Escherichia coli (ATCC® 13706™) is Susceptible to Retapamulin, an Antimicrobial Semisynthetic Derivative of Pleuromutilin

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BACKGROUND

Antibiotic resistance is a global threat to human health and is increasing at unprecedented rates. Bacterial infections are becoming more difficult to treat[1].

Wastewater (WW) is identified as a reservoir for pathogens that carry antibiotic resistant genes. Additionally, WW contains traces of antibiotics which can lead to resistance of multiple classes of antibiotics[2,3].

Escherichia coli (E. coli) (strain ATCC 13706) is commonly found in WW and has the potential to become resistant to antibiotics. Previously, retapamulin exhibited antimicrobic effects against Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* (MRSA)[4] and Gram-negative *H. influenzae*[5].

OBJECTIVE & HYPOTHESIS

Given the inhibitory effects of retapamulin on Gram-positive and negative bacteria, we explored the antimicrobial properties of retapamulin on *E. coli* (strain ATCC 13706). **We hypothesized that retapamulin inhibits the growth and survival of *E. coli*.**

METHODOLOGY

We performed a Kirby Bauer disk diffusion assay (Figure 1) to determine the zone of inhibition of *E. coli* against retapamulin.

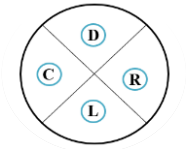

Figure 1: Kirby Bauer disk diffusion set-up. Retapamulin (R) was the test solution. Ciprofloxacin (C) and levofloxacin (L) were used as positive controls and DMSO (D) as a negative control.

We also conducted a minimum inhibitory concentration (MIC) assay (Figure 2) as well as a minimum bactericidal concentration (MBC) test to determine the inhibitory and lethal concentrations of retapamulin on *E. coli*.

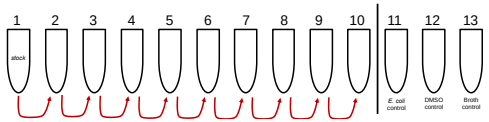

Figure 2: MIC assay set-up. A 1:2 serial dilution was performed using test tubes 1 to 10 with a 25,000 µg/ml stock solution. Test tubes 11 to 13 were used as the positive and negative controls.

RESULTS

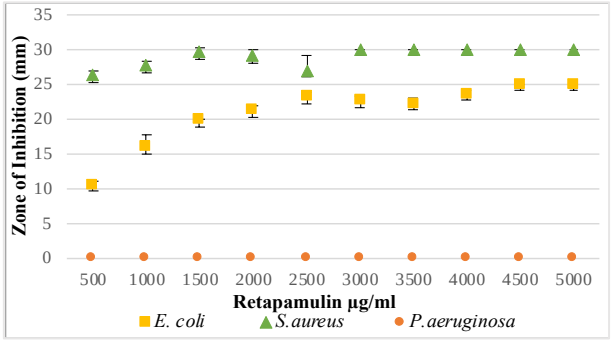

Figure 3: Antibiotic susceptibility of the test organisms when subjected to various concentrations of retapamulin for 24 hours. Results determined by the Kirby Bauer antibiotic assays (Average mm, n=3) (p-value = 4.73×10^{-58}).

Table 1: MIC of *E. coli* after being subjected to various concentrations of retapamulin for a 24 h incubation period at 37°C. Positive (+) = turbidity indicating growth of *E. coli*, whereas negative (-) = inhibition of *E. coli* (p-value = 0).

Retapamulin (µg/ml)	Set 1	Set 2	Set 3	
500	-	-	-	
250	-	-	-	← MIC
125	+	+	+	
62.5	+	+	+	
31.25	+	+	+	
15.63	+	+	+	
7.81	+	+	+	
3.91	+	+	+	
1.96	+	+	+	
0.98	+	+	+	

Table 2: Observed MBC of *E. coli* after being subjected to various concentrations of retapamulin based on the MIC results for a 24 h incubation period at 37°C. Positive (+) = visible colonies of *E. coli*, whereas negative (-) = no visible colonies of *E. coli* (p-value = 0).

Retapamulin (µg/ml)	Set 1	Set 2	Set 3	
500	-	-	-	← MBC
250	-	+	+	
125	+	+	+	
62.5	+	+	+	
31.25	+	+	+	
15.63	+	+	+	
7.81	+	+	+	

DISCUSSION

This study shows that *E. coli* (strain ATCC 13706) is inhibited by retapamulin. The inhibition of *E. coli* growth increases with increasing concentrations of retapamulin starting at 500 µg/ml, however, inhibition plateaus at 2500 µg/ml (Figure 3). Although the concentrations are different in other bacterial species as observed in previous studies, they show similar inhibition profiles to *E. coli*[6].

The observed MIC value for retapamulin against the *E. coli* strain tested in this study is 250 µg/ml (Table 1). Previously, it was observed that *H. influenzae* has a MIC with a range of 0.25-2 µg/ml. In comparison, *E. coli* requires a higher dose of retapamulin to inhibit the growth[6].

The observed MBC value for retapamulin against the *E. coli* strain tested in this study is 500 µg/ml (Table 2). The MBC values have not been published for other Gram-negative bacterial species (i.e., *H. influenzae, B. fragilis*, and *M. catarrhalis*). The MBC value is higher than the MIC value because we need a higher concentration of retapamulin to kill the bacterium versus to inhibit its growth.

CONCLUSION

In this study, *E. coli* (strain ATCC 13706) displayed susceptibility to retapamulin. Further studies are required to determine cytotoxicity of retapamulin. Proper documentation can be retained to study antibiotic resistant *E.coli* with similar sequences to the strain I tested to determine if they will be resistant or susceptible to retapamulin.

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