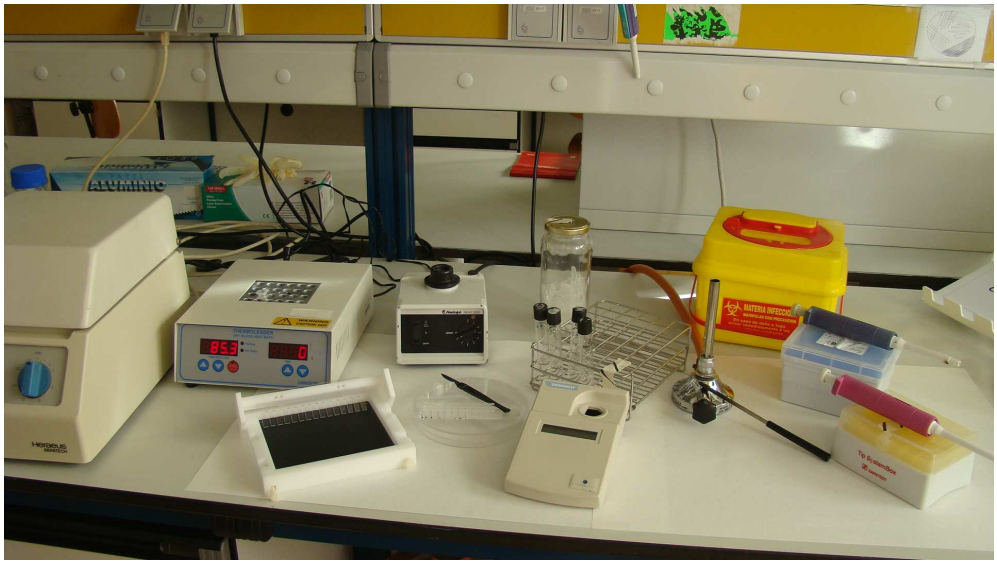
PROTOCOL 4. <u>TYPING OF BACTERIAL PATHOGENS USING PULSED FIELD GEL ELECTROPHORESIS (PFGE)</u>

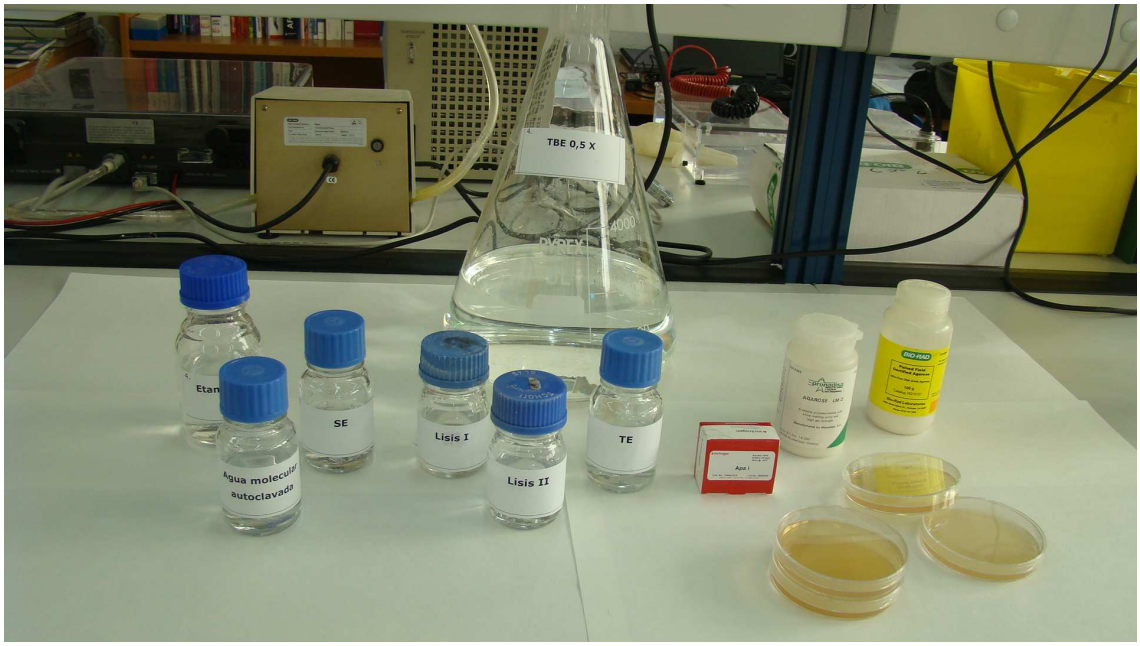
Materials

- Bacterial culture on an agar plate
- Molecular Grade agarose
- Low-melting point (LMP) agarose
- PFGE grade agarose
- Restriction enzymes (*Apa* I for *A. baumannii*)
- Inoculating loop
- Incubator
- Eppendorf tubes (vol 1,5 ml), sterile estériles
- 50 ml tubes, sterile
- Pipettes and tips, sterile
- Cutter
- Plug mould
- Gel tray
- Comb
- Microwave oven
- Centrifuge
- Espectrophotometer
- PFGE equipment (chamber, power suply…..)
- Transilluminator



Solutions

- Molecular grade water, autoclaved
- SE buffer: 75 mM NaCl, 25 mM EDTA (pH 7.5)
- Lysozyme (10 mg/ml): disolve in 10 mM Tris-Cl (pH 8.0) just before use.
- Proteinase K (50 mg/ml): disolve in 50 mM Tris (pH 8.0), 1.5 mM calcium acetate. Keep at -20ºC.
- Lysis solution I: 6 mM Tris, 100 mM EDTA, 1M NaCl, 0,5 % w/v Brij 58, 0,2 % w/v sodium deoxycholate, 0,5 % N-lauroylsarcosine, 1mM $MgCl_2$.
- Lysis solution II: 1 % w/v N-lauroylsarcosine, 0,5 M EDTA (pH 9.5).
- TE: 10 mM Tris, 10 mM EDTA (pH 7.5)
- 70 % ethanol
- TBE 0,5 X
- Molecular Weight Marker PFGE
- Gel stain solution: Gel Red



Procedure (for A. *baumannii*)

4.A. Preparation of unsheared DNA

Day 1:

- Incubate *A. baumannii* overnight in nutrien broth at 37ºC

Day 2:

- Set waterbath to 50-56ºC and clean block moulds with etanol and distilled water.
- Prepare 2% LMP agarose in TE buffer and place in waterbath
- Pellet 3 ml overnight broth in 2 ml eppendorf and resuspend in 300 µl TE or SE buffer. (concentration of 2,3-2,7 Mc Farland).
- Prepare samples one by one. Keeping the sample and agarose in the waterbath, add 300 µl agarose to the sample, mix by pipetting and inmediatly transfer to moulds (2/sample). Set moulds at 4ºC.
- Transfer plugs to sterile 5 ml tubes, add 3 ml lysis solution I + 0.5 mg/ml lysozyme, and incubate overninght at 37ºC.

Day 3:

- Remove lysis solution I , add 3 ml of lysis solution II + 0.5 mg/ml proteinase K, and incubate overnight at 56ºC.

Day 4:

- Wash plugs in 3 ml TE for 30 min at 4ºC. Repeat three times.

4.B. DNA Restriction

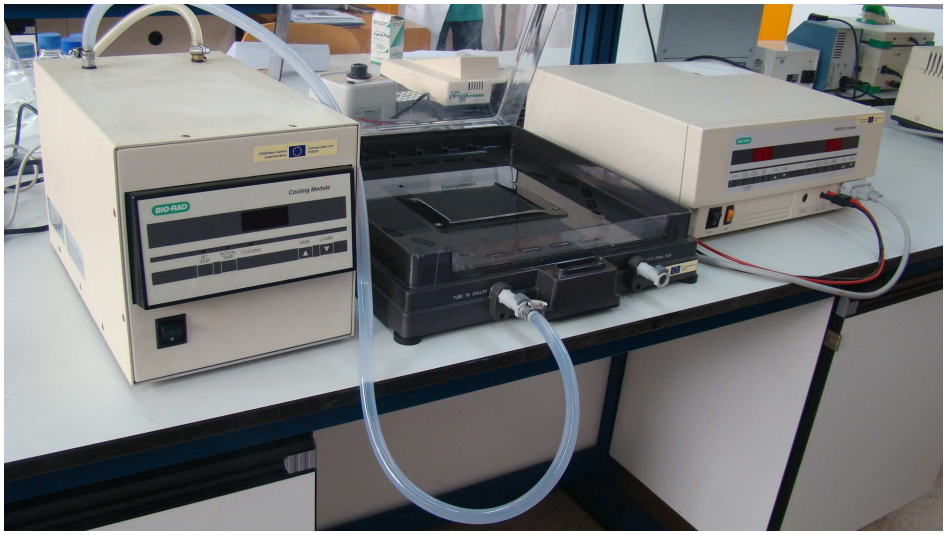
Day 1:

- Prepare 1X *Apa* I reaction buffer with sterile distilled water
- Cut 3mm portion of plug, cover with 100 µl fresh TE buffer in 0.5 ml eppendorf, incubate at 4ºC for 1 hour.
- Wash plugs with 100 µl washing buffer for 20 min.
- Repeat washes in 1X *Apa* I buffer for 20 min
- Replace with 100 µl fresh 1X *Apa* I buffer and 20 U Apa I and incubate at 30 ºC 4-5 hours (or overnight)
- If not inmediatly used, keep plugs at 4ºC in TE (up to a week)

4.C. Separation of DNA fragments

Day 1:

- Prepare 1.5 L 0.5X TBE
- Prepare 1.5% PFGE agarose gel in 100 ml 0.5 TBE. Add Gel Red.
- Load digested plugs into wells, with standard strain at either en of gel and every 5 lanes. Seal wells with leftover LMP agarose.
- Run conditions: 14ºC; ramping 5-30 sec; 200 V; run time 30 h



Day 2:

-Visualise under UV illumination, photograph and save image.