

High level resistance to aminoglycosides (HLAR) in Group B Streptococci (GBS): an underestimated aspect.

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Background

GBS is intrinsically resistant to aminoglycosides. High-level aminoglycosides resistance (HLAR) abrogates the enhanced bactericidal activity of the combined use of beta-lactam plus aminoglycoside that is used as empirical therapy for neonatal sepsis and in case of highly invasive and difficult to sterilize GBS or enterococcal infections such as endocarditis, meningitis or infections associated with medical devices. Nevertheless, GBS surveillance screening schemes usually do not include aminoglycosides susceptibility testing. No aminoglycosides breakpoints to be used for beta-haemolytic streptococci are present in the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Only the French EUCAST reports breakpoints for gentamicin and streptomycin for beta-haemolytic streptococci (TABLE 1).

Methods

One hundred-nine GBS strains isolated from different sources in adult disease were tested for both gentamicin and streptomycin by disk diffusion (disk content for gentamicin 120 µg and streptomycin 300 µg). Gentamicin resistance was confirmed also by MIC determination and by PCR for the presence of *aac(6′)-Ie-aph(2″)-Ia* gene

Results

High-level gentamicin resistance (HLGR) was identified in six GBS strains (5.5%) according to the interpretative criteria for zone diameter for enterococci of CLSI. EUCAST and French EUCAST criteria could not be used due to a different recommended gentamicin disk content (30 µg for EUCAST and 500 µg for the French EUCAST). Gentamicin resistance was confirmed by the presence of the gentamicin resistance *aac(6′)-Ie-aph(2″)-Ia* gene and MIC values > 1024 µg/ml. High-level streptomycin resistance (HLSR) rate was highly variable depending on the chosen definition criteria. The screening identified eleven resistant GBS strains (10%) according to CLSI for enterococci, seventy-five GBS strains (69.4%) according to EUCAST for enterococci and all but one GBS strains (108 strains) according to the French EUCAST for beta-haemolytic streptococci (TABLE 2).

Conclusions

- HLAR amongst GBS may emerge as a potential problem and threaten an effective therapy. The abolition of the synergistic action of the beta lactam-aminoglycoside regimen due to possibly emerging HLAR GBS strains could have an impact on the treatment of neonatal sepsis and meningitis, their resolution and the risk of recurrence because of unsuccessful eradication.
- It is advisable to introduce an antibiotic susceptibility testing scheme of GBS clinical isolates that includes HLAR.
- To this aim, the definition and harmonisation of breakpoints for the definition of HLAR is needed and advisable.

References

Creti R *et al.* Creti R, Imperi M, Berardi A, Angeletti S, Gherardi G. Laboratory breakpoints for assessing high level gentamicin resistance in *Streptococcus agalactiae*: it is the time for a consensus. Clin Microbiol Infect. 2022 May 5:S1198-743X(22)00219-1. doi: 10.1016/j.cmi.2022.04.011.

TABLE 1. Interpretative criteria for gentamicin and streptomycin susceptibility test recommended for enterococci and beta-haemolytic streptococci

	EUCAST		CA-SFM EUCAST		CLSI	
	HLGR	HLSR	HLGR	HLSR	HLGR	HLSR
<b>Enterococci</b>						
Microdilution broth (mg/L)	>128	>512	>128	>512	>500	>1000
Disk diffusion zone diameter in mm (disk content)	<8 (30µg)	<14 (300µg)	<8 (30µg)	<14 (300µg)	= 6 (120µg)	= 6 (300µg)
Agar dilution (mg/L)	na	na	na	na	>500	>2000
<b>Beta-haemolytic streptococci</b>						
Microdilution broth (mg/L)	na	na	>256	>512	na	na
Disk diffusion zone diameter in mm (disk content)	na	na	<17 (500µg)	<19 (300µg)	na	na
Agar dilution (mg/L)	na	na	na	na	na	na

EUCAST: European Committee on Antimicrobial Susceptibility Testing  
CLSI: Clinical and Laboratory Standards Institute  
CA-SFM: Comité de la Antibiogramme de la Société Française de Microbiologie  
HLGR: high-level gentamicin resistance  
HLSR: high-level streptomycin resistance  
na: not available

TABLE 2

serotype (n) *	EUCAST (enterococci)		CA-SFM EUCAST (beta-haemolytic streptococci)		CLSI (enterococci)		<i>aac(6')-Ie-aph(2'')-Ia</i> gene	gentamicin MIC
	disk diffusion method **		disk diffusion method **		disk diffusion method **			
	HLGR	HLSR	HLGR	HLSR	HLGR	HLSR		
Ia (21)	na	R (14)	na	R (20)				
Ib (10)	na	R (9)	na	R (10)	R (1)	R (4)	1	> 1024 µg/ml
II (8)	na	R (5)	na	R (8)				
III (40)	na	R (23)	na	R (40)		R (6)		
IV (9)	na	R (8)	na	R (9)	R (4)	R (1)	4	> 1024 µg/ml
V (19)	na	R (15)	na	R (19)	R (1)		1	> 1024 µg/ml
IX (1)	na		na	R (1)				
NT (1)	na	R (1)	na	R (1)				
TOT. 109		TOT. 75		TOT. 108	TOT. 6	TOT. 11	TOT. 6	

\* determined by both latex agglutination (SSI Diagnostica) and multiplex PCR (Imperi et al. J Microbiol Methods 2010;80:212-214 )