DETERMINING THE MINIMUM INHIBITORY CONCENTRATION OF LINCOMYCIN ANTIBIOTICS FOR PAENIBACILLUS LARVAE AND THE CONTROL OF AMERICAN FOULBROOD IN HONEYBEES

Antibiotic resistance and Honeybees, what we can do to help.

Authors

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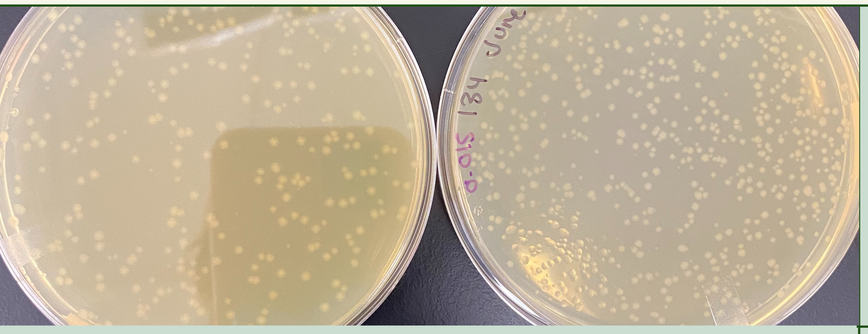


01. Introduction

Paenibacillus larvae (*P. larvae*) is a spore-forming bacteria able to infect honeybee larvae causing the honeybee disease American Foulbrood (AFB). Larva exposed to the bacteria through contaminated food become infected. The bacteria will eventually fully consume and decompose the larva, leaving a brown “ropy” mass containing millions of P. larvae spores (Genersch, 2010). These spores can remain pathogenic for over 35 years through an array of temperature and environment changes (Ebeling, 2016).

As of December 2018, 3 antibiotics oxytetracycline, tylosin, and lincomycin have been approved for veterinary use by prescription only to treat AFB in Canada. Oxytetracycline has been used the longest by beekeepers for AFB control though there has been an increase in resistant strains of P. larvae over the past two decades (Thompson et al., 2003). Though P. larvae is not pathogenic to humans, antibiotic resistance in hives can lead to antibiotic overuse, potentially leading to trace amounts of the antibiotics being found in honey.

By controlling the ways which consumers can obtain antimicrobials, the potential development of resistance in pathogens and other organisms may be limited, and treatments will remain effective when necessary. However, this option is only a temporary fix, allowing more time to develop and research other possible antibiotics (Ebeling, 2016).

02. Objective

Determine the Minimum Inhibitory Concentration of Lincomycin for *P. larvae*

Since lincomycin hydrochloride is the most recently approved antibiotic in Canada for treatment of AFB, there is a lack of knowledge regarding its use, specifically the minimum inhibitory concentration (MIC). The MIC is defined as the lowest concentration of an antibiotic needed to inhibit growth of a microorganism after incubation (Andrews, 2002).

Determining MICs helps optimize the efficacy of the antibiotics we use and this research would help provide insight into novel ways we can treat foulbrood in our hives nationally. This research would also help other labs and research facilities in screening for AFB; establishing the MIC would help develop updated screening practice to detect resistance.

The objective of this project was to determine the MIC of lincomycin for the control of AFB, specifically in the treatment of 10 wild strains isolated from Alberta and one reference P. larvae strain. Beyond the minimum inhibitory concentration, the concentrations required to inhibit 50%, and 90% of growth were also determined for each individual strain that was tested.

03. Methodology

A total of 10 P. larvae isolates from across Alberta, as well as one reference P. larvae strain, were used in this study (Table 1) Each isolate was streak plated onto MYPGP petri plates and incubated for 72 hours at 35 ℃. 2000 mg of lincomycin hydrochloride with a potency of 850 mg/mg was diluted in 170 mL of nano pure water to make a stock solution of 10000 mg/L.. MYPGP medium was maintained at 60 ℃ until the antibiotic was incorporated. The final medium was poured into 25mL petri dishes with final lincomycin concentrations of 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, and 8 mg/L. For control, MYPGP without lincomycin was also prepared. Isolated colonies were picked from streak plates of each bacterial strain and suspended in nuclease-free water to make a heavy suspension. A diluted suspension equivalent to the McFarland standard 0.5 was made in nuclease-free water. That light suspension was serially diluted to 10-2 in water and 25 uL of it was spread-plated onto the Lincomycin plates in triplicates for each concentration of lincomycin (including MYPGP + 0 mg/L lincomycin). After spread plating, the plates were incubated at 35 C for 72 hours and CFUs were counted.

Table 1. Preliminary antimicrobial discs diffusion zones (diameter) of P. larvae strains (mm). Ssusceptibility to oxytetracycline represented by inhibition zone larger than or equal to 20 mm and resistance represented by inhibition zone less than 20 mm.

	AFB isolate	Oxytetracycline	Lincomycin
1	AB 001	0	31
2	AB 002	34	42
3	AB 046	44	11
4	AB 048	0	10
5	AB 052	0	22
6	AB 060	0	11
7	AB 086	0	38
8	AB 134	0	25
9	AB 138	38	39
10	AB 145	0	41

04. Results

The MIC was 0.25 mg/L for all 11 AFB isolates as no growth was present past 0.125 mg/L lincomycin. Despite the great difference in CFUs on the plates without antibiotics between different P. larvae isolates, the concentration at which growth was completely inhibited was consistently 0.25 mg/L. Strains that displayed zones of inhibition less than 20 mm for the tetracycline antibiotic disc test, indicated antibiotic resistance. *However, the MIC of lincomycin for the inhibition of growth of these strains was equal to that of all the other strains used in this study. Thus, these findings support the use of lincomycin for the control of tetracycline resistant strains of P. larvae.* The concentration required to inhibit 50% of growth was consistently less than or equal to the concentrations needed to inhibit 90%, which was expected.

The concentration needed to inhibit 100% of bacterial growth was consistently 0.25 µg/mL, which supports the other data in this study; that lincomycin is effective at controlling growth for tetracycline resistant strains of P. larvae. These results are congruent with previous studies done on susceptibility of P. larvae to novel antibiotics, where researchers also reported low MIC values suggesting the bacteria is susceptible to antibiotics other than oxytetracycline

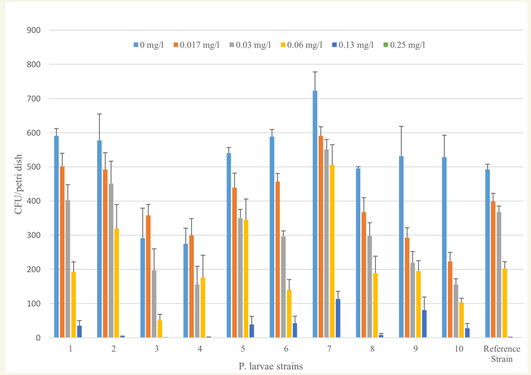


Figure 1. Inhibition effect of increasing lincomycin concentrations on P. larvae growth. P. larvae strains were plated in triplicates onto MYPGP media containing varying concentrations of lincomycin (0, 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, and 8 mg/L). Plates were then incubated at 35 oC for 72 hours and CFUs were counted. The concentration able to fully inhibit growth for each strain was noted as the MIC.

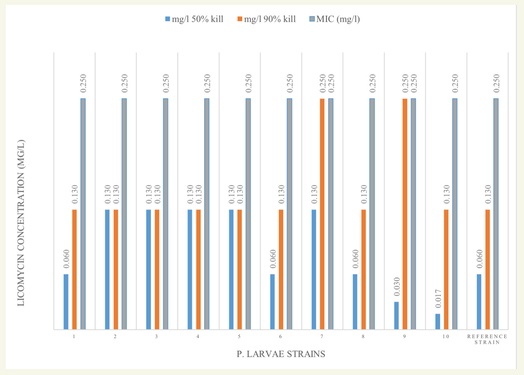


Figure 2. Concentration of lincomycin to inhibit 50, 90 and 100% (MIC) of growth of P. larvae strains.

07. Reference

Andrews, J. M. (2002). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 49(6), 1049-1049.

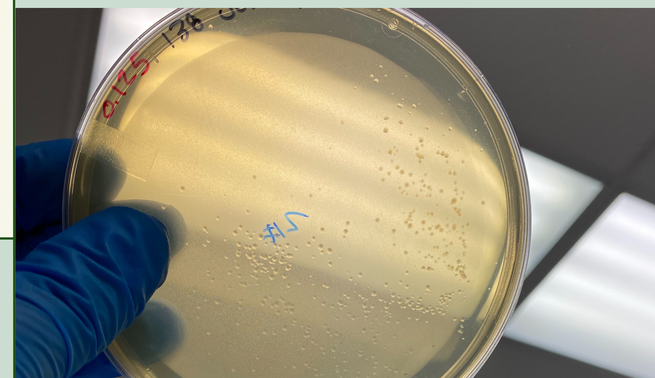
Ebeling, J., Knispel, H., Hertlein, G., Fünfhaus, A., Genersch, E. (2016). Biology of Paenibacillus larvae, a deadly pathogen of honey bee larvae. Applied microbiology and biotechnology, 100(17), 7387–7395. https://doi.org/10.1007/s00253-016-7716-0

Genersch E. (2010). American Foulbrood in honeybees and its causative agent, Paenibacillus larvae. Journal of invertebrate pathology, 103 Suppl 1, S10–S19.

Thompson, T. S., Noot, D. K., Calvert, J., & Pernal, S. F. (2003). Determination of lincomycin and tylosin residues in honey using solid-phase extraction and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Journal of chromatography A, 1020(2), 241-250.

06. Conclusion

The MIC of lincomycin for the control of P. larvae was found to be 0.25 µg/mL. The low value indicated that the chosen P. larvae strains analyzed were highly susceptible to the antibiotic lincomycin. Based on the results of this study, out of the strains tested there are no resistant or intermediate strains of P. larvae to lincomycin at 0.25 mg/L. Through this study, we have determined the minimum inhibitory concentration of lincomycin for the control of American Foulbrood. And we have shown that lincomycin is effective alternative in controlling oxytetracycline resistant AFB



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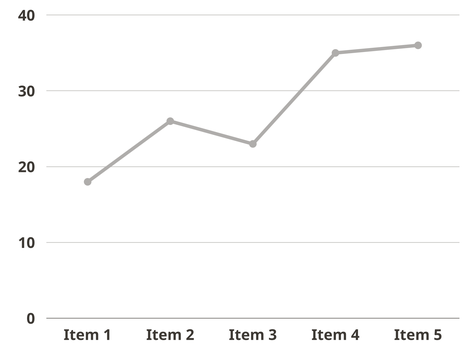
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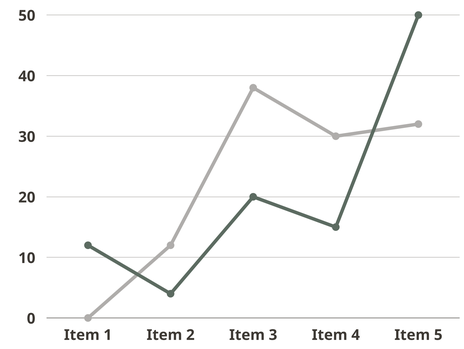
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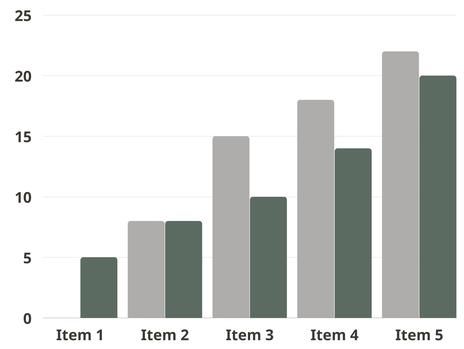
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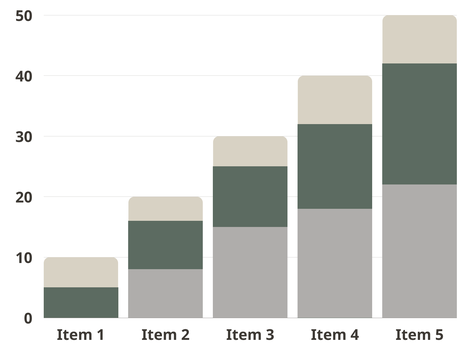
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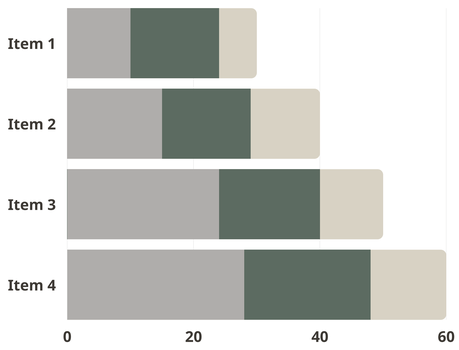
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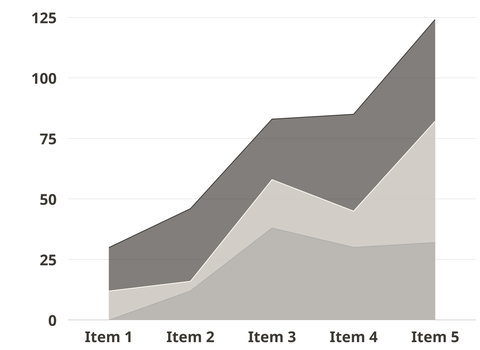
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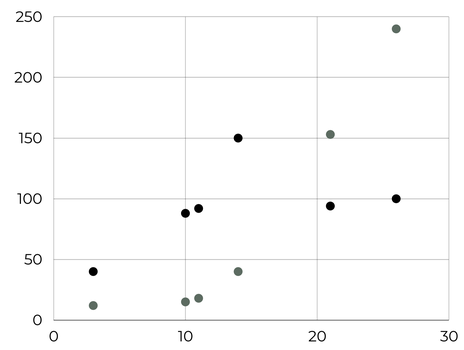
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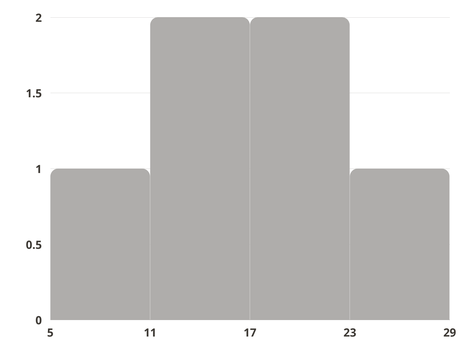
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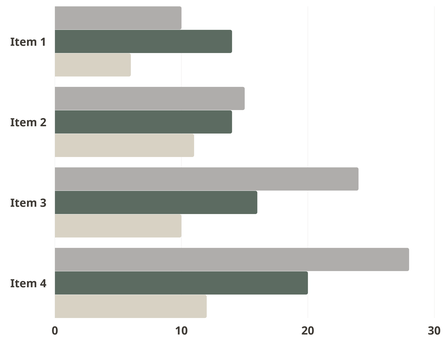
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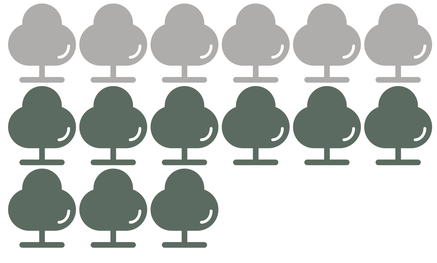

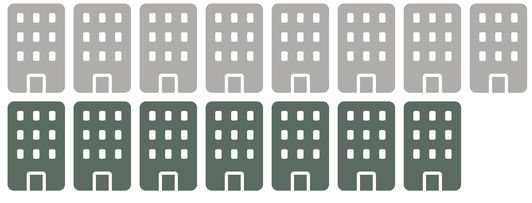

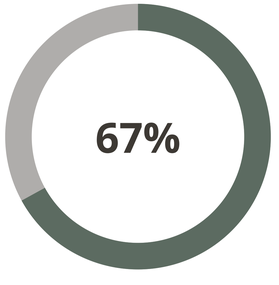
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