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Gentamicin Protection assay (Intracellular Survival Assay) for *Salmonella* Typhimurium/Typhi

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Salmonella invades the epithelial cells by inducing bacterial uptake, whereas phagocytes readily take up the bacteria by phagocytosis. Intracellular *Salmonella* is resistant to gentamicin, but extracellular bacteria are susceptible to the antibiotic, therefore, adhesion, invasion, and bacterial replication can be measured using this protocol. Inside the cells, *Salmonella* proliferates and the rate of proliferation can be determined by enumerating the bacterial CFU from the infected cells.

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1 Epithelial cells were infected with mid-log phase culture of bacteria grown in LB (OD600 0.3)

OR

phagocytes were infected with overnight culture (OD600 0.3). The multiplicity of infection (MOI) of 10 was used in each case.

2 Bacterial attachment to host cells was enhanced by centrifuging at 600 rpm for 10 min.

3 After 25 min of infection, cells were washed three times with 1x PBS and treated with gentamicin (100 µg/ml in complete media) for 1 hour and then maintained with 25 µg/ml gentamicin for the rest of the experiment.

4 0.1% Triton-X 100 (v/v in 1x PBS) was used to lyse the cells and the lysate was plated on Salmonella-Shigella (SS) agar for S. Typhimurium strains and Wilson Blair (WB) agar for S. Typhi strains.

5 For adhesion assay:
The cells were washed three times with 1x PBS and lysed immediately after the initial 25 min incubation. The percent adhesion was calculated with respect to the pre-inoculum used for infection.

6 For invasion assay:
The cells were washed three times with 1x PBS and lysed after incubation in 100 µg/ml gentamicin treatment (i.e. 1 hour post infection) and percent invasion was calculated with respect to the pre-inoculum used for infection.

7 For intracellular survival assay (ICSA):
The cells were washed three times with 1x PBS and lysed at 2 hours and 18 hours post infection. CFU at 18 hours was divided by CFU at 2 hours to obtain fold replication of the intracellular bacteria.

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