To what extent can E. coli develop resistance to Manuka honey?

Matthew Muller

University High School, Parkville, Wurundjeri Country

Imagine you lived in a world where the most common deadly infection had no cure, where every little cut, scrape or bruise could lead to your death. Well, this world is quickly becoming a reality.

You see, antibiotics, the wonder drug that has cured nearly all bacterial infections since the 1940s are rapidly becoming ineffective due to the rising threat of Antibiotic resistance. According to the World Health Organisation in 2023, antibiotic resistance, where bacteria evolve methods of becoming immune to antibiotics due to the overuse in health and agriculture, is quickly becoming one of the biggest threats in medicine and led to the deaths of 5 million people in 2019.

To combat this spread of antibiotic resistance, numerous alternatives have become popular in both natural and modern medicine with one such being Manuka Honey. Manuka is known to have many powerful antimicrobial properties against a range of Pathogenic organisms. Further research by Schell et al, 2022 found Manuka is effective against antibiotic resistant bacteria in topical and intestinal uses. Yet research into whether bacteria can develop resistance to MH has not formed a conclusive consensus, with numerous investigations finding opposing or differing data.

To investigate the fundamental question of whether bacteria can become resistant to MH, an experiment was conducted so more research can expand current evidence for whether bacterial resistance to MH can form. This experiment exposed E. coli to increasing solutions of MH with selected concentrations of 5%, 10%, 20% and 40% to assess whether bacteria can grow in higher than lethal concentrations of MH. For this method, the lethal concentration of MH was determined to be 20%, as according to Tan et al., 2009, it was found to inhibit at least 95% of E. coli growth. Further, E. coli was used in this method as it is a relatively safe bacteria meaning minimal harm would be caused to researchers according to Lim et al., 2013, additionally according to Cooper (2000) E.coli is a well-known and highly researched organisms, meaning investigations on E.coli are likely to be more reliable.

The hypothesis that led this investigation was if E. coli bacteria are exposed to increasing MH concentrations, then such E. coli will be able to survive in higher then lethal amounts of MH, as bacteria would undergo random mutations becoming resistant. Methods used in this experiment are derived from both the Kirby-Bauer disk susceptibility test as well as the Harvard medical school experiment on antibiotic resistance. Both tests are well regarded to investigate how susceptible a bacterial colony is to an antibiotic, as well as how bacteria develop immunity to such antibiotics.

Experimental trials in this investigation were repeated 5 times to ensure reliability of results. This experiment will also have 3 control trials with two negative controls of Ampicillin and Vinegar and one positive of Nutrient Agar, which is a jelly that harbours bacterial growth. The negative controls will assure that the E. coli strain has not been exposed to abnormal environments such as antibiotics or an acidic environment which may lead to a different genetic make-up of a standard E. coli strain. Meanwhile the positive controls assure normal bacterial growth in the nutrient agar.

MH and control solutions were plated with their respective concentrations and incubated at 37℃ for 5 days. At the end of the 5-day growth period, plates were taken out of the incubator and analysed via visual inspection.

Both Vinegar and Agar control trials saw growth, with the Vinegar having colony growth up to the 5% ring and the Agar having unimpeded growth in the entire plate. The Ampicillin trial saw no growth in any concentration including 0% ring. The Experimental trials labelled as plates 1 to 5 all saw growth into 20% concentration, with plates 2, 3 and 4 seeing growth into the 40% concentration. Furthermore plates 2, 3 and 4 also saw potential contamination, but bacterial growth in surrounding areas were unaffected.

The low concentration required to eliminate E. coli in VIN control ensures the reliability of the e. coli strain, as between 2.5% to 5% vinegar required to eliminate E. coli according to Entani et al., 1998 and Yagnik et al., 2018. Further, the successful growth of bacteria in the positive control Agar plates affirms the reliability of the E. coli strain used. Meanwhile, the absence of growth in negative ampicillin control may indicate inaccuracies in the setup of the control trial, which would be due to spreading of solutions into the 0% concentration leading to no growth.

The growth of E. coli colonies in the 40% concentration in 3 out of the 5 experimental trials indicates presence of MH resistance either developed during the experiment or present in the original sample which is supported by Bischofberger et al. (2021) and Camplin and Maddocks (2014). However, due to the presence of the contaminant, which is likely to be a fungus due to the presence of hair-like filaments which are unique to fungus may have been impacted according to CD Genomics, n.d. and UNC Lineberger, n.d results. Yet, due to E. coli growth in the surrounding areas of the fungi, it is unlikely to have impeded any E. coli growth. However, further research should be conducted into whether Fungi are able to detoxify MH due to its presence in higher concentrations of MH.

Finally, limitations present in this investigation may have affected the results of the experiment. This includes the inability to prevent the spread of MH concentrations throughout the plate which may have been the cause of the complete prevention of growth in the ampicillin control. Other limitations such as the potentially differing amounts of solution absorbed by the paper, supported by Tao et al., 2020, which may have altered the results of the investigation.

This investigation was guided by the fundamental question of whether E. coli could develop resistance to MH. The study was led by the hypothesis that, if E. coli bacteria are exposed to increasing rings of MH concentration, then E. coli will be able to survive in MH concentrations above the estimated lethal concentration. This research was conducted to increase the pool of available research on the topic of MH resistance, allowing for precautions to take place in the use of MH reducing chances of MH resistant infections.

The experiment overall found that E. coli populations were able to survive in higher than lethal solutions of MH, indicating that the use of MH for medicinal and holistic purposes is believed to increase the chances of MH resistance. Therefore, the use of MH should be restricted to necessary purposes, primarily for use as an antimicrobial, similar to such restrictions with modern-day antibiotics. However, due to limitations and existing contradictory data, more research should be conducted to ensure this conclusion, as well as to investigate whether other organisms, such as the fungus that grew in 3 of the 5 plates, can detoxify or become resistant to MH.

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