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

XVIth LISSSD
September 2005

 # 155

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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*.**
  - **By investigating *in vitro*, the potential synergism and killing kinetics of penicillin and gentamicin (ratio 1:1), at different concentrations, against strains of GBS recently isolated in Belgium.**

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

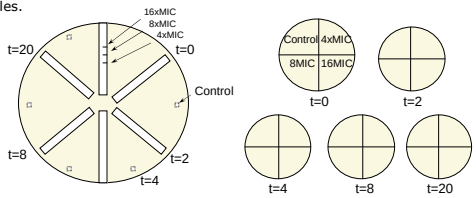
Benzylpenicillin MICs and gentamicin MICs were determined for all GBS and *E.faecalis* strains by the Etest method (AB Biodisk), respectively on Muller Hinton agar with and without 5% of sheep blood.

MATERIAL & METHODS

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- Excess fluid pipetted and drained, plate then dried 15 minutes in an incubator.
3- Six Etest strips of P applied using a templates.
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 levels of the known MICx4, MICx8 and MICx16, 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 at t=2. Master plate reincubated and the t=2 CFU plate incubated.
8- Procedure repeated at incubation intervals 4 h, 8 h, and 20 h.
9- After overnight incubation, colonies per quadrant enumerated.
10- 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 0.016 – 256 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 positionned onto 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

Penicillin MICs

The range of P-MICs for the 6 isolates of GBS is 0.032-O.O47 mg/L.

For *E.faecalis* the MICs were respectively 1.5 and 6 mg/L for the isolates with a LLR or a HLR to G.

Synergy testing

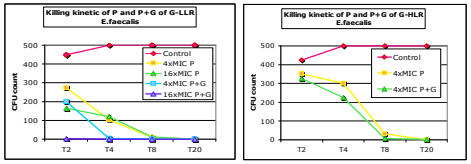
A synergistic effect of 2 antibiotics was considered when the MIC of drugs in the combination is >= 2 dilutions lower than the MIC of the most active drug. "Indifference" was reported when MIC of drugs in the combination was within +/- 1 dilution compared to single drugs.

"Indifference" was observed either for all GBS or E.*faecalis* strains: P+G in combination were not more effective than P alone.

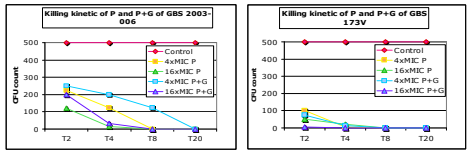
Kinetic experiments

Comparison of killing of different strains by P or P+G. Representative data are shown in the following figures. Growth of bacteria occurred in the absence of antibiotic (control), killing was complete with P >= 4xMIC in 10 to 20 h.

E.faecalis: killing by P+G occurred at a much rapid rate for G-LLR but no significant difference for G-HLR strains.



GBS: no accelerated killing was observed for any GBS strain with the combination P+G, even at 16xMIC, compared with P alone. On the contrary, unexpected, killing was reduced at T2 for 3 isolates and even at T8 for 1 isolate with the combination P+G.



DISCUSSION AND CONCLUSION

For our kinetics studies and investigation for synergism, 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 results with 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 in vitro study, very far from the pharmacodynamic and pharmacologic conditions of clinical *in vivo* use of a combination therapy, just show that any β-lactam in combination with G does not demonstrate necessarily the expected synergism to kill GBS.

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

→ It did not show any synergism or accelerated killing.

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.

→ Further evaluation should be performed on these strains with other ratio or other β-lactams, as ampicillin, in combination with gentamicin.