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BRIEF REPORT

Study of Antibiotic Resistance in *E. coli* against Conventional Medicines as Compared to Natural Herbs

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HIGHLIGHTS

A new study found that tea tree oil was nearly as effective as prescription antibiotics in treating bacterial colonies, which showed low resistance to this natural substance.

ABSTRACT

The rising Incidence of antibiotic resistance has been increasing at an exponential rate in recent years, and since it is often caused by rampant misuse of drugs such as penicillin, many bacterial species such as *E. coli* have been able to mutate and gain multi-drug resistance and extended drug resistance (XDR) to form what is otherwise known as superbugs. The main purpose of our research was to investigate the efficacy of conventional medicines as compared to natural herbs in treating pathogenic bacterial colonies, so as to find an alternative to reduce antibiotic resistance. This study conducted an experiment and analysed the effectiveness of the conventional medicines and natural herbs, in treating an *E. coli* culture (K-12 strains). The experiment was carried out over a span of 14 days, with readings taken on every consecutive 2-day period. The efficacy was measured with respect to the zone of inhibition, and the results were compared for any form of resistance developed. The allopathic drug azithromycin, as hypothesized, was found to be the most effective in treating the *E. coli* culture, but a few resistant strains were observed. However, natural herbs like tea tree oil were not far behind, and only a few colonies of *E. coli* showed resistance against them. Erythromycin and garlic were not found to be as effective. This study answers questions on alternatives to conventional medicines and resistance to different substances. Further studies are however needed to establish the mechanism of resistance to these substances and to develop preventative measures.

KEYWORDS

Biology, antibiotic resistance, natural herbs, *E. coli*, conventional medicines

INTRODUCTION

The word antibiotic emerges from antibiosis, which means "against life" (Davies & Davies, 2010). From the onset of the first antibiotic, penicillin, developed by Alexander Fleming in 1928, antibiotics have occupied a central stage in today's society (Davies & Davies, 2010). Antibiotics such as penicillin, erythromycin, neomycin, and others are known to treat countless bacterial as well as fungal infections (Martinez, 2014). However, recent studies show that these microbes are gaining resistance against certain drugs, and this process is increasing at an exponential rate (Munita & Arias, 2016; Mwangi et al., 2019).

When the first antibiotic, penicillin, was developed, it was sold over the counter, and therefore everyone was able to procure it (Davies & Davies, 2010). However, rampant misuse of such drugs caused certain bacterial populations to undergo mutations and thereby gain resistance to this drug. Today the heavy use of antimicrobial drugs continues not only in human therapeutics but also in livestock production, leading to continuous contamination of our environment (CDC, 2021; Eliopoulos, 2003). Indiscriminate use

has led to the emergence and spread of antimicrobial drug resistance in microbes including dangerous pathogens leading to an imbalance in the micro-biota of our system and the environment (Benguigui & Salvatierra-González, 2000).

The World Health Organization defines antibiotic resistance as a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics; pathogens resistant to multiple antibiotics are considered multi-drug resistant (MDR) or, more colloquially, superbugs (Wiviott Tischler, 2013; Christaki et al., 2019). Antibiotics, once considered miracle drugs, have lost their extensive credibility. There are now hardly any antibiotics left to treat multi-drug–resistant (MDR), extended drug-resistant (XDR), and total drug resistant (TDR) pathogens. Even synthetic antibiotics, such as sulfa drugs, are not as effective with growing strains of sulphonamide-resistant *bacillus* (Eguale et al., 2015). In addition, there are no alternatives to antibiotics at hand or any insight at present. In spite of this, there are no policies to regulate antimicrobial drugs.

According to the CDC, "Each year in the U.S., at least 2.8 million people are infected with antibiotic-resistant bacteria or fungi, and more than 35,000 people die as a result" (CDC, 2013) In addition to this, certain sections of the population including those above the age of 60, children below the age of 18, and those with existing co-morbidities are likely to be at higher risk (Alós, 2015). Many medical advances are dependent on the ability to fight infections using antibiotics, including joint replacements, organ transplants, cancer therapy, and treatment of chronic diseases like diabetes, asthma, and rheumatoid arthritis (CDC, 2013), and therefore these might also be hampered, due to an increasing population of resistant pathogens (Gray & Wenzel, 2020).

In recent years of research on ways to minimize microbial resistance to antibiotics, many have sought to investigate natural sources as alternatives in treating resistant populations of different pathogens (Gray & Wenzel, 2020). There has also been a growing use of these alternatives and thus the United States Congress created the National Center for Complementary and Alternative Medicine (NCCAM) (Nahin & Straus, 2001).

Recent research has found that alternative medicines are usually used to treat the body and the mind wholly, as opposed to conventional medicines that are used to treat one specific medical condition (Onder & Liperoti, 2016). These alternative medicines are seen to be widely used for a variety of medical conditions, such as flu, diarrhoea, or pain. Without enough research in this field, these medicines are open to false speculations (Nahin & Straus, 2001). They are rarely tested and often clear proof of benefits is lacking. However, there has been enormous research conducted on the conventional medicines and thus they are supported by scientific evidence. Although herbal or complementary medicines are safe, they can also cause negative effects on the body such as fatigue, nausea, vomiting, or seizures (Onder & Liperoti, 2016). There have also been reported cases of hepatotoxicity or liver damage, and life-threatening anaphylactic reactions to these alternatives. Often, these might also obstruct the conventional medicines from acting on the body, when most needed (Onder & Liperoti, 2016). However, as mentioned earlier, most conventional medicines are prone to resistance, and thus these alternatives must also be explored. Therefore, although herbal/complementary medicines might be promising, further research on them is still needed.

In our experiment, cultures of the K-12 strain of *E. coli* were used. *E. coli* is an important group of bacteria for study, since *E. coli* is known to develop resistance to certain beta lactam antibiotics such as erythromycin, and the protein biosynthetic pathway along with the nucleic acid structures are well-known. *E. coli* is commonly found around the gut area of humans and it is a pathogen known to be associated with diarrhoea, urinary tract infections, and meningitis. The particular strain was chosen because it is one of the most sensitive strains, and thereby we sought to determine if it can develop resistance to herbs and to conventional medicines. Since there are fewer previously known cases of resistance to this particular strain; it was best-suited for the purpose of this research.

In this study, we sought to ascertain if natural plant-based sources could provide any efficacy for the treatment of pathogens as compared to conventional medicines. Here, different cultures of *E. coli* were left exposed to different substances such as turmeric, tea tree oil, erythromycin, and azithromycin. One of the petri dishes containing the *E. coli* culture was left untreated to serve as a control. It was hypothesized that conventional medicines should have the capacity to annihilate the culture more rapidly than plant-based sources. We hope others will research this area to provide better treatment for curing microbial resistance and to prevent future pandemics.

MATERIALS AND METHODS

Preparation of the Culture Medium

All beakers and petri dishes were first cleansed and sterilized. The agar was melted using a microwave and then added onto 5 petri dishes, in equal proportion using 12.5 gm of Luria-Bertani broth in 500 ml of distilled water added to 7.5 gm of agar. The set so formed was allowed to

cool, and then the culture of *Escherichia coli* was transferred onto the petri dish. The *E. coli* colony was allowed to grow for 24 hours. In the preparation of the natural sources, which were assumed to act as antibiotics, tea tree oil was mixed and a separate solution of turmeric was prepared. The same was done for the medicinal drugs, azithromycin and erythromycin, which were dissolved in distilled water, separately (500 mg of each in 50 ml of distilled water).

After this, blank disks were soaked in each sample of the antibiotic prepared, and they were allowed to dry. Afterwards, an equal amount of disk was inoculated using sterile swabs of cotton, and then the disk was placed at near the center of the petri dish, with the help of tweezers. This was carried out in a laminar flow as shown in Figure 1. One of the petri dishes containing the bacterial culture was left untreated, and was considered as control. The lid was covered, and the prepared samples were labelled and placed in an incubator at about 37 degree Celsius.

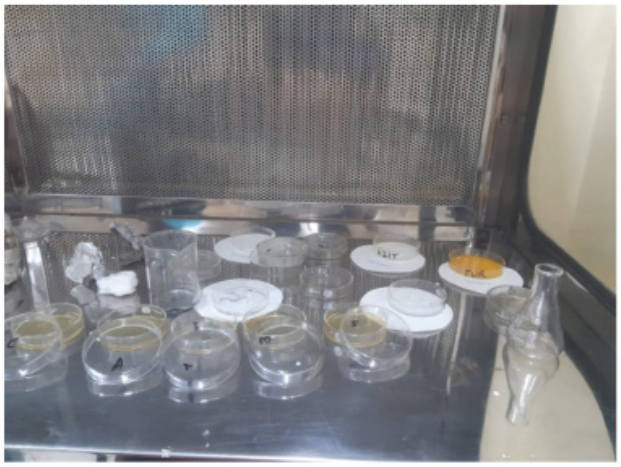

Figure 1. Experimental setup, under the laminar flow, before the disks were inoculated with the *E. coli* strains.

Readings

The growth of the *E. coli* strains was checked after every two consecutive days. The growth was determined on the basis of the zone of inhibition diameter, which was calculated with the help of a vernier caliper. The zone of inhibition is the area around the antibiotic with no growth. It was also assumed that most of the calculated area is circular and this was calculated for 2 weeks. A bar graph was plotted based on the data obtained. All graphs were made with the help of Meta Charts.

RESULTS AND DISCUSSION

The petri dishes including the one used as a control were evaluated for bacterial growth at regular intervals. The consecutive diametric zone of inhibition was calculated, and plotted on a table as well as on a bar graph (Figure 2, Table 1). The graphs represent the zone of inhibition of each sample, over consecutive days. These samples were analysed against the control medium, whose readings also were taken. The final graph (Figure 3) represents the final zone of inhibition of each sample, at the end of the second week.

After careful analysis of these data, it was observed that initially the area around the antibiotic discs, especially those exposed to the conventional medicines, were clear. The ones treated with the natural sources tea tree oil and turmeric also showed significant increase in zone of inhibition. In other areas which were not in direct contact with the antibiotic disc, the result was fewer colonies.

However, over time there was decreased efficacy of the samples treated against erythromycin and azithromycin, as the areas which were once devoid of growth during days 2 to 6 showed some growth in new colonies. These were the strains of the bacterial population that had gained resistance against the drug. Tea tree oil was the only natural source that was closest to erythromycin, and over the full course resistance against it was not developed. Diametric zones of inhibition were initially quite high for those treated with azithromycin and erythromycin but there were resistant strains as well. It was also found that erythromycin is comparatively weaker in treating *E. coli* than tea tree oil.

Discussion and Analysis

Resistance to drugs has been a major problem for health professionals and patients. Since the onset of the first antibiotic resistance to penicillin in the year 1947, the growth of superbugs has been rampant in the health sector. Millions of cases have been reported in recent years with regard to XDR, TDR, MDR, and other such strains. The CDC has also reported these cases on their scale of "urgent to watch" list, summarized in Table 2 (CDC, 2013).

Drug overuse is a deterrent to the efficacy of antibiotics. Resistance happens over time, when a particular bacterium incorporates mutations against a particular drug. This is usually associated with the plasmid DNA of the bacteria (Li et al., 2019) and is passed on to the other individuals in the colony. The plasmid DNA can be transferred between different bacteria, and therefore a bacterium can become resistant to multiple antibiotics at once by picking up a single plasmid. Genes that influence virulence are also found frequently on plasmids (ReAct, 2019). Genes which provide protection against antibiotics or metals are commonly found in the same plasmid, a phenomenon called co-selection (ReAct, 2019). This is also the reason why it is difficult to reverse resistance once it has been established in a bacterial colony. It would therefore not be enough to

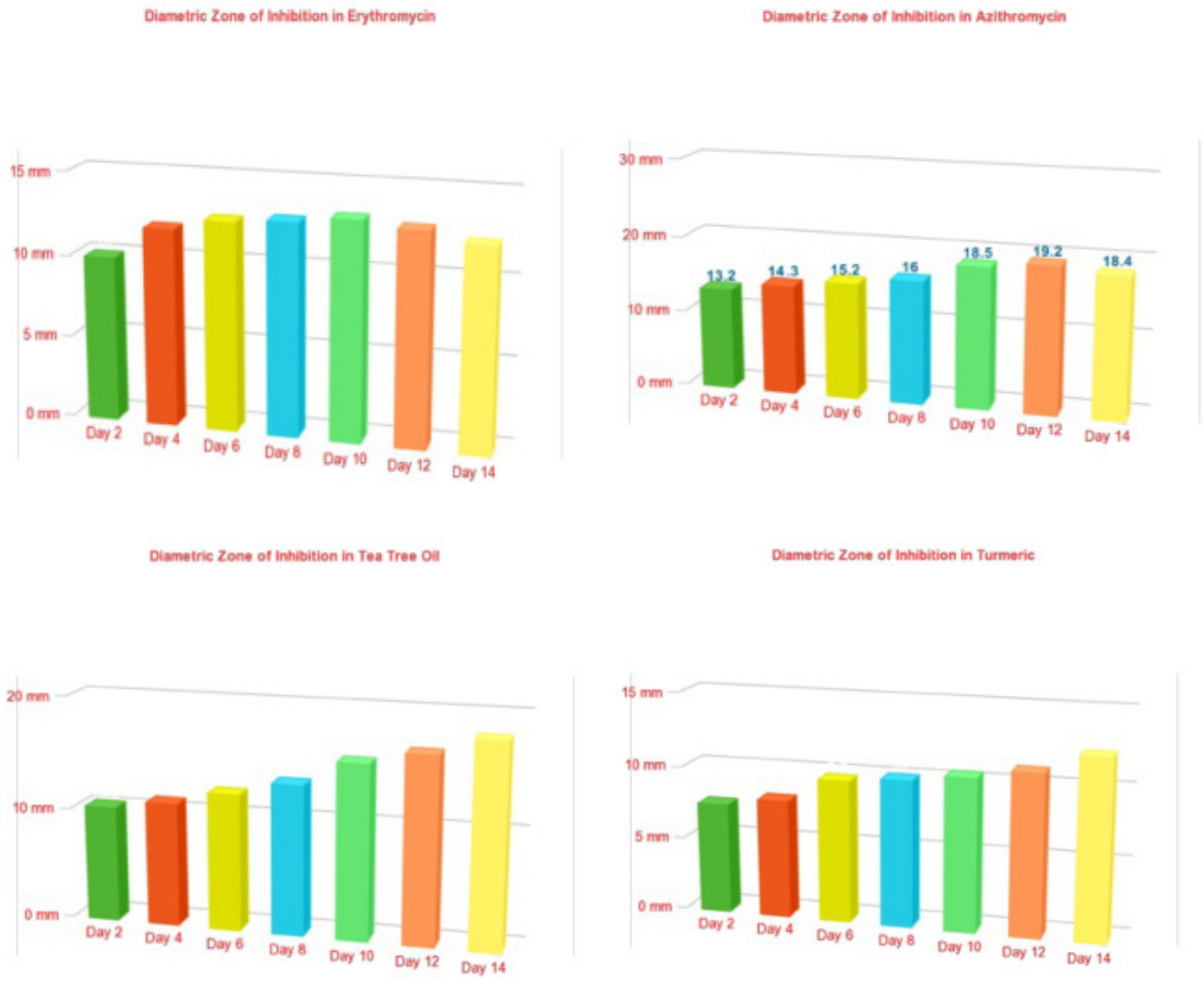

Figure 2. Diametric zone of inhibition in different culture media over a span of 14 days. Readings were taken after every 2 consecutive days.

TABLE 1. Diametric zone of Inhibition of *E. Coli* Colony over a period of 14 days.

The reading was taken on every consecutive day (after a skip of 1 day). There were significant differences between turmeric and erythromycin and tea tree oil and azithromycin, but this difference became minimal by day 14. Erythromycin and turmeric were not found to be very effective.

Type of Antibiotic	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Erythromycin	10 mm	11.9 mm	12.6 mm	12.8 mm	13.2 mm	12.8 mm	12.2 mm
Azithromycin	13.2 mm	14.3 mm	15.2 mm	16 mm	18.5 mm	19.2 mm	18.4 mm
Tea tree oil	10.3 mm	11 mm	12.2 mm	13.4mm	15.6 mm	16.7 mm	18.2 mm
Turmeric	7.5 mm	8 mm	9.7 mm	10 mm	10.4 mm	11 mm	12.3 mm
Control	0	0	0	0	0	0	0

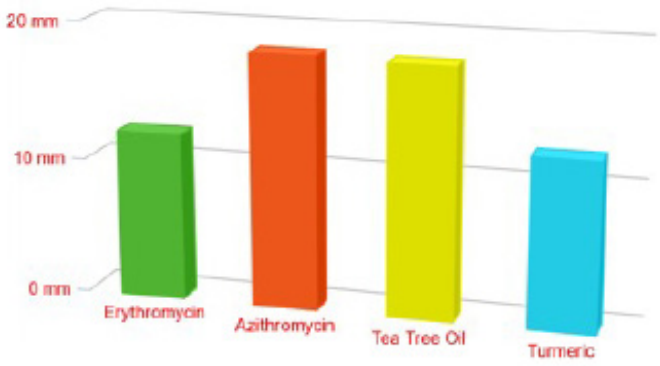

Figure 3. Final diametric zone of inhibition after a span of 14 days; the final zone of inhibition.

just stop the use of a particular antibiotic, as the resistance mechanism is linked to other antibiotics as well (Onder & Liperoti, 2016). This experiment has shown the very same phenomenon where the *E. Coli* strains had taken up resistance to a particular drug, and this resistance has been passed down to the rest of the colony as well, thereby showing considerable growth in the colonies of resistant strains.

Based on the results of this experiment, we can draw two conclusions. Antibiotic resistance happens over a period of time, and once shown in a bacterium is passed on to the rest of the colony. The next conclusion is that some natural herbs can act as an antibiotic. The results have shown that there is less resistance to natural herbs, and even though they take time to act, they might be a promising alternative to medicinal drugs.

These conclusions and data support the original hypothesis that drugs like erythromycin or azithromycin act at a faster rate than natural herbs, but there might be a significant increase in resistant strains when overused. This is so because these herbs are known to not only act on the bacterium but also help in enhancing the body's immune system. Previous research studies have shown that polyphenols (a micronutrient) that we obtain from these herbs help in promoting the growth of the essential bacterial colony needed, at the expense of pathogens (Cardona et al., 2013).

The results indicated that patients should minimize the intake of antibiotics and take them only when prescribed by a medical professional. And a switch to natural herbs may provide significant protection, similar to medicinal drugs. As noted in this study, *E. Coli* doesn't tend to show significant resistance to natural herbs despite prolong exposure to the herbs. Some natural herbs (which are foods) can be considered as medicine themselves (Garcia et al., 2012).

The results of this research agree with past studies examining antibiotic resistance in different bacterial strains. These studies have shown that natural sources such as garlic and tea tree oil have a significant impact on enhancing the human immune response as well as serving as an antibiotic to certain strains of the bacterial population. However, further data are needed to establish how natural herbs are an effective measure against resistance.

Owing to constraints in resources, this experiment could not be repeated by us. As a result, the data, especially on bacterial growth, might be skewed. However, this study still indicates that natural herbs can act as a promising alternative to medicinal drugs (Garcia et al., 2012).

TABLE 2. Bacteria and Fungi Listed in the 2019 AR Report, CDC. This data was taken from the CDC report, wherein different resistant strains were weighted over a scale from Urgent to Watch (CDC, 2013).

Urgent	Serious	Concerning	Watch List
Carbapenum-resistant *Acinetobacter*	Drug-resistant *Campylobacter*	Erythromycin-resistant Group A *Streptococcus*	Azole-resistant *Aspergillus fumigatus*
Candida auris	Drug-Resistant *Candida*	Clindamycin-Resistant Group B *Streptococcus*	Drug-resistant *Mycoplasma Genitalium*
Clostridioides difficile	Vancomycin-resistant *Enterococci*		Drug-resistant *Bordetella pertussis*
Drug-resistant *Neisseria gonorrhoeae*	Drug-resistant Tuberculosis		

IMPLICATIONS AND APPLICATIONS

Antibiotic resistance mainly occurs due to overuse of drugs, and this can be minimized with the use of natural alternatives to medicinal drugs. Although in this experiment azithromycin was the most effective antibiotic, tea tree oil was not far behind. Some herbs may serve as promising antibiotics in the future. However, it is necessary that further research be carried out in this field to better explain the phenomenon and the molecular basis of resistance to both conventional medicines and natural herbs, alongside subsequent studies on other pathogens.

It is also of interest whether the current findings of this research can be generalized to other strains or bacterial populations, including the BM2195 *E. coli* strain, which is known for extensive antibiotic resistance due to its ability to inactivate many antibiotics. Also, tea tree oil cannot be taken orally and parenterally and is only given topically. Thus, this limits the clinical scope of the findings. However, the bactericidal components of tea tree oil could be harvested and incorporated into future drugs, which can be modified to be administered safely either orally or parenterally.

Research on other herbs that may show in-vitro effectiveness as tea tree oil might also be the next step. Future research on the comparative analysis of sulfa drugs against herbs such as tea tree oil is also the need of the hour.

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