The Effects of EDTA and Chlorhexidine as Antibiotic Adjuvants Against *Bacillus cereus* and *Escherichia coli*

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ABSTRACT

Introduction: Antibiotic resistance is an emerging, worldwide epidemic—so much that the World Health Organization believes that a post-antibiotic era is approaching because of its prevalence. Concerningly, no new major types of antibiotics have been brought to the consumer market in nearly 30 years. However, recent studies have shown promise of aiding the effectiveness of currently available antibiotics by using adjuvants to boost antibacterial activity. **Objectives:** This study aims to assess the potency of two potential adjuvants, ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX), in restoring antibiotic efficacy against bacteria that have developed antibiotic resistance mechanisms. EDTA is currently used in the clinical setting for a number of treatments, including lead poisoning, and has shown some activity against bacterial cell envelope structure. CHX is used as a common clinical antiseptic/disinfectant and disrupts the integrity of bacterial membranes. If low concentrations of EDTA or CHX are used, it is hypothesized that their combination with antibiotics will increase the susceptibility of bacteria to the antibiotic versus using the antibiotic or adjuvant alone. **Methods:** The disc diffusion method was used to assess the antimicrobial properties of EDTA, CHX, antibiotics (Tetracycline, Cephalothin, Ampicillin, or Amoxicillin), or combination treatments. Zone of inhibition measurements (mm) against *Bacillus cereus* and *Escherichia coli* were used to determine the efficacy of each treatment. **Results:** EDTA alone resulted in no zone of inhibition (ZOI) against both bacteria. CHX alone was more effective against the gram-positive bacteria *B. cereus* (18.8mm) versus the gram-negative bacteria *E. coli* (13.5mm). *B. cereus* exhibited increased ZOIs when using various antibiotics combined with one of the two adjuvants: Tetracycline (EDTA added 6.3mm; CHX added 1.1mm), Ampicillin (EDTA added 3.9mm; CHX added 9.1mm), Cephalothin (CHX added 1mm), and Amoxicillin (CHX added 1.45mm). EDTA was found to slightly decrease the ZOI with Cephalothin (decreased by 0.45mm) and Amoxicillin (decreased by 1mm). In contrast, combining the adjuvants with Tetracycline (TE) or Cephalothin (CF) against *E. coli* displayed decreased zones of inhibition: TE 27.2mm, TE+EDTA 21.9mm, TE+CHX 26mm; CF 22.1mm, CF+EDTA 21.1mm, and CF+CHX 20.5mm. **Conclusion:** Several combination adjuvant treatments showed increased inhibition against *B. cereus.* Surprisingly, the use of EDTA and CHX as antibiotic adjuvants against *E. coli* resulted in decreased efficacy of the antibiotics tested. Together, this initial data suggests that EDTA and CHX are good candidates for further exploration as antibiotic adjuvants against gram-positive bacteria; however, EDTA and CHX may be contraindicated when using antibiotics against gram-negative bacteria.

INTRODUCTION

- *Bacillus cereus* is a gram positive bacteria associated with common foodborne illnesses (1). *Escherichia coli* is a gram negative bacteria associated with numerous human infections, see figure 1 (2).
- **Antibiotic resistance** occurs when bacteria possess the ability to withstand the effects of an antibiotic designed to kill or inhibit them, see figure 2 (3).
- Potentiators of antibiotic activity are known as **antibiotic adjuvants (**4).
    - blocking resistance mechanism(s)
    - boosting antibiotic inhibitory mechanism(s)
- Ethylenediaminetetraacetic acid (EDTA) is a chemical that binds certain metal ions. The efficacy of EDTA as an adjuvant is promising after a recent study successfully used this salt as an adjuvant (5):
    - Possible EDTA mechanisms of action: Perturbation of the cell membrane, prevention of biofilm formation, and increased biofilm susceptibility in gram negative microorganisms.
- Chlorhexidine (CHX) is a common disinfectant and antiseptic. It is used for cleaning wounds, surgical instruments, and preventing dental plaque (6).
    - Possible CHX mechanisms of action: Perturbation of the cell membrane

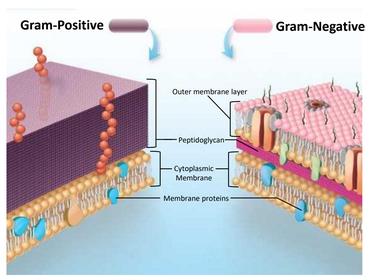

Figure 1. Gram positive and Gram negative bacterial cell envelopes shown. Modified from (7).

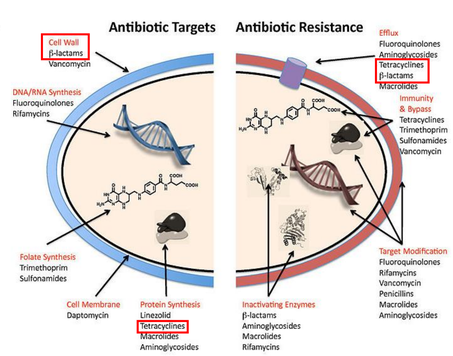

Figure 2. Common antibiotic targets and antibiotic resistance mechanisms shown. Red boxes indicate type of antibiotics tested with adjuvants. Figure modified from (8).

HYPOTHESIS

Combination treatments of antibiotic, CHX, and EDTA will boost antibiotic response to the microorganism and increase ZOIs.

METHODS AND MATERIALS

- Determined whether bacteria were resistant, intermediate, or susceptible to various antibiotics by measuring zones of inhibition on Kirby Bauer plates.
- Antibiotics that produced zones of inhibition that measured close to but not within the susceptible range in mm, using the standard Kirby Bauer, were used for experimentation with adjuvants.
    - Tetracycline 30 (TE30) and Cephalothin 30 (CF30) were tested against *E. coli* since they were intermediate, almost susceptible
    - TE30 and CF30 were tested against *B. cereus* since they were intermediate, almost susceptible, on the Kirby Bauer; Ampicillin 10 (AM10), and Amoxicillin 30 (AMC30) were tested because they were resistant, almost intermediate, on the Kirby Bauer
- The disc diffusion method was used to determine the efficacy of combination adjuvant [ethylenediaminetetraacetic acid (EDTA) or chlorhexidine (CHX)] treatments (antibiotic + 20 µL H_2O (control), antibiotic + 20 µL EDTA, antibiotic + 20 µL CHX, 20 µL EDTA, and 20 µL CHX) against *E. coli* (a gram negative bacteria) and *B. cereus* (a gram positive bacteria).
- Zones of inhibition diameter were measured in millimeters
- Everything was grown on Trypticase soy agar (TSA) plates in room temperature.

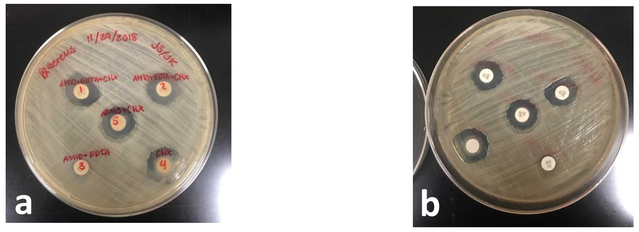

Figure 3. Example of *Bacillus cereus* lawn on a TSA plate. Antibiotic AM10 discs with and without adjuvants (CHX or EDTA) are shown. Zones of inhibition (ZOI) are indicated as a clearing around the disc. The larger the ZOI the better the antibiotic is at inhibiting the growth of the bacterium. Labels are shown in 1a. Plate lid is removed in 1b.

RESULTS

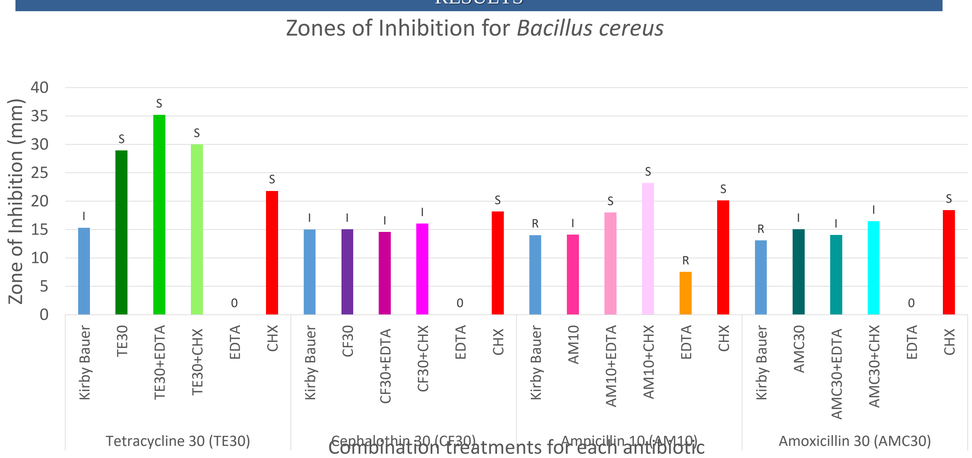

Figure 4. Average zones of inhibition (in mm) for Kirby Bauer and each combination treatment tested against *B. cereus.* Includes whether the bacteria was susceptible (S), intermediate (I), or resistant (R).

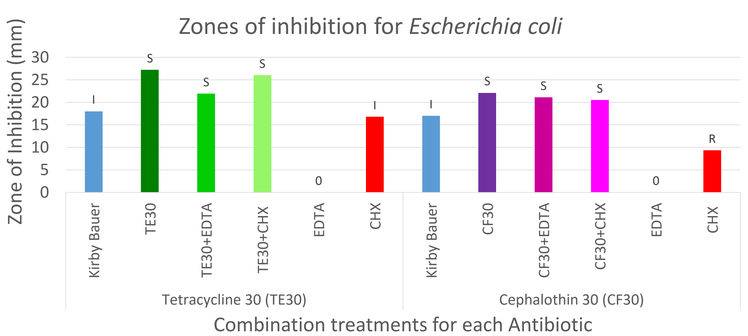

Figure 5. Average zones of inhibition (in mm) for each combination treatment tested against *E. coli*. Includes whether the bacteria was susceptible (S), intermediate (I), or resistant (R).

DISCUSSION

- CHX demonstrated the potential to be an antibiotic adjuvant against Gram positive bacterium *B. cereus* with increased ZOIs (TE30 +1.1mm, AM10 +9.1mm, CF30 +1mm, and AMC30 +1.45mm).
- Combination adjuvant treatments against Gram negative bacterium *E. coli* consistently lowered ZOIs: (TE30+EDTA -5.3 mm, TE30+CHX -1.20mm; CF30+EDTA -1.0 mm, CF30+CHX -1.6mm).
- EDTA by itself was ineffective against both gram positive and gram negative bacteria.
- Interestingly, chlorhexidine alone was more effective at killing *B. cereus* than 3 out of the 4 antibiotics tested.
- Observed differences between *E. coli* and *B. cereus* with the antibiotic adjuvants can likely be explained based on differences in cell envelope structure between Gram negative and Gram positive bacteria and the mechanisms of action used with CHX and EDTA.
    - CHX alone has shown differences in killing Gram – and Gram + bacteria (6).
- CHX is used commonly in dental practices (9) and my be combined with antibiotics during oral surgery (10).

SUMMARY

This initial data suggests that **EDTA and CHX** are good candidates for **further exploration as antibiotic adjuvants against gram-positive bacteria**; however, EDTA and CHX may be contraindicated when using antibiotics against gram-negative bacteria.

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