KILLING KINETICS OF CLINICAL ISOLATES OF GROUP B STREPTOCOCCI (GBS) ISOLATED IN BELGIUM FOR PENICILLIN ALONE OR IN COMBINATION WITH GENTAMICIN

BACKGROUND
Associated with high morbidity and mortality, severe GBS infections, either in neonates or in adults, should be treated promptly with antimicrobial agents alone or in combination characterized by both a good diffusion at the site of infection and a short bactericidal lag time. Group B streptococci are uniformly susceptible to penicillin or ampicillin at concentrations usually achieved in blood or cerebrospinal fluid. To accelerate the killing of these organisms, Penicillin (P) or another β-lactam given in combination with an aminoglycoside is usually recommended to start the therapy. Gentamicin (G) MICs of GBS recently isolated in Belgium range from 16 to 256 mg/L. These observed gentamicin MICs are often higher than G-MICs for E. faecalis with low-level resistance to G (LLR), but lower than the G-MICs of E. faecalis with a high-level of resistance to G (HLR).

OBJECTIVE
To determine conditions required for eradication of GBS in vitro.

MATERIAL & METHODS

GBS Strains
6 Belgian strains (stored at –70°C at the Belgian Reference lab. for GBS): - invasive strains isolated, in 2002-2003, either from neonates or adults, - selected either for their known low G-MIC or higher G-MIC (16 to 128 mg/L).

Control strains for P+G synergism testing
As positive and negative control: 2 strains of Enterococcus faecalis either with low or high level of resistance (LLR or HLR) to gentamicin (LLR or HLR).

Determination of MICs
Benzylpenicillin MICs and gentamicin MICs were determined for all GBS and E. faecalis by the Etest method (AB Biodisk), and respectively on Muller Hinton agar with and without 5% of sheep blood.

Kinetic studies: killing curve (KC) determination
Performed according to an Etest-AB Biodisk original procedure. Each isolate was tested twice.
1. KC master plate prepared by flooding a 14 cm agar plate with a 1:10 dilution of the 0.5 McFarland inoculum suspension.
2. Excise fluid pipetted and drained, plate then dried 15 minutes in an incubator.
3. Six Etest strips of P applied using a template.
4. A growth control area (3 x 3 mm) sampled with a 1ml loop and then streaked in the “control” quadrant of CFU (colony forming units) t=0 plate.
5. Along one Etest strip at the level of the known MICs, MICx2 and MICx4, areas sampled and streaked in the respective CFU quadrants.
6. Master plate reincubated and the CFU t=0 plate incubated.
7. After 2 h, master plate taken out the incubator and sampled as previously, along another Etest strip: the control and MIC multiples t=1.
8. Master plate reincubated and the t=2 CFU plate incubated.
9. Procedure repeated at incubation intervals 4 h, 8 h, and 20 h.
10. After overnight incubation, colonies per quadrant enumerated.
11. Numbers of CFU/9 mm2 plotted vs. sampling time for the control and MIC multiples.

Synergy experiments
Determination of MICs: Combination test performed according to an Etest-AB Biodisk original procedure.
1. Plate inoculated as for determination of MICs of individual drugs, then a strip of G (range 0.004-128 mg/L) placed on the agar surface, its position marked and plate left for 1 hour on the bench.
2. G strip removed and P strip positioned on the imprint of the first antibiotic to have a ratio of 1:1, the gradients then superimposed. Plate immediately incubated overnight at 35°C.
3. Reading of the MIC of the combination.

Kinetic studies: test performed as described above but with a previous step: G strips left for 1 h upon the plate, further removed and replaced with the P strips. Killing times observed for P+G respectively compared to killing times observed with P alone.

RESULTS

For our kinetics studies and investigation for synergy, we performed the testing according to original AB Biodisk procedures. The expected results observed with the strains of E. faecalis validated, to some extent, the procedures used for our studies. But the observed higher G-MIC (16 to 128 mg/L) for GBS strains were quite disappointing:
- As no synergy at all was demonstrated with the combination P+G (1:1) used, independently of values of Gentamicin-MICs.
- And moreover the killing was reduced at T2 for half of the isolates of GBS by comparison with P alone.

This limited in vitro testing for killing of Belgian GBS isolates by P and by the combination P+G (1:1).

DISCUSSION AND CONCLUSION

To determine which combination and ratio of antimicrobial agents could be used to shorten the killing time of GBS and to recommend wisely which regimen should be administered to treat patients with invasive GBS infections.
- It did not show any synergism or accelerated killing.
- Further evaluation should be performed on these strains with other ratio or other β-lactams, as ampicillin, in combination with gentamicin.