Effects of Diphenhydramine on E. coli

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Human Microbial Flora

- About 10% of the biomass on the body is symbionts
- Comprised of all the microorganisms that reside in or on the human body
- Most species are non-pathogenic, commensals or mutualists
- Almost 1000 species of bacteria live in the gut of an average human body.
- Vast majority of drugs tested on human cells- Microflora?
Diphenhydramine

- Antihistamine
- Used to relieve allergies
- Hinders the assembly of histamines that are produced during an allergic reaction
- Found in medicines like Genahist, Sominex, Unisom and Benadryl
Benadryl

- Over-the-counter drug used to relieve allergy symptoms such as watery eyes and runny nose
- Diphenhydramine concentration of 12.5 mg./5mL
- Contains sodium benzoate, sodium chloride, sucrose, and glycerin
- Targets acetylcholine (histamine) to prevent allergic reactions
- Taken orally in 5mL to 10 mL doses
Escherichia coli (E. coli)

- Ubiquitous gram-negative bacteria
- Inhabits intestines of most mammals
- Most strains harmless and often beneficial
- Some pathogenic
- Lab strain DH Alpha used as model
Problem

- Does diphenhydramine significantly affect E. coli survivorship?
Recent Study

- 2008 study- examined effects of diphenhydramine on bacterial survivorship in propofol
- Significant inhibitory effect found on growth of bacteria (Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida albicans) at concentrations greater than 1%
Purpose

- To determine if diphenhydramine has a significant effect on E. coli survivorship
Hypotheses

Null Hypothesis - Diphenhydramine WILL NOT significantly reduce E. Coli survivorship

Alternative Hypothesis - Diphenhydramine WILL significantly reduce E. coli survivorship
Materials

- Benadryl
- E. Coli DHS Alpha
- LB Agar Plates + Media
  (1% Tryptone, 0.5% Yeast extract, 1% NaCl)
- Test tubes
- Sterile fluid (100mM KH2PO4, 100mM K2HPO4, 10mM MgSO4, 1mM NaCl)
- Petri dishes
- Pipettes
- 50 Klett Spectrophotometer
- Side arm flask
- Pipette tips
- Spreader bars
- Ethanol
- Bunsen Burner
- Vortex
- Incubator
Procedure

1. E. Coli was grown overnight in sterile LB media
2. A sample of the overnight culture was added to fresh media in a sterile sidearm flask
3. The culture was incubated at 37 degrees Celsius until a density of 50 Klett spectrophotometer units was reached. (Cell density approximately $10^8$ cells/mL)
4. The culture was diluted in a sterile dilution fluid to a concentration of approximately $10^5$ cells/mL
5. The selected experimental variables were diluted with sterile dilution fluid to the chosen concentrations to a total of 9.9 mL.
Procedure (continued)

6. 0.1 mL of cell culture was then added to the test tubes, yielding a final volume of 10 mL. and a cell density of approximately $10^3$ cells/mL.

7. The solution was mixed by vortexing.

8. After vortexing to evenly suspend cells, 0.1 aliquots were removed from the tubes and spread on LB agar plates.

9. The plates were incubated at 37 degrees Celsius for 24 hours.

10. The resulting colonies were counted. Each colony is assumed to have risen from one cell.
Concentration of Benadryl in test tubes

<table>
<thead>
<tr>
<th></th>
<th>0%</th>
<th>0.1%</th>
<th>1%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. Coli</strong></td>
<td>0.1 mL</td>
<td>0.1 mL</td>
<td>0.1 mL</td>
<td>0.1 mL</td>
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<tr>
<td><strong>Sterile Fluid</strong></td>
<td>9.9 mL</td>
<td>9.89 mL</td>
<td>9.8 mL</td>
<td>8.9 mL</td>
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<tr>
<td><strong>Benadryl</strong></td>
<td>0 mL</td>
<td>0.01 mL</td>
<td>0.1 mL</td>
<td>1 mL</td>
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<tr>
<td><strong>Total (mL)</strong></td>
<td>10 mL</td>
<td>10 mL</td>
<td>10 mL</td>
<td>10 mL</td>
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</table>
## Data

<table>
<thead>
<tr>
<th>Trial</th>
<th>0%</th>
<th>0.1%</th>
<th>1%</th>
<th>10%</th>
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<tbody>
<tr>
<td>Trial 1</td>
<td>358</td>
<td>297</td>
<td>341</td>
<td>311</td>
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<tr>
<td>Trial 2</td>
<td>374</td>
<td>313</td>
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<td>344</td>
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<td>Trial 3</td>
<td>402</td>
<td>336</td>
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<td>347</td>
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<td>Trial 4</td>
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<td>Trial 5</td>
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<td>Trial 7</td>
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<td>Trial 8</td>
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<td>385</td>
<td>370</td>
<td>296</td>
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Benadryl effects on E. Coli

Colony Count

Benadryl Concentration

P-value = 0.456053
## ANOVA

### SUMMARY

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
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<td>Column 2</td>
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<td>2749</td>
<td>343.625</td>
<td>3555.982143</td>
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<td>353.375</td>
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<td>336.5</td>
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### ANOVA

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<tr>
<th>Source of Variation</th>
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<th>MS</th>
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<td>Total</td>
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</tr>
</tbody>
</table>
Conclusion

- The data supports the acceptance of the null hypothesis that diphenhydramine would not significantly reduce E. coli survivorship.
- The ANOVA statistical analysis yielded a p-value of 0.456053, which is significantly greater than the 0.05 cutoff for accepting the null hypothesis.
Limitations

- Unknown effects of secondary compounds in Benadryl
- E. coli colonies could have been too small to count
- E. coli colonies could have been too close together to count
- Only one type of test (liquid pulse exposure) used to assess effect
- Slight deviations in plating times
- Only one exposure time
Extensions

- Different medications containing diphenhydramine
- Pure diphenhydramine could have been tested
- Other bacterial models
- Synergistic effects
- Different exposure
References