Table 3L. Tests for Detecting High-Level Aminoglycoside Resistance in *Enterococcus* spp.[a] (including disk diffusion)

Test	Gentamicin HLAR			Streptomycin HLAR		
Test method	Disk diffusion	Broth microdilution	Agar dilution	Disk diffusion	Broth microdilution	Agar dilution
Medium	MHA	BHI[b] broth	BHI[b] agar	MHA	BHI[b] broth	BHI[b] agar
Antimicrobial concentration	120-µg gentamicin disk	Gentamicin, 500 µg/mL	Gentamicin, 500 µg/mL	300-µg streptomycin disk	Streptomycin, 1000 µg/mL	Streptomycin, 2000 µg/mL
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16-18 h	24 h	24 h	16-18 h	24-48 h (if susceptible at 24 h, reincubate)	24-48 h (if susceptible at 24 h, reincubate)
Results	6 mm = resistant 7-9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: • R = > 500 µg/mL • S = ≤ 500 µg/mL	Any growth = resistant	> 1 colony = resistant	6 mm = resistant 7-9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: • R = > 1000 µg/mL (broth) and > 2000 µg/mL (agar) • S = ≤ 1000 µg/mL (broth) and ≤ 2000 µg/mL (agar)	Any growth = resistant	> 1 colony = resistant

Table 3L. (Continued)

Test	Gentamicin HLAR			Streptomycin HLAR		
Additional testing and reporting	Resistant: is not synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin) that is also susceptible. If disk diffusion result is inconclusive: perform an agar dilution or broth dilution MIC test to confirm. Strains of enterococci with ampicillin and penicillin MICs ≥ 16 µg/mL are categorized as resistant. However, enterococci with penicillin MICs ≤ 64 µg/mL or ampicillin MICs ≤ 32 µg/mL may be susceptible to synergistic killing by these penicillins in combination with gentamicin or streptomycin (in the absence of high-level resistance to gentamicin or streptomycin, see CLSI M07[1]) if high doses of penicillin or ampicillin are used. Enterococci possessing higher levels of penicillin (MICs ≥ 128 µg/mL) or ampicillin (MICs ≥ 64 µg/mL) resistance may not be susceptible to the synergistic effect.[2,3] Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.					
QC recommendations – routine[c]	*E. faecalis* ATCC®[d] 29212: 16-23 mm	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212: 14-20 mm	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible
QC recommendations – lot/shipment[e]		*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant		*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; CSF, cerebrospinal fluid; h, hour(s); HLAR, high-level aminoglycoside resistance; IQCP, individualized quality control plan; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Footnotes

a. Other aminoglycosides do not need to be tested, because their activities against enterococci are not superior to gentamicin and streptomycin.

b. Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably with BHI media.

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Daily if the test is performed less than once per week and/or if an IQCP justifying less frequent QC has not been developed
- Less frequent than daily if the test is performed at least once per week and an IQCP has been developed

d. ATCC® is a registered trademark of the American Type Culture Collection.

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

Table 3L
Tests for High-Level Aminoglycoside Resistance in *Enterococcus* spp.

Table 3L. (Continued)

References for Table 3L

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

2 Torres C, Tenorio C, Lantero M, Gastañares MJ, Baquero F. High-level penicillin resistance and penicillin-gentamicin synergy in *Enterococcus faecium*. *Antimicrob Agents Chemother*. 1993;37(11):2427-2431. doi:10.1128/AAC.37.11.2427

3 Murray BE. Vancomycin-resistant enterococci. *Am J Med.* 1997;102(3):284-293. doi:10.1016/S0002-9343(99)80270-8