

Differences in Antibiotic Susceptibility of *Corynebacterium pseudotuberculosis* Grown Planktonically or as a Biofilm

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Introduction

- Corynebacterium pseudotuberculosis* (Cptb) is a gram positive, facultative intracellular bacteria.
- Cptb is the causative agent of caseous lymphadenitis (CLA) in sheep and goats but can also cause chronic infections in other animals and humans.
- Infections caused by Cptb are difficult to control because of the lack of adequate diagnostic assays and poor responses to antibiotic therapy.
- Cptb is susceptible to several classes of antibiotics *in vitro*, but for unknown reasons is generally resistant to *in vivo* drug therapy.
- In vivo* resistance may be due to the inability of drugs to penetrate encapsulated lesions or the formation of extracellular biofilm-like communities by Cptb.
- Biofilms are defined as attached communities of bacteria that produce an extracellular matrix and express phenotypic resistance to antibiotics.

Objective

To demonstrate that Cptb expresses phenotypic drug resistance by forming extracellular biofilm-like communities *in vivo* and *in vitro*.

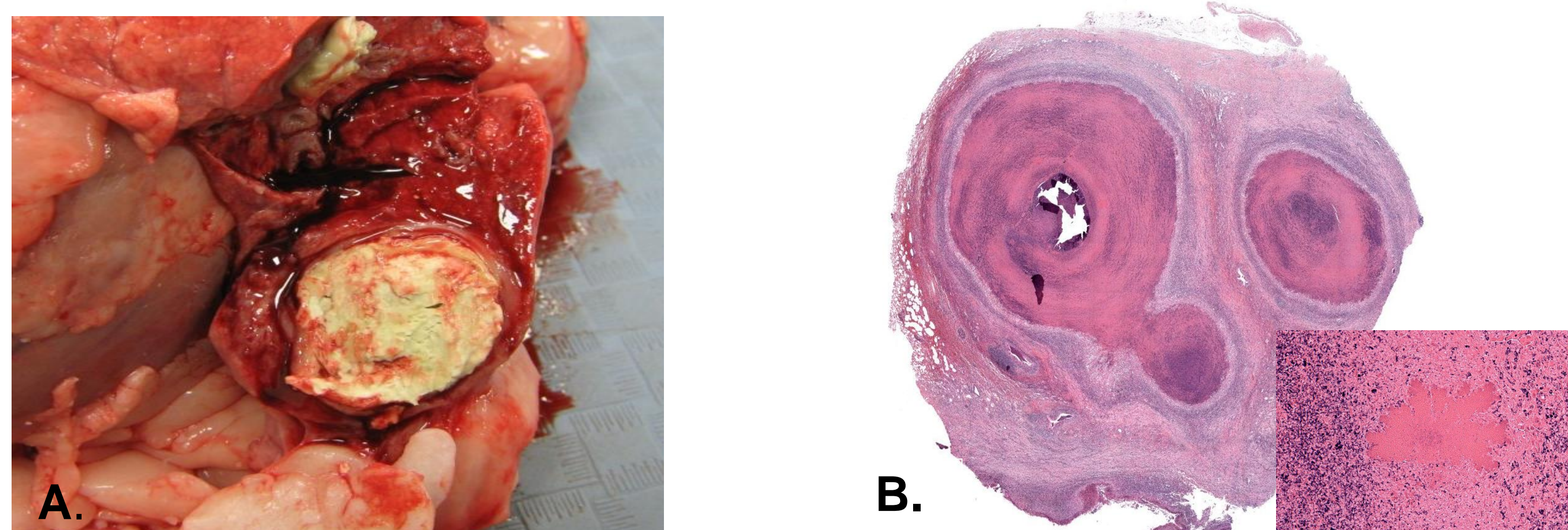


Figure 1. A. Gross Cptb lesion in the lung of a sheep with CLA. B. Histologic section showing encapsulation of a necrotic CLA lesion. Inset: Extracellular biofilm-like community of Cptb. H&E stain.

Materials and Methods

- Cptb was isolated from a sheep with CLA and grown in tryptic soy broth (TSB) with (planktonic) or without (attached) 0.05% Tween-80 at 37°C for 24-27 hours.
- Cptb was cultured planktonically to a concentration of 2-5x10⁵ CFU/ml then transferred and treated in a 96 well plate.
- For the biofilm-like communities (attached), the Cptb was cultured and treated attached to the bottom of a 96 well plate.
- The MIC of ampicillin (4 µg/ml) and rifampin (4 ng/ml) for Cptb was determined on planktonic cultures.
- Cultures were treated with serial dilutions of ampicillin or rifampin from 16 µg/ml to 1 µg/ml and 0.0156 µg/ml to 0.001 µg/ml, respectively.
- After 24 hours of treatment, the CFU corresponding to the MIC for each drug was determined by plating on TSB + 5% sheep blood agar plates.

Results

	Planktonic	Attached
Control (no drug)	1.07x10 ⁵ CFU/ml	3.75x10 ⁴ CFU/ml
Ampicillin (4 µg/ml)	TFTC (1 CFU)	2.55x10 ⁴ CFU/ml
Rifampin (4 ng/ml)	3.48x10 ⁴ CFU/ml	3.4x10 ⁴ CFU/ml

Table 1: CFU of planktonic and attached Cptb treated with ampicillin and rifampin.

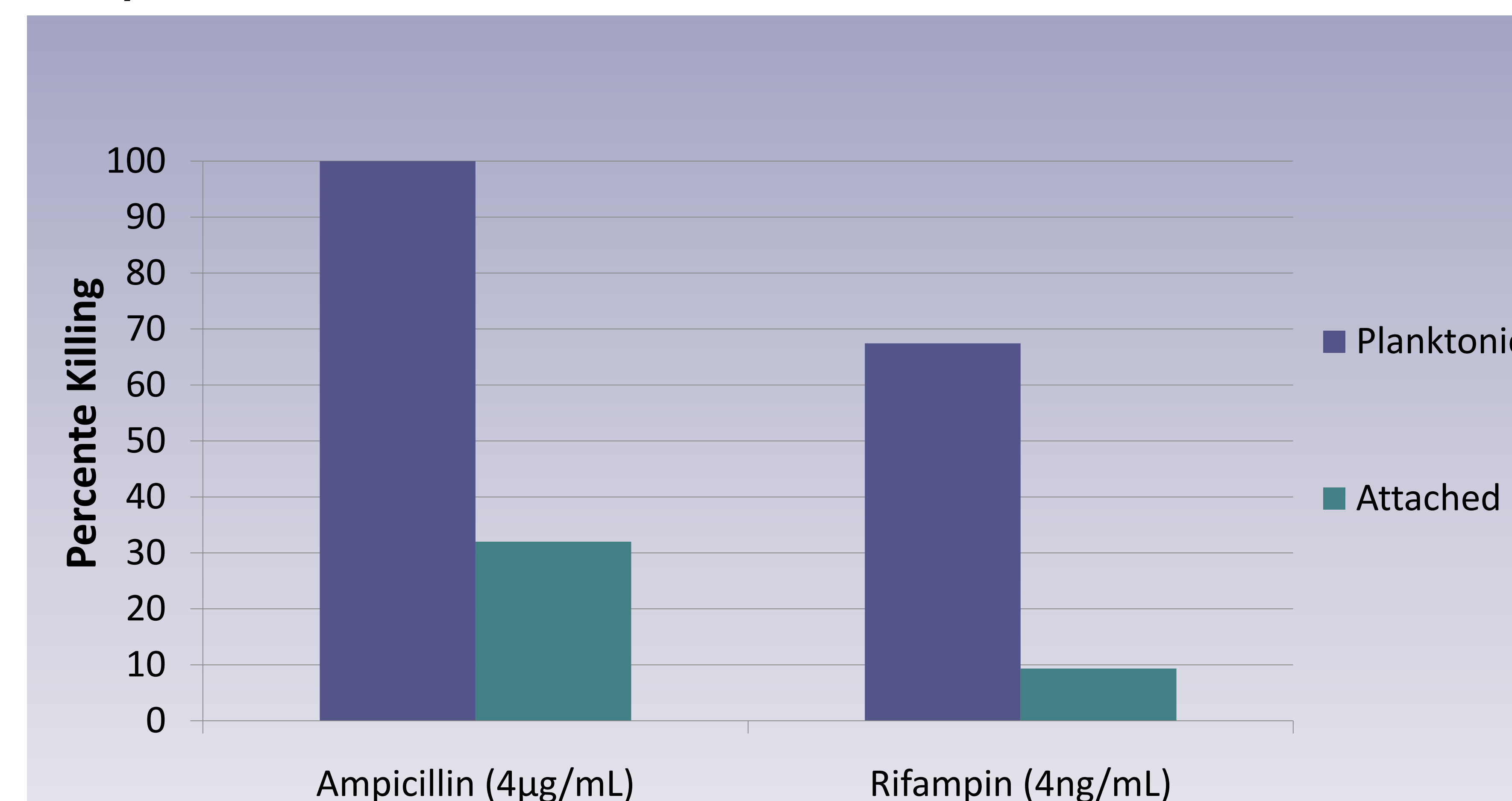


Figure 2: Percent killing of planktonic and attached Cptb by ampicillin and rifampin.

Conclusions

- Cptb forms extracellular biofilm-like communities *in vivo*.
- Cptb can exist *in vitro* as attached biofilm-like communities.
- Attached biofilm-like communities of Cptb express increased antibiotic resistance compared to bacteria grown planktonically.
- Cptb cultured planktonically or as a biofilm is more susceptible to rifampin.
- The MIC based on planktonic growth does not accurately reflect antibiotic susceptibility of Cptb grown as a biofilm.

Acknowledgements

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