12	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH13	vanA, aac-aph	256	>256	6 mm	S	S
E. faecium SMH14	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH15	vanA, aac-aph	≥1024	>256	6 mm	S	S
E. faecium SMH16	vanA, aac-aph	256	>256	6 mm	S	R
E. faecium SMH17	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH18	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH19	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH20	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH21	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH22	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH23	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH24	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH25	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH26	vanA, aac-aph	≥1024	>256	6 mm	R	R
E. faecium SMH27	vanA, aac-aph	≥1024	>256	6 mm	R	R

for strains with MICs >128 mg/L and this MIC level should be accepted as high level gentamicin resistance, which indicates acquisition of a resistance mechanism. BSAC also agrees with EUCAST recommendation. CA-SFM accepts ≤256 mg/L as susceptible, while for CLSI <512 mg/L is susceptible.[1–4] MIC testing for gentamicin is useful only to evaluate the presence of β-lactam-aminoglycoside synergy. *E. faecium* isolates with MIC >128 mg/L, this synergy is broken. Our study showed that some of the strains with *aac-aph* gene reported as susceptible by VITEK and PHOENIX. We believe that the concentration used by automates to detect gentamicin-resistant enterococci should be re-evaluated.

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