Induction of Tigecycline Resistance in *Acinetobacter baumannii*

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[Abstract] Multidrug resistance *Acinetobacter baumannii* (*A. baumannii*) (MDRAB) has emerged as a serious threat in hospitals in recent years. Currently, there are few antibiotics, including tigecycline, available to treat infections caused by MDRAB effectively. Both tigecycline-resistant and tigecycline-susceptible isogenic strains of MDRAB are valuable in understanding the mechanisms underlying tigecycline resistance. To get the isogenic strains in the laboratory, we describe a protocol for induction of tigecycline resistance in *A. buamannii* by serial passage to plates with tigecycline of different concentrations. The minimal inhibitory concentration of *A. baumannii* by tigecycline was determined according to the protocol "Minimal Inhibitory Concentration (MIC) Assay for *Acinetobacter baumannii*" (Lin *et al.*, 2014b).

Materials and Reagents

1. *A. baumannii* (ATCC, catalog number: 17978)
2. Tigecycline (Wyeth, catalog number. 0220620-09-7)
3. Tryptone (Pronadisa, catalog number: 1612)
4. Yeast extract (Pronadisa, catalog number: 1702)
5. NaCl (MDBio, catalog number: 101-1647-14-5)
6. 99% glycerol (Honeywell International, Riedel-deHaen, catalog number: 5523)
7. Lysogeny broth (LB) (see Recipes)
8. 20% glycerol (see Recipes)

Equipment

1. 50 ml polystyrene culture tubes (sterile)
2. 37 °C shaking and static incubators
3. Multichannel pipette (volume ranges 10 µl-1,000 µl)

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Procedure

1. Fetch the *A. baumannii* ATCC 17978 stock stored at -80 °C, scrape the surface of the frozen stock, and plate it on a LB agar.
2. Put the LB agar in the 37 °C incubator overnight.
3. Dissolve a single colony of *A. baumannii* from the overnight culture in 3 ml LB broth and incubate overnight at 37 °C, 220 rpm.
4. On day 1, 3 ml of LB broth containing tigecycline at the MIC, which is determined by the protocol "Minimal Inhibitory Concentration (MIC) Assay for *Acinetobacter baumannii*" (Lin *et al.*, 2014b), is inoculated with 30 µl broth containing *A. baumannii* (step 1), and the cultures are incubated at 37 °C with shaking (220 rpm) overnight. *Note: The choice of the volumes 30 µl and 3 ml is aimed to keep 1:100 dilutions. If other volumes or dilution ratios are used, it may lead to the change of tigecycline resistance induction time.*
5. On day 3, 30 µl of the culture is transferred to 3 ml of LB broth containing tigecycline at 8x MIC (step 2), and the cultures are again incubated at 37 °C with shaking (220 rpm). If no bacterial growth is noted from step 1, lower the tigecycline concentration to half of the 8x MIC, then repeat step 1.
6. On day 5, 30 µl of the culture is transferred into LB broth containing tigecycline at 16x MIC (step 3), and the cultures are again incubated at 37 °C with shaking (220 rpm).
7. This passaging is repeated on day 7 (step 4).
8. On day 9, aliquots (0.5 ml) of the cultures are mixed with 0.5 ml 20% glycerol and stored at -80 °C until use.
9. Daily passaging in tigecycline-free LB is conducted for 30 days after the tigecycline resistant strain is obtained to make sure the induction of tigecycline resistance is successful and long-lasting.

Recipes

1. LB
   10 g tryptone
   5 g yeast extract
   5 g NaCl
   Fill to 1 L with ddH_2O
   Sterilized by autoclaving at 121 °C for 15 min
2. 20% glycerol
   20 ml 99% glycerol fill to 100 ml with ddH_2O
   Sterilized by autoclaving at 121 °C for 15 min

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