

# Sciential

McMaster Undergraduate Science Journal



**ISSUE 3 - NOVEMBER 2019**

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# DEAR READER,

We take infinite pride and pleasure in welcoming you to Issue 3 of *Sciential*! We are at the intersection of innovation and challenges in the realm of health research. Scientific advancements and novelty continue to tackle the global burden caused by debilitating diseases. This release cultivates the marvellous efforts of our authors to communicate their findings and directions on the most prevailing conditions, and offer an ingenious lens on the outlook on gaps, incentives, and solutions towards accelerating the relevant research.

Issue 3 opens with a remarkable original research manuscript by Dominique Tertigas and Gemma Barber, reporting on their methodological groundwork in determining the rate of development of antibiotic resistance. Succeeding, it is our privilege to display our featured academic literature review by Suhaila Abdelhalim and Lyan Wadood, embarking us on a journey about the evolution of cataracts surgery. Following, Caitlin Reintjes and Yona Tugg authored a review manuscript prefacing the considerations for chemotherapy in platinum resistant, high-grade serous ovarian carcinoma. Further, we are delighted to showcase our debut infographic contrived by Razam Samara, Amama Khaizad, and Lyan Wadood, imparting us on the causes, global burden, and stewardship in relation to *Mycobacterium tuberculosis* antimicrobial resistance. With our utmost pleasure, this issue also features Dr. Ayesha Khan's interview conducted by Tressa Mastroianni highlighting Dr. Khan's pedagogical approach to community-oriented education. Upcoming, Pouriya Sadeghighazichaki reports on a novel outlook on the prevention of Alzheimer's disease pathogenesis and progression through a characterized biological "marker", *Porphyromonas gingivalis*. Following, Ishita Paliwal describes an ingenious prospect of cancer treatment through the implementation of induced pluripotent stem cell-based vaccines. The issue further encloses an original citizen science approach on education and management of Dengue virus in underprivileged geographical areas around the world. Proudly, we are lastly serving you with an opinion piece delivered by Duha Sikander considering the legitimacy of complementary and alternative therapeutic options used for infertility treatment today.

We hope that Issue 3 will captivate your attention with the spectrum of health-related pieces that could not be possible without the unprecedented talents of our authors and the unconditional commitment of our Editorial and Creative boards. It is a true endowment to appreciate the boundless efforts of our Senior Editors, Amama Khaizad and Ishita Paliwal, and Creative Director, Youssef El-Sayes, who have exhibited excellence in their leadership, workflow management, and competence in considering and enhancing our submissions. We are gratified to acknowledge our Senior Advisors team: Dr. Veronica Rodriguez Moncalvo, Dr. Katie Moisse, Dr. Kimberley Dej, and the distinguished Science Librarian, Abeer Siddiqui, for their exquisite advice, encouragement, and affirmation in our accomplishments that have increased *Sciential's* outreach and impact. Finally, we thank our unequivocal sponsors, Science Initiative Fund (SIF) from the McMaster Science Society, for their generous allocation of funds to support our enterprise. It is with paramount pleasure, on behalf of *Sciential's* Team, to invite you to indulge in Issue 3, and we sincerely wish you will find delight in reading our journal.



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# Determining the Rate of Development of Antibiotic Resistance to Streptomycin and Doxycycline in *Escherichia coli*

## ARTICLE INFORMATION

Received: 16 October 2019  
Accepted: 18 November 2019  
Published: 29 November 2019

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## ABSTRACT

Antibiotic resistance is a pressing issue in the medical field today. It is important to understand the development of bacterial resistance to implement effective preventative measures against antibiotic resistant bacteria. This study investigated the rate at which *Escherichia coli* (*E. coli*), a common pathogen, developed resistance to streptomycin and doxycycline, as Oz et al. (2014) showed differing levels of resistance in *E. coli* to these two antibiotics. The development of antibiotic resistance was measured by adding *E. coli* to 96-well plates in the presence of increasing doses of doxycycline, streptomycin, or a combination treatment. Successive generations were added to the same treatments to see whether they would grow at higher concentrations of antibiotic. The change in minimum inhibitory concentration for streptomycin and doxycycline was determined as the bacteria became increasingly resistant to each antibiotic. The fastest rate of antibiotic resistance was observed for streptomycin, with doxycycline resistance exhibiting a slower rate of development. The rate of resistance development for the combination treatment was the slowest, potentially due to small differences in target domains. Some cross-resistance was also observed. This study provides a small-scale methodological basis and preliminary insight on antibiotic resistance trends for two antibiotic classes and a combination treatment.

**Keywords:** Antibiotic resistance, *E. coli*, selective pressures, cross-resistance

## INTRODUCTION

Antibiotic resistance is becoming more prevalent, resulting in diminished effectiveness of many antibiotics.<sup>1</sup> Antibiotic resistance occurs when bacteria evolve to survive in the presence of antibiotics designed to eliminate them. This occurs for several reasons, including the overuse of antibiotics both for treatment in humans and livestock.<sup>2</sup> Antibiotics are becoming less effective against bacterial infections as bacteria evolve and become resistant to antibiotic treatments.<sup>2</sup> In order to combat this issue and create new antibiotics that prevent the development of antibiotic resistance, it is important to understand how the rates of antibiotic resistance in bacteria differ between antibiotics and how this relates to the mechanisms of antibiotic

resistance. This study attempted to provide a small-scale methodical basis, adapted from Oz et al. (2014), to investigate the development of antibiotic resistance by comparing the rate of resistance development for two antibiotics in *Escherichia coli* (*E. coli*).

Oz et al. analyzed the levels of resistance of *E. coli* under various selective pressures to 22 antibiotics by measuring the minimum inhibitory concentration (MIC) of the antibiotics daily for 21 days.<sup>3</sup> MIC is the lowest effective dose of a drug. Knowing the MIC is vital to appropriately prescribe treatment doses so that all bacteria are eliminated. Breakpoints are also crucial to investigate, and they are correlated with MIC values. Breakpoints represent the concentration of antibiotic at which the bacteria are no longer susceptible to the antibiotic.<sup>4</sup> If the MIC is greater than or equal to

the breakpoint value, the bacteria are considered resistant to the antibiotic. Therefore, it is essential to ensure that the MIC of the antibiotic remains below the breakpoint value to ensure effective treatments.

For this study, two antibiotics, streptomycin and doxycycline, were chosen as Oz et al. showed that *E. coli* displayed differing levels of resistance to these antibiotics after 21 days.<sup>3</sup> The antimicrobial targets of these antibiotics are involved in protein synthesis.<sup>5</sup> Specifically, tetracyclines, including doxycycline, are thought to prevent binding of tRNA to the A site of the ribosome; and aminoglycosides, including streptomycin, are thought to cause misreading and premature termination of mRNA translation, thereby acting against the bacteria to reduce its effects on the host.<sup>5</sup> Doxycycline is commonly used for the treatment of acne,<sup>6</sup> and can also be used to prevent malaria.<sup>7</sup> Streptomycin is used in the treatment of tuberculosis,<sup>8</sup> as well as other serious infections like the plague.<sup>9</sup> The aim was to replicate the results of Oz et al. through an adapted method and gain insight on the relationship between antibiotic class and the rate of development of antibiotic resistance.

There are several ways by which antibiotic resistance can occur in bacteria. One such mechanism is mutational resistance, where bacteria develop genetic mutations that overcome the activity of the drug, thereby leading to predominance of resistant bacteria.<sup>10</sup> These genetic mutations can lead to the decrease in drug uptake in the cell; activation of pumps or efflux mechanisms to remove the drug from the cell; alteration of metabolic pathways; or the modification of the antimicrobial target to decrease its affinity for the drug.<sup>10</sup> Another way bacteria can become resistant is by acquiring foreign DNA that contain antibiotic resistance genes through horizontal gene transfer, which can be accomplished through transformation, transduction or conjugation.<sup>10</sup> Transformation is the uptake of naked DNA from the environment. Transduction occurs when part of a host bacterium's genome is passed to another bacterium by a bacteriophage. Conjugation involves transfer of genetic material between bacteria via direct contact.<sup>10</sup> Bacteria are known to become resistant to streptomycin by using aminoglycoside modifying enzymes that alter the hydroxyl or amino groups of the antibiotic.<sup>10</sup> Doxycycline is possibly susceptible to a different mechanism of resistance where bacteria develop resistance through efflux pumps.<sup>10</sup>

The resistance mechanism that bacteria develop to one antibiotic may allow them to become resistant to another antibiotic at the same time through a process known as cross-resistance.<sup>3,11</sup> The opposite can also occur, whereby bacteria become more susceptible to other antibiotics upon developing resistance to one antibiotic, which is a phenomenon known as collateral sensitivity.<sup>12</sup> In this study, cross-resistance was determined using antibiotic disks and agar plates. This is

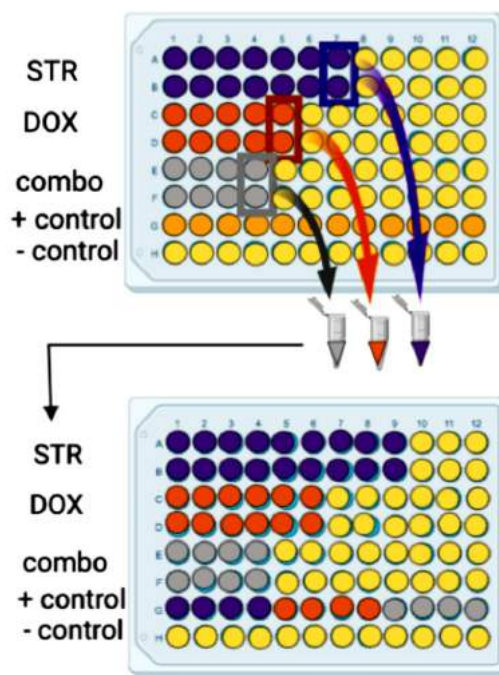
known as the disk diffusion method, used in both hospitals and laboratories, to determine the susceptibility of bacteria to the effects of antibiotics.<sup>13</sup> Cross-resistance is problematic because it can cause bacteria to become resistant to antibiotics they have never been exposed to, which hastens the process of antibiotic resistance as a whole. In order to understand the phenomenon of cross-resistance, chemogenomic profiling has been used.<sup>11</sup> This method uses drug-mutant interactions and gene fitness to determine the mechanism of action of drugs. Similarities between chemogenomic profiles may be a strong predicting factor for cross-resistance.<sup>11</sup>

This study focused on comparing the rate of development of antibiotic resistance in *E. coli* to streptomycin, doxycycline, and a combination treatment. In addition to measuring rate of resistance, cross-resistance was also measured since understanding cross-resistance poses implications for the design of new antibiotics.

## METHODS

### Antibiotic Resistance Study

*E. coli* was exposed to one of three treatments: streptomycin (STR), doxycycline hyclate (DOX), or a combination of the two antibiotics (both obtained from Sigma-Aldrich). Minimum inhibitory concentration (MIC) values were determined in 96-well plates with each antibiotic added in duplicate (n=2) (Figure 1, Table 1).



**Figure 1. Visual representation of the plating procedure for generations zero and one of the antibiotic resistance study.** Schematic diagram of the process of isolating bacteria from MIC/2 (mg/L)

wells which were then grown and added to a new 96-well plate. The respective treatment colours in this diagram refer to bacterial growth after a 24-hour incubation period. All bacteria were added to wells at an optical density at 610 nm between 0.08 and 0.10 to ensure an equal starting point so that growth could accurately be determined. Yellow wells represent no bacterial growth. This process was repeated for eight generations. Figure created using BioRender.

Difco™ Nutrient Broth was added to all wells: 50 µL in rows A-G and 100 µL in row H. Row H acted as a negative control, as it contained a solution of nutrient broth only, so no bacterial growth was expected. 50 µL of STR at a concentration of 80 mg/L was added to well 12 of the first two rows. This concentration was changed to 5120 mg/L as the *E. coli* continued to grow at higher concentrations, likely due to their development of a resistance mechanism. 50 µL of DOX at a concentration of 512 mg/L was added to well 12 of the next two rows. For the combination treatment, the highest concentration consisted of 40 mg/L of STR and 256 mg/L of DOX. For this treatment, 25 µL of the specified STR concentration and 25 µL of the specified DOX concentration were added into well 12 of the next two rows. Two-fold serial dilutions were performed from well 12 to well 1 of rows A to E so that each well would have a concentration half of that in the well to their right. For the initial well set-up, 50 µL of wild type *E. coli* with an optical density at 610 nm between 0.08 and 0.10 was added to all wells in rows A to G (Figure 1). Row G acted as a positive control, as it contained 50 µL nutrient broth and 50 µL wild type *E. coli* and would represent optimal bacterial growth used as a comparison for growth in treatment wells. After all components were added, the plate was incubated at 37°C for 24 hours, at which point it was read to determine the MIC of each antibiotic.

A scale was created from zero to four, where zero referred to no growth and four referred to maximum growth seen in the positive control well. The MIC was defined as the lowest concentration where no bacterial growth was observed. Bacteria were isolated from the MIC/2 wells, or the highest antibiotic concentration with bacterial growth. This is represented as the well the farthest to the right that had bacterial growth (see Figure 1 where growth is represented by colour). Bacteria isolated from the same treatment were combined and centrifuged, the supernatant was removed, and the pellet was resuspended in Difco™ Nutrient Broth and grown overnight in a water bath at 37°C. These bacteria were then used to repeat the plating procedure outlined above. Bacteria were added to the same treatment from which they had been isolated. For example, bacteria isolated from the wells treated with STR were used for the next generation of STR treatment. Isolated bacteria from each treatment were also added to four wells in the positive control row. This procedure was repeated for eight generations.

## Statistical Methods

Percent difference comparing the final generation MIC and initial generation MIC for both replicates of each treatment were determined. A one-way ANOVA was performed in order to test whether at least one pair of treatments was statistically different. If the ANOVA yielded significant results, a post hoc Tukey test was performed to determine which treatments were significantly different from each other. The statistical analysis was performed using R software.

## Cross-Resistance Study

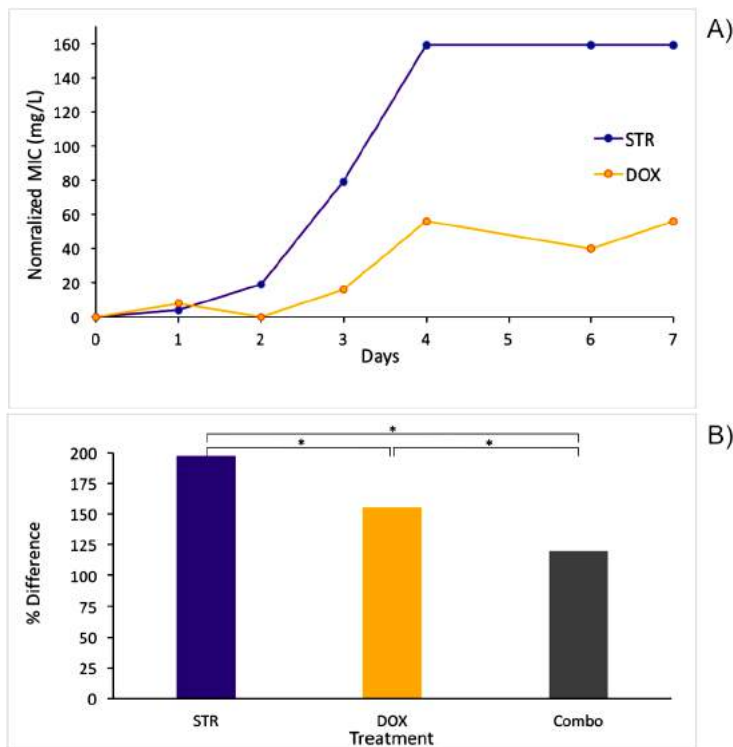
Cross-resistance was tested by plating 100 µL of the final generation of each bacteria treatment and the control (initial wild type *E. coli*) on four different petri dishes containing Difco™ Nutrient Broth agar. Each plate was split into quadrants, and an antibiotic disk was placed in each quadrant. The disks used were 10 µg streptomycin (S10), 10 µg penicillin (P10), 30 µg chloramphenicol (C30), and 5 µg tetracycline (Te5) (Figure 3A). Penicillin and chloramphenicol were used in addition to streptomycin and doxycycline as they belong to different classes of antibiotics, β-lactams and chloramphenicols respectively.<sup>5</sup> If the antibiotic was effective against the bacteria, a ring of inhibition would be seen where there was no bacterial growth. A larger ring suggests a more effective antibiotic and a smaller ring or lack of a ring suggests antibiotic resistance. These plates were incubated for 24 hours at 37°C and diameter of inhibition was manually measured in centimetres.

## RESULTS

**Table 1. Recorded MIC (mg/L) for the first and last generations of the three antibiotic treatments.** For the combination treatment, the volume of each antibiotic was half the volume of the independent treatments.

Treatment	Initial MIC (mg/L)	Final MIC (mg/L)
Streptomycin (STR)	0.94	160
Doxycycline (DOX)	8	64
Combination (STR/DOX)	0.32/2	1.25/8

Bacteria treated with STR became resistant at a faster rate than the bacteria treated with DOX (Figure 2A). This was determined by comparing the change in MIC of each generation to the initial MIC. When analyzing the percent difference (comparing the final and first generations' MIC) for each treatment, the difference in MIC was statistically significant (P<0.05) between STR and DOX, STR and combination, and DOX and



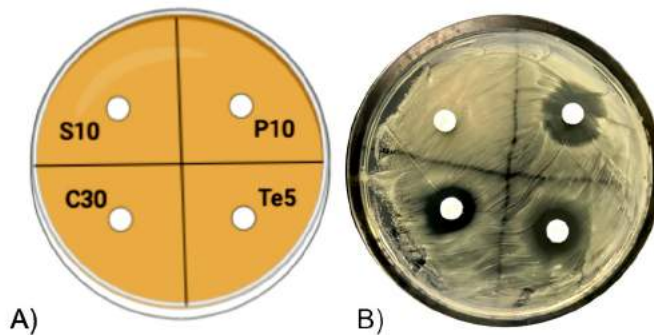
**Figure 2. Change in MIC for STR, DOX and combination treatments.** **A)** Normalized MIC of STR and DOX treatments over eight generations. STR treated bacteria developed resistance at a faster rate than DOX treated bacteria. **B)** Percent difference of final and initial MIC of all three treatments (n = 2) over eight generations (\* = P < 0.05). The percent difference was statistically significant between all treatments indicating that STR treated bacteria become resistant the fastest, followed by DOX treated bacteria and the combination treated bacteria respectively.

combination (Table 1, Figure 2B). This further confirms that STR-treated bacteria become resistant at a faster rate than DOX-treated bacteria. It also shows that the combination-treated bacteria become resistant at an overall slower rate compared to STR and DOX-treated bacteria.

The resistance of these bacteria was further confirmed in the cross-resistance test as STR-treated bacteria were resistant to the S10 antibiotic disk and DOX-treated bacteria were resistant to the Te5 antibiotic disk (Figure 3B, Table 2). This means that STR-treated bacteria thrived in the presence of streptomycin and DOX-treated bacteria thrived in the presence of tetracycline, the class of antibiotics to which doxycycline belongs. This test also showed some incidence of cross-resistance; DOX-treated bacteria become resistant to streptomycin and penicillin. This can be seen by comparing the diameter of inhibition of the control to the diameter of inhibition of DOX-treated bacteria (Table 2).

Bacteria from all treatments became more susceptible

to C30 in comparison to the control. There was no inhibition by this antibiotic disk on the control, but all other treatments were inhibited to some degree by C30 (Table 2). P10 and Te5 were more effective against STR-treated bacteria than the control. The combination treatment exhibited almost no changes from the control, indicating that very little resistance occurred in these bacteria.



**Figure 3. Set-up and results of the cross-resistance study.** **A)** Schematic diagram of the set-up of the cross-resistance test plate with four different antibiotic disks: 10 µg streptomycin (S10), 10 µg penicillin (P10), 30 µg chloramphenicol (C30) and 5 µg tetracycline (Te5). Four of these plates were prepared, each for a different treatment. **B)** Image of STR-treated bacteria plate with the same orientation as in A. No ring of inhibition is seen around the S10 antibiotic disk, but all other antibiotics had an effect. Figure created using BioRender.

**Table 2. Results of the cross-resistance study.** Diameter of inhibition is measured as the diameter of the area around the antibiotic disk where no bacterial growth was observed. A higher diameter of inhibition indicates a higher efficacy of the antibiotic or a lower level of resistance of the bacteria.

Treatment	Diameter of Inhibition for each Antibiotic (cm)			
	S10	P10	C30	Te5
Control	1.0	0.8	0	0
STR-treated bacteria	0	1.9	1.6	1.3
DOX-treated bacteria	0	0	2.3	0
Combination-treated bacteria	1.2	0.8	2.3	0

Overall, these results showed bacteria that become resistant to DOX are most likely to become resistant to other antibiotics, and bacteria treated with a combination treatment are least likely to become resistant to other antibiotics, regardless of class.

## DISCUSSION

It was observed that bacteria become resistant to STR at a faster rate than they become resistant to DOX. Trends similar to those observed in this study were seen by Oz et al., demonstrating that this specific small-scale method was able to replicate their results.<sup>3</sup>

The faster rate of resistance of STR-treated bacteria is possibly due to a difference in mechanism or amplification of resistance between these two antibiotics.<sup>14</sup> DOX and STR belong to different classes of antibiotics: tetracyclines and aminoglycosides, respectively.<sup>5</sup> Both of these antibiotics are known to have a mechanism of action involving the 30S subunit of the ribosome, which impacts protein synthesis.<sup>5</sup> Although they have similar antimicrobial targets, their rates of resistance may differ based on distinct mechanisms of antibiotic resistance, such as the development of efflux pumps or modification of the 30S ribosomal subunit.<sup>14</sup> It is also important to note that this study only looked at the rates of antibiotic resistance using *E. coli*; other species of bacteria may show different trends.

The results of this study showed that the combination treatment using both STR and DOX had the slowest rate of development of antibiotic resistance. A possible explanation for this is that it may be more difficult to become resistant to two different antibiotics at the same time as the bacteria could require development of multiple mechanisms of resistance. Other studies have also found that combination treatments are effective in reducing antibiotic resistance in bacteria.<sup>11</sup> However, research about the effectiveness of combination antibiotic treatments in comparison to monotherapies remains inconclusive.<sup>15</sup> Additionally, there is an increased risk of adverse side effects and a higher cost associated with combination treatments, making them a less attractive option.<sup>16</sup>

While the rate of resistance is important to consider, breakpoint values are also essential. Breakpoints are universal values defined as the concentration at which bacteria become resistant to an antibiotic based on their MIC.<sup>4</sup> For STR, this is 32 mg/L,<sup>17</sup> which was surpassed by day 3. For DOX, a tetracycline, it is 16 mg/L,<sup>17</sup> which was reached on day 4. The combination treatment never surpassed either of these breakpoint values. This supports the notion that a combination treatment of these two antibiotics will lead to a slower rate of resistance than a treatment with a single antibiotic.

As mentioned previously, in some cases the bacteria developed cross-resistance and in other cases the bacteria became more susceptible to antibiotic treatments. These results can potentially be explained by the differences in mechanism and/or amplitude of resistance between each treatment.<sup>5</sup> Specifically, the results illustrated that DOX-treated bacteria become resistant to streptomycin (S10) and penicillin (P10) disks. Streptomycin and doxycycline target protein synthesis as their mode of action, while penicillins target the cell wall of the bacteria.<sup>5</sup> Cross-resistance to streptomycin by the DOX-treated bacteria can be explained by general mechanism similarities. However, penicillins have a different mechanism of action. These conflicting results suggest it is not merely simi-

larities in chemical structure and the general mode of action that can be used to predict cross resistance. Lázár et al. (2014) found that chemogenomic profiling is the strongest predicting factor of cross-resistance.<sup>11</sup> Chemogenomic profiles of these antibiotics in *E. coli* may provide further insights on the drug's mechanism of action, which may allow for a more in depth analysis of the cross-resistance results. However, further replications of these studies are necessary to confirm the validity of results.

This research provides a simple method to study antibiotic resistance; however, it is important to recognize the limitations of this study. Although trends were observed, there is no clear explanation for why these trends were observed. In order to dissect their meaning, whole genome sequencing of the *E. coli* at multiple generations would be required to determine mutations that may have developed to cause antibiotic resistance.<sup>18,19</sup> This would help confirm if there is a relationship between the rate at which resistance is acquired, antibiotic class and underlying mechanisms of resistance. Understanding these relationships would allow for the creation and implementation of antibiotic treatments that prevent rapid development of antibiotic resistance. Future steps would also include chemogenomic profiling to determine if the observation of cross-resistance could be due to similarities in chemogenomic profiles. Despite a low statistical power due to the small sample size (n=2) for each treatment, this analysis provides a methodological basis for future studies with larger sample sizes. Therefore, this study should be replicated in order to produce results with increased power. These results may also differ if replicated *in vivo* rather than *in vitro*, so further investigation is required to determine how these interactions might change when other factors, such as varied metabolism of the antibiotic,<sup>20</sup> are present. If these trends were to be analyzed based on data collected in a clinical setting, it would be of interest to determine the degree to which differing mechanisms of resistance are responsible for the development of resistance, as compared to other factors such as prescription doses and frequency. Other strains of bacteria may develop resistance at different rates from *E. coli* so further testing would be required to assess the trends of antibiotic resistance in other bacteria.

## CONCLUSION

This study showed that *E. coli* developed resistance to streptomycin at the fastest rate, followed by doxycycline and then the combination treatment. In some cases, cross-resistance was also observed, however further replications and analyses are required to draw conclusions about the cross-resistance results. Although this study only investigated these two antibiotics, it would be beneficial to know the rates of resistance of bacteria to other antibiotics as well to allow



for an in depth comparison between antibiotic class and antibiotic resistance. There are also many implications for this study that can be applied to a clinical setting. This study demonstrated that combination treatments can reduce the prevalence of antibiotic resistant bacteria. Research comparing monotherapies and combination antibiotic treatments currently remain inconclusive and controversial.<sup>15</sup> The question that remains unanswered is the underlying cause of varying resistance rates. Understanding the mechanisms of resistance and how this relates to the structure and mechanism of each antibiotic can be achieved through whole genome sequencing. Determining which structures, classes and chemogenomic profiles of antibiotics lead to slower rates of resistance in bacteria could allow for the creation of new antibiotics that would induce slower rates of resistance.

## ACKNOWLEDGEMENTS

We thank our second year Enrichment Project supervisor, Mr. Russ Ellis, Integrated Science Program Laboratory Coordinator at McMaster University for giving access to the Integrated Science laboratory, providing materials as well as helping us develop and execute our experiment. We would also like to thank Ms. Linda Ejim for her contributions to our protocol and Dr. Eric Brown for his insights on our research and results.

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## APPENDIX A

### Analysis of Variance Table

Response: percent difference

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Antibiotic	2	0.60472	0.30236	7606.4	2.768e-06

Residuals 3 0.00012 0.00004

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

### Tukey multiple comparisons of means

95% family-wise confidence level

	diff	lwr	upr	p adj
DOX-COMBO	35.55556	32.92093	38.19018	0
STR-COMBO	77.67140	75.03677	80.30603	0
STR-DOX	42.11585	39.48122	44.75047	0

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

# The History, Present, and Future of Age-Related Cataracts Surgery

## ARTICLE INFORMATION

Received: 31 August 2019  
Accepted: 1 October 2019  
Published: 29 November 2019

**Senior Editor**  
Amama Khairzad

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## ABSTRACT

Twenty years ago, WHO and IAPB introduced an initiative called ‘The Right to Sight’, which set out to eliminate avoidable blindness universally by 2020. Age-related cataracts is a major contributor to treatable blindness worldwide and is increasing in global prevalence due to the growing proportion of individuals over 65 years of age. Cataracts refers to opacification of the lens inside the eye and clinically presents as a painless blurring and clouding of vision. From couching operations in 1200 B.C. to modern phacoemulsification, different approaches have been used to tackle this ancient disease over the centuries. Treatment today mainly involves surgery to replace the opaque lens with an artificial intraocular lens. Cutting-edge research into future therapies include investigating accommodating intraocular lenses, which hope to postoperatively restore accommodation. With the target year 2020 approaching, it is necessary to initiate discussion on age-related cataracts. This paper will provide a brief overview of this disease, discuss developments in treatment, and review innovations currently being pursued in the field.

**Keywords:** Age-related cataracts, ophthalmology, ROS, phacoemulsification, IOL, aging

## INTRODUCTION

Age-related cataracts refers to opacification of the lens inside the eye. It is a major cause of treatable blindness worldwide, and it is responsible for approximately 50% of the population’s visual impairment.<sup>1</sup> With an increasing global aging population, age-related cataracts is set to become more prevalent and exert a greater burden on health care resources.<sup>2</sup> In 1999, the International Agency for the Prevention of Blindness (IAPB) along with the World Health Organization (WHO) aimed to eliminate all avoidable blindness in the world by the year 2020. A large proportion of this blindness is due to age-related cataracts. They aimed to achieve this goal by investing in necessary infrastructure that would contribute to providing effective and accessible treatment, including greater investment in the equipment required to conduct treatments.<sup>3</sup> However, the proportion of individuals receiving cata-

ract surgery in developing countries remains low.<sup>4</sup> Cataracts has been surgically treated using different methods over the years. With an inequality of access to treatment and 2020 approaching, it is important to review the advances that have been made in cataract surgeries. This review will outline developments in cataract surgery over the centuries and compare the different surgical techniques. This paper benefited from the review article written by Asbell et al. (2005).<sup>5</sup>

## SYMPTOMS

Age-related cataracts is the most common form of cataracts and usually develops in individuals over the age of 50.<sup>5</sup> Symptoms develop gradually and are characterized by a painless blurring of vision and eventually functional blindness in mature white cataracts, where an individual may only discern light.<sup>5</sup> Symptoms may also include glare, monocular double vision, and near-

sightedness.<sup>5,6</sup> The type of cataract depends on its location in the lens fibres of the eye, which are the main cell type in the lens. Nuclear cataracts (NC) results from opacification in the centre of the lens due to hardening of the inner lens layers. Cortical cataracts involves newer lens fibres, and spoke-like opacities can form in the outer layers. Finally, posterior subcapsular cataracts (PSC) are grain-like opacities that form in the back of the lens.<sup>5</sup> This cataract type causes the greatest reduction in visual acuity.<sup>7</sup>

## PATHOPHYSIOLOGY

Cataracts refers to opacification of the lens within the eye. The lens is responsible for bending light to focus on near and far objects, accordingly. This function is facilitated by the lens structure. It is layered, with cells composed of a few organelles to keep it transparent and many proteins that are precisely arranged to bend light. These characteristics contribute to the formation of cataracts, where the lack of organelles prevents protein turnover and the layered structure results in the accumulation of older cells. Over the lifetime of an individual, exposure to reactive oxygen species (e.g. from diet or UV radiation) contributes to cellular and protein damage.<sup>8</sup> The lens cells have antioxidant defence mechanisms that can target these oxidative species. Specifically, superoxide dismutase, catalase, and glutathione peroxidase are enzymes that can neutralize molecules with the potential to form these harmful species.<sup>9</sup> When this damage overwhelms the lens' protective antioxidant defence mechanisms, proteins aggregate and form opacities in the lens.<sup>10</sup>

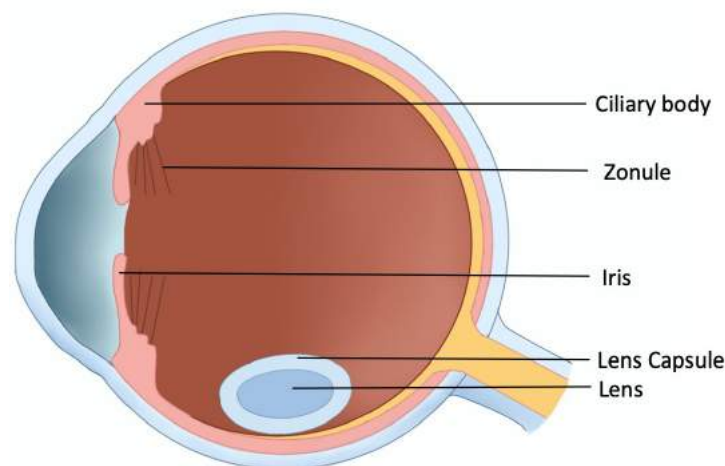
Both environmental factors and genetics play a role in age-related cataracts. The Age-Related Eye Disease study found that older individuals all had increased risk of cataracts, showing that older age is correlated with the development of cataracts.<sup>11</sup> In addition, smoking and UV exposure is associated with cataract formation.<sup>12,13</sup> Twin studies found that monozygotic twins had higher concordance of cataracts compared to dizygotic twins, suggesting a role of heritability in cataract development.<sup>14</sup>

## SURGICAL TREATMENTS - HISTORY

Age-related cataracts can only be treated surgically. However, if diagnosed early, a patient is recommended to postpone surgical treatment until their visual symptoms become severe enough to impair normal day-to-day function. Initially, there are smaller opacities in the eye that do not significantly interfere with light refraction. As the opacities become larger and the cataract further develops, reparative surgery is conducted. This helps patients avoid complications from surgeries that may only result in minimal improvements in vision.<sup>5</sup>

## Couching

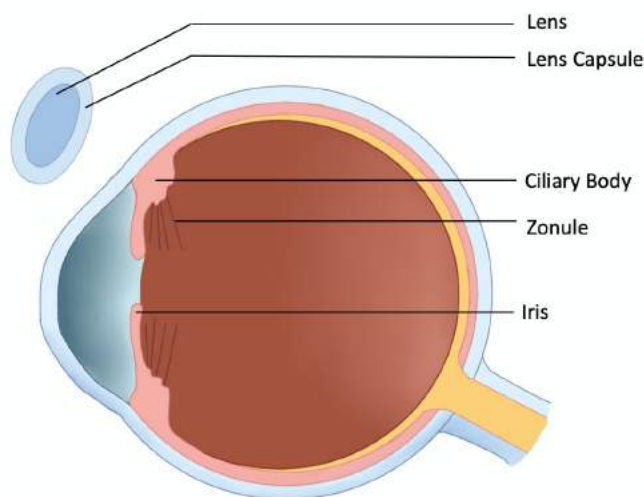
The earliest surgical method used to treat cataracts was couching.<sup>5</sup> A 1200 B.C. wall painting in an Ancient Egyptian tomb depicts what could be interpreted as a couching operation, where an individual is portrayed inserting a long, needle-like object into the eye of a worker. Another Ancient Egyptian temple constructed between 1479-1425 B.C. contains stone engravings that depict an array of long, sharp instruments, which many have speculated to be the tools used to perform couching surgeries.<sup>15</sup> Couching involves inserting a thin surgical instrument (e.g. a sharp needle) into a patient's pars plana and dislodging the capsule that the lens sits within. The lens falls from its original position, into the vitreous cavity, and out of the patient's visual field (Figure 1). Couching has also been non-invasively performed by repeatedly hitting a patient's affected eye, using the force of a blunt object to physically dislodge the lens.<sup>16</sup> Postoperatively, the characteristic symptoms of cataracts are eliminated and vision may improve. However, in many cases, vision still remains comparatively poor.<sup>16</sup> Preoperatively, a patient with mature white cataracts would only be able to differentiate between light and dark; however, postoperatively, they would be able to see how many fingers an individual is holding up, with blur. Couching is considered a dangerous and imprecise technique. Despite having low success and high complication rates, it is still used to treat cataracts in areas with limited access to medical resources. In one study, researchers surveyed individuals 40 years and older over a thirty-month period to determine which cataracts treatments they underwent.<sup>16,17</sup> They found that of 583 eyes that underwent cataract surgery in Nigeria, 249 (42.7%) were done by couching, and 73.1% of those couching eyes had poor visual acuity.<sup>16</sup>



**Figure 1. Couching Procedure.** This figure depicts the outcome of a couching operation used to dislodge a cataractous lens out of the visual field.

## Intracapsular Cataract Extraction

In the 18th century, the technique of intracapsular cataract extraction (ICCE) was introduced as a safer alternative to couching.<sup>5</sup> This involved the creation of a large incision at the limbus, which is the connection between the cornea and sclera. The whole lens and the capsule it sits in are then extracted without replacement, and the patient remains aphakic, or without a lens (Figure 2).<sup>18</sup> A set of suspensory ligaments called zonules hold the lens system in place by connecting the lens capsule to the eye's ciliary muscles. Alpha-chymotrypsin, a proteolytic enzyme, is sometimes injected into the eye during surgery in order to dissolve these zonules and facilitate capsular extraction.<sup>20</sup> Similar to couching, after severe cataracts, vision will improve compared to vision pre-surgery, but it will remain relatively poor. Therefore, thick aphakic glasses that negatively impact peripheral vision can be postoperatively provided to patients as part of the treatment.<sup>2,18</sup> A study by Schemann, Bakayoko, and Coulibaly (2000)<sup>19</sup> compared the outcomes of ICCE and couching in patients from Mali. Despite the two procedures being similar in cost, ICCE patients experienced better visual outcomes. 5.3% of ICCE patients compared to 0% of couching patients achieved good acuity, 76.8% compared to 29.1% had poor acuity, and 17.9% compared to 70.9% became blind. Approximately half of those patients who underwent couching were unaware of alternative or more effective options. This indicates that initiatives to educate surgeons and raise awareness within communities about the existence of other cataract surgery techniques are important to pursue. Intracapsular cataract extraction is still performed today but only when necessary, as in for instance, cases where the posterior capsule is unstable due to weakened zonules.<sup>5</sup>



**Figure 2. Intracapsular Cataract Extraction.** This figure depicts the outcome of the intracapsular extraction of a cataractous lens and its surrounding capsule.

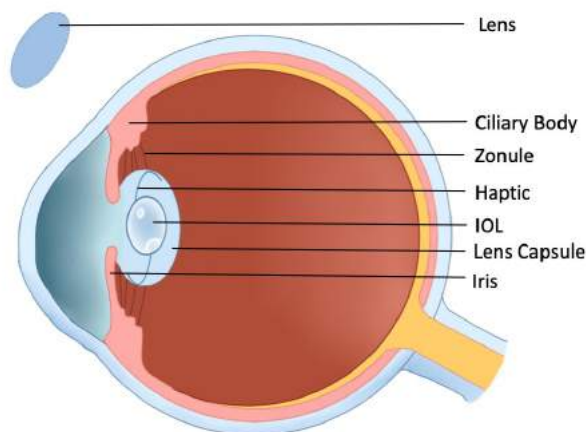
## SURGICAL TREATMENTS - PRESENT

### Extracapsular Cataract Extraction

During the late 20th century, the surgical technique of extracapsular cataract extraction became widespread. This method involved the removal of the aged, opaque lens and implanting an artificial intraocular lens (IOL) to replace it.<sup>21</sup> Therefore, the patient is no longer aphakic postoperatively, unlike with intracapsular cataract extraction or couching. In extracapsular cataract extraction, the surgeon creates an 11 mm limbal incision between the cornea and the sclera. Anterior capsulorrhexis, also known as anterior capsulotomy, is then performed, where the anterior capsule is removed, leaving behind the posterior capsule and the zonular attachments that hold it in place. The surgeon then extracts the lens nucleus and suctions out what is left of the lens cortex. In this manner, the integrity of the posterior lens capsule is maintained. An artificial IOL that has also been designed to correct for a patient's refractive errors can then be accurately maneuvered into place (Figure 3). This refractive correction provides patients with sharp acuity for distant vision, unlike with aphakic patients; reading glasses may be provided for near vision.<sup>5</sup>

IOLs are artificial lenses that are usually composed of polymethylmethacrylate if they are rigid and silicone or acrylic if they are foldable.<sup>5,22</sup> Hennig, Puri, Sharma, Evans, and Yorston (2014)<sup>22</sup> compared the use of rigid IOLs to the use of foldable IOLs in cataracts patients post-surgery. Visual outcomes in both groups were similar, but slightly better in the rigid IOL group. Furthermore, rigid IOLs are significantly cheaper than foldable ones. However, the incidence of posterior capsule opacification, a postoperative complication, was approximately 1.5 times higher in the rigid IOL group. Rigid IOLs require the creation of a 5 mm sclerocorneal tunnel incision that they can fit through. On the other hand, foldable IOLs allow for a smaller 2.5 mm corneal incision that is easier to create. Foldable IOLs are more widely used in developed countries, possibly due to the appeal of a smaller incision. However, in regions with limited access to medical resources, the low cost of rigid IOLs makes them preferable, especially when the visual outcomes are similar to those after the implantation of a foldable IOL. Two other types of IOLs currently exist: the posterior chamber IOL and the anterior chamber IOL. Usually, cataract surgery involves replacing the lens with a posterior chamber IOL. However, in cases where the posterior lens capsule is considered too unstable to hold the IOL in place or after an ICCE, an anterior chamber IOL is used instead. It is placed in front of the iris, where it can be held into place by attached "hooks" known as haptics.<sup>5</sup> Astigmatism is a condition characterized by an irregular corneal surface that prevents the sharp focus of light on the

retina and results in blurred vision. Toric IOLs that correct for astigmatism are also available and implanted in patients when necessary.<sup>23</sup>



**Figure 3. Extracapsular Cataract Extraction.** This figure depicts the outcome of extracapsular extraction of a cataractous lens without its capsule and its replacement with an artificial IOL.

### Phacoemulsification

Currently, the most commonly-used cataract surgical technique is phacoemulsification: an altered form of extracapsular cataract extraction that was introduced by Kelman in 1967.<sup>24-26</sup> Similar to the traditional ECCE, phacoemulsification involves the creation of a corneal incision, an anterior capsulotomy, and the replacement of the cataractous lens with a new IOL. Phacoemulsification differs from ECCE in the introduction of the phacoemulsification probe. After capsulorrhexis is performed, this probe is inserted into the opaque lens through a corneal incision. Instead of extracting the lens nucleus as one solid mass, the probe is used to transmit high-frequency ultrasound waves that emulsify the lens nucleus and allow for its suction through the same probe.<sup>2,24</sup> This is more colloquially known as the “divide and conquer” step. Once the old lens is suctioned out, a new IOL is inserted into the posterior lens capsule. With the advent of thinner phacoemulsification tools that can extract the lens in pieces and foldable IOLs, the corneal incision was significantly reduced to a size of 3 mm that does not require stitches. This may reduce complications such as suture-induced astigmatism.<sup>5</sup> De Silva, Riaz, and Evans (2014)<sup>2</sup> conducted a meta-analysis of studies that compared patient outcomes after phacoemulsification and patient outcomes after ECCE. Patients who underwent phacoemulsification achieved better uncorrected and corrected visual acuity than patients who underwent ECCE. Complication rates, such as iris prolapse, cystoid macular oedema and posterior capsular opacification, were also lower in the phacoemulsification groups. While phacoemulsification is surgically more expensive, ECCE may cost more overall, including the costs of extra postoperative follow-ups and treatments

for complications.<sup>2</sup>

### Femtosecond Laser-Assisted Cataract Surgery

The most recent development in cataract surgery is the more expensive and less commonly used Femtosecond Laser-Assisted Cataract Surgery (FLACS) technique. FLACS differs from phacoemulsification in that lasers largely automate the surgery by performing the incision, anterior capsulotomy, and fragmentation of the aged, opaque lens before its ultrasound emulsification and suction with the phacoemulsification probe.<sup>21</sup> Compared to previous techniques, FLACS lends the surgeon more precision and can reduce the risk of complications. Chen, Chen, He, and Yao (2016)<sup>25</sup> conducted a meta-analysis of studies that investigated FLACS outcomes and found multiple advantages associated with the technique compared to phacoemulsification. Specifically, FLACS was found to be a safer option that presents better visual outcomes, more accurate surgical performance, and reduces complications caused by surgical damage to the corneal endothelium. FLACS patients also experienced a faster recovery time, and consequently, better uncorrected distant visual acuity. Corrected distant visual acuity was also found to be better one-week post-surgery in the FLACS group. However, in the long-term, no significant difference was detected between the two groups. Nevertheless, FLACS is expensive, and the benefits it lends can be considered marginal in comparison to the cost of implementing it.<sup>25</sup> It is less commonly widespread compared to traditional phacoemulsification.<sup>25</sup>

### Viscoelastics

The introduction of viscoelastics to intraocular surgery in 1980 significantly contributed to the safety and efficacy of modern cataract surgeries.<sup>27</sup> Viscoelastics are transparent, fluid substances that are regularly injected into the eye throughout the duration of modern cataract operations. The main purpose behind the use of viscoelastics in cataract surgery is protection. Corneal edema presents one of the more common early postoperative complications to cataract patients.<sup>28</sup> Normally, corneal endothelial cells help regulate the fluid content of the cornea and maintain transparency by transporting excess fluid into the aqueous humor of the eye.<sup>29,30</sup> During surgery, some of these corneal endothelial cells can become damaged or lost, which reduces function and results in corneal swelling or corneal edema. This interferes with corneal transparency and results in poor vision.<sup>31</sup> Viscoelastics minimize friction and damage to the corneal endothelium while removing and replacing the lens, and therefore, they significantly reduce the incidence of complications such as bullous keratopathy, a severe form of corneal edema.<sup>32-34</sup> Additionally, they serve to fill the eye so as to hold ocular structures in place. This allows the surgeon more space to accurately maneuver tools and im-

mensely facilitates the surgery.<sup>34</sup>

## SURGICAL TREATMENTS - FUTURE

### *Accommodating Intraocular Lenses*

The lens capsule is connected to ciliary muscles that regularly relax and contract, causing the lens to alter its convexity. This process, known as accommodation, is crucial for the functioning of the natural lens and accurately focuses light on the retina to allow for sharp vision at all distances, according to Helmholtz's theory.<sup>35</sup> However, because extracapsular cataract extraction involves performing an anterior capsulotomy, the capsule and the entire lens system is unable to partake in accommodation.<sup>36</sup> Furthermore, cataracts patients are fitted with artificially constructed and comparably stiff IOLs, which additionally limit their ability to accommodate.<sup>36</sup> To compensate for this, IOLs are corrected in a manner that would allow the patient to have focused distant vision, and they are then provided with reading glasses that aid with near vision.<sup>5</sup> Alternatively, multifocal IOLs that are engineered to confer focused vision at different distances may be used. While they allow for a certain degree of accommodation, they do not possess the same range of focus that a natural lens does and may reduce contrast sensitivity.<sup>37</sup> Moreover, these multifocal "pseudoaccommodating" lenses are not as financially accessible as regular, unaccommodating IOLs.<sup>38</sup>

Due to these limitations in accommodation, current research efforts are focused on creating a transparent, optical substance that can be injected into the capsule once the opaque lens has been removed - without performing a capsulorrhexis.<sup>39</sup> Ideally, the liquid would be malleable enough to adequately and dynamically respond to the shifting capsule as it changes shape. This would accommodate for focused vision at all distances, provided that the ciliary muscles have not compromised their contractile function, as may be the case in older patients.<sup>39,40</sup> These lenses are known as accommodating IOLs and could potentially replace regular IOLs as the standard treatment for cataracts and revolutionize ocular surgery.

The challenges facing the successful development and application of accommodating IOLs include the optical material leaking through the opening made in the capsule and postoperative capsule opacification. Posterior capsule opacification is the most common late postoperative complication.<sup>41</sup> After extracapsular cataract extraction is conducted, whether phacoemulsification was performed or not, lenticular fibers may not have been completely extracted and remain in the periphery of the posterior lens capsule. These cells then proliferate and migrate into the center of the posterior capsule before undergoing opacification. This results in symp-

toms that echo those experienced when the individual was suffering from cataracts (e.g. low visual acuity). For this reason, posterior capsule opacification is also known as "secondary cataracts". To treat this complication, a neodymium: YAG laser-automated posterior capsulotomy is performed as a way to clear the patient's visual field and allow a transparent passage for light to enter the eye. Currently, there are no established ways to prevent posterior capsule opacification from occurring.<sup>41</sup> With an accommodating IOL, posterior capsule opacification would reduce visual acuity and prevent effective accommodation. Furthermore, a laser capsulotomy to treat posterior capsule opacification might result in the leaking of the optical substance and a compromise of the accommodating IOL structure.<sup>42</sup>

Another goal for these accommodating IOLs is to correct for a patient's refractive errors and eliminate the need for visual correction with glasses or contact lenses, also known as emmetropia.<sup>42</sup> Multiple accommodating IOL designs and methods of administration have been proposed and remain under research investigation. In a study by Hao et al. (2012)<sup>36</sup>, researchers tested the effectiveness of a polysiloxane gel as an alternative to traditional IOLs and found that the gel fully recovers the accommodation range of human cadaver eyes. Ideally, this research aims to achieve a point where accommodating IOLs can be clinically utilized in human cataract surgeries, and patients can postoperatively enjoy sharp, uncorrected vision at all distances. More research on accommodating IOL design, application, and long-term outcomes is needed.

## CONCLUSION

Approximately 50% of the world's blindness is caused by age-related cataracts, and people across the globe and across centuries have been faced with the disease. Technological advances over time have allowed the treatment of cataracts to evolve: from the couching method used in ancient times to phacoemulsification, the most common surgical method used to treat cataracts today. Research into future therapies remains active and looks into developing accommodating IOLs to replace regular ones and lend the patient emmetropia through restoring lens accommodation.

With a greater understanding of age-related cataracts and more research into viable therapeutics, the disease can be effectively combated. Strategies to make treatments more cost-effective, accessible and convenient will bring us closer to the Right to Sight Initiative of eliminating avoidable blindness. However, this goal is far from being reached. A large number of individuals in developing countries are unaware of cataract treatment options available to them and tend to undergo couching, which is the least safe and least effective technique. Without disseminating the relevant infor-

mation and advocating for access to safe treatments in developing countries, surgical advances in the field are inconsequential in these regions. Therefore, it is integral to train specialists on these surgical techniques and inform potential patients on their treatment options. Implementing these measures along with providing the equipment required to conduct these surgeries can help reduce inequalities in treatment.

## ACKNOWLEDGEMENTS

This work did not receive funding. There are no conflicts of interests.

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# Considerations for Chemotherapy Treatment in Platinum Resistant High-Grade Serous Ovarian Cancer

## ARTICLE INFORMATION

Received: 14 October 2019  
Accepted: 17 November 2019  
Published: 29 November 2019

**Senior Editor**  
Amama Khairzad

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## ABSTRACT

Ovarian cancer is considered to be the most fatal type of any gynecological cancer. Prognosis for the disease is poor, with a median survival of only thirty-two months following diagnosis and a five-year survival rate of only 39%. Many of the most lethal ovarian cancer cases are classified as part of the high-grade serous ovarian cancer (HGSOc) subtype, which is the most aggressive form of the disease. The primary concern with regards to treatment is that nearly 30% of patients will develop a resistance to forms of platinum chemotherapy, which is the main method of treatment. This suggests that a one-size fits all approach cannot be taken to treat ovarian cancer, and that further research must be done to understand how to treat the patients who present with platinum resistance. This literature review examines the mutations within two susceptible loci, specifically, the p53 and BRCA1/2 genes, in order to understand how platinum resistance develops and why it is present in some patients. The objectives of this review are to characterize the underlying genetic mechanisms affecting platinum resistance, specify the biomarkers associated with those mechanisms, and describe alternative methods for approaching the treatment of ovarian cancer on an individual scale.

**Keywords:** Ovarian cancer, platinum resistance, HGSOc

## INTRODUCTION

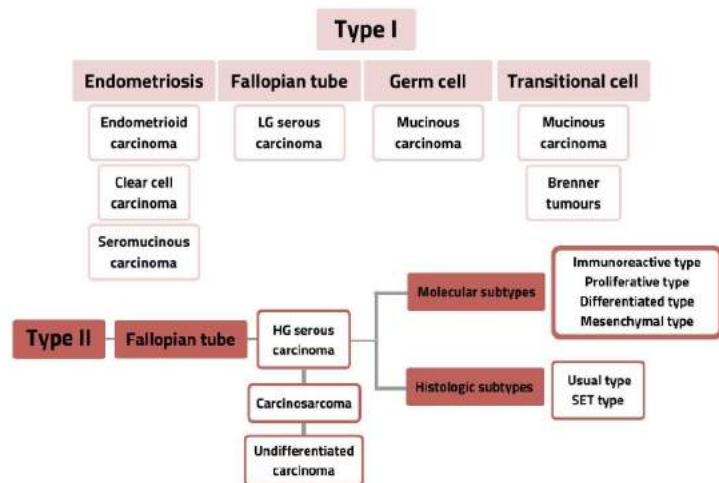
In the broad sense, ovarian cancer refers to a cancer that originates near or within the ovaries, or on the outer layer of the ovary.<sup>1</sup> As is typical of many cancers, the disease can then spread throughout the body. Ovarian carcinomas usually migrate to local regions such as the pelvis and abdomen, but can eventually metastasize to distant areas of the body.<sup>2</sup> Data suggests that in 2017, around 2,800 women in Canada developed ovarian cancer and 1,800 of these women died as a result.<sup>3</sup> Prognosis is poor, with a five-year survival rate of around 39% and a median survival of thirty two months following diagnosis.<sup>4</sup>

Ovarian cancers can be subdivided into type I and type II cancers, as shown in Figure 1, each with a unique presentation.<sup>2</sup> Type I cancers have a large, unilateral

growth that is typically low grade.<sup>5</sup> These low grade tumours appear similar to healthy cells at the microscopic level and typically grow at a much slower rate than high grade cancerous cells.<sup>6</sup> Patients with type I tumours usually have a higher survival rate, accounting for only 10% of ovarian cancer deaths. Type II cancers are typically much more lethal. They are high grade, present an abnormal morphology under a microscope, and proliferate at a faster rate.<sup>6</sup> Overall, tumour volume in type II ovarian cancers tends to be lower than in type I tumours, but this disease is much more aggressive and results in 90% of recorded ovarian cancer deaths.<sup>5</sup> Unfortunately, over 75% of ovarian cancer cases are advanced stage type II tumours at the time of diagnosis.<sup>5</sup>

The most common type of ovarian cancers that are diagnosed are type II carcinomas known as serous cancers.<sup>5,7</sup> Although they were believed to arise from the





**Figure 1.** Characterization of type I and type II ovarian cancers.<sup>5</sup>

epithelium of the ovary, researchers now believe that they originate from secretory cells of the fallopian tubes.<sup>7,8</sup> When considering all types of ovarian cancer, the most aggressive type is high-grade serous carcinomas (HGSOCs) and most patients with advanced stage tumours present with this cancer type.<sup>7</sup> For this reason, the diagnosis, treatment, and challenges associated with this type of cancer will be discussed in more detail in an attempt to remodel how the medical community views and treats high-grade serous carcinoma.

HGSOC and other types of ovarian cancers are notable for being difficult to detect. Symptoms often do not present distinctively until later stages of the disease,<sup>9</sup> and one challenge in diagnosis is differentiating between symptoms of the cancer and conventional function in women, such as menstrual cycle-related pain in the uterus<sup>10</sup>. Around 95% of women reported symptoms in advance of their diagnosis, with common concerns including abdominal pain, pelvic and urinary problems, or issues pertaining to the gastrointestinal tract.<sup>10</sup> These are typical symptoms of many other diseases, making early diagnosis of HGSOC rather challenging.

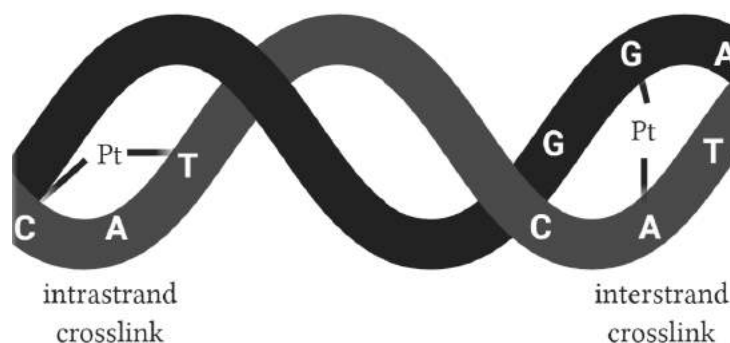
## CONVENTIONAL TREATMENT

This cancer has a great level of heterogeneity, thereby requiring different types of treatments depending on the individual.<sup>11</sup> The most common treatment methods are surgery and chemotherapy. Hormonal therapy and radiation therapy are also used infrequently, due to the fact that they require a small target area, whereas ovarian cancer tends to spread to multiple organs.<sup>11</sup>

Chemotherapy treatment commonly consists of a combination of carboplatin and paclitaxel; however, many other chemotherapeutic agents have been used to date.<sup>12</sup>

Carboplatin is a compound comprised of platinum and organic functional groups. Its main mechanism of action to kill cancer cells is through crosslinking DNA, or creating bonds between nucleotides that do not normally exist.<sup>13</sup> In the cell, carboplatin is activated and forms crosslinks through reactive platinum complexes that bind to nucleophilic groups in DNA, forming intrastrand and interstrand DNA crosslinks, as shown in Figure 2. The crosslinks result in apoptosis because that part of DNA is no longer functional.<sup>13</sup>

Carboplatin is more commonly used for treatment than cisplatin, another platinum compound that acts through crosslinking DNA due to cisplatin's propensity to cause nephrotoxicity – toxic activity in the kidneys that can lead to renal failure.<sup>13</sup> However, carboplatin induces myelosuppression in high doses, which causes reduced bone marrow activity and a decrease in both red and white blood cells.<sup>13</sup> Paclitaxel is usually administered with carboplatin to prevent microtubule depolymerization, however, it can cause nerve damage in high doses.<sup>13</sup>



**Figure 2.** Mechanism of intrastrand and interstrand crosslink formation in platinum-based chemotherapeutics (adapted).<sup>12</sup> Figure created using BioRender.

## RESPONSES TO TREATMENT AND PLATINUM RESISTANCE

Initial response rates in patients undergoing chemotherapy treatments of carboplatin and paclitaxel tend to be 60–80%.<sup>12</sup> However, many patients that initially respond well to platinum-based chemotherapy treatments eventually develop some level of platinum resistance and either stop responding to treatment or relapse because the platinum is no longer able to effectively form crosslinks.<sup>14</sup> Platinum resistance in chemotherapy is measured by “platinum-free interval” (PFI), which is defined as the time period between the final treatment and the point at which the patient relapses.<sup>15</sup> If the relapse occurred more than six months after the completion of treatment, it is considered platinum sensitive, but if the patient relapsed sooner than six months, it is characteristic of platinum resistance.<sup>14,15</sup>

To better understand the mechanisms of platinum re-

sistance in serous ovarian cancer and guide future treatments, the Cancer Genome Atlas Project performed a genomic analysis of DNA samples in diseased subjects.<sup>16</sup> Part of the difficulty in treating HGSOV is the great deal of heterogeneity within the tumours and in patient responses; however, genomic data has provided some similarities. It was found that TP53 mutations are present in nearly all ovarian cancers, with a prevalence rate of 96%.<sup>16</sup> While less common, breast cancer gene (BRCA) mutations were found in 20% of DNA samples.<sup>16</sup> Many other genes have an influence on the molecular biology of ovarian cancer, including NF1, CDK12, over 100 copy number aberrations, and 168 genes associated with promoter methylation events. CCNE1, NOTCH, FOXM1, *src*, and E2F3 signaling also affect the molecular biology of the cancer.<sup>16</sup> The role of BRCA will now be highlighted because it could be significant in determining treatment regimes. Mechanisms of platinum resistance in TP53 will also be discussed in this review due to the prevalence of mutations in the gene.

### BCRA1 AND BCRA2 MUTATIONS RELATING TO PLATINUM-RESISTANCE

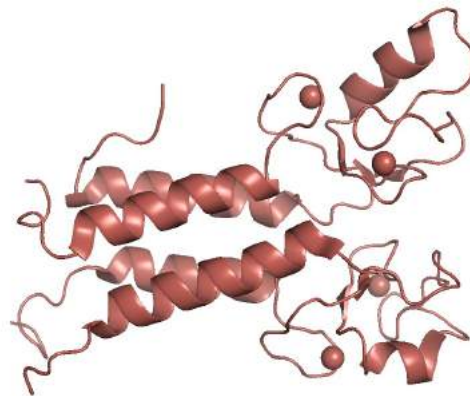
BRCA1 and BRCA2 genes are both implicated in the development of ovarian cancer, but can also influence an individual's treatment regimen for HGSOV. Mutations in these genes increase an individual's likelihood of developing ovarian cancer. For those with mutations in BRCA1, the likelihood of developing ovarian cancer in an individual's lifetime is nearly 40%; the probability lowers to 11-18% for mutations in BRCA2.<sup>17</sup> However, despite an increased propensity for the development of carcinoma with these mutations, the prognosis is often better than those with wild-type BRCA1/2, which is the non-mutated gene form.<sup>18</sup>

BRCA1 is located on the long arm of chromosome 17 at position 21. Specifically, it is located at base pairs 43,044,294 to 43,125,482.<sup>19</sup> BRCA2 is located on the long arm of chromosome 13 at position 12.3, spanning base pairs 32,315,479 to 32,399,671.<sup>19</sup> Both genes are thought to have an "ovarian cancer cluster region" (OCCR) where an increased prevalence of mutations may lead to the development of ovarian cancer.<sup>19</sup> In the body, BRCA1 gene products are involved in tumour suppression and DNA repair.<sup>20</sup> The gene has an N-terminal RING domain that undergoes protein interactions, as shown in Figure 3.<sup>21</sup> The C terminus has BRCA1 C-terminus (BRCT) domains, which researchers have also uncovered in other proteins that are involved in DNA repair. Mutations in BRCA1 likely cause improper folding of the BRCT domain or change the interface where dimerization occurs.<sup>21</sup>

Similarly, BRCA2 gene products also play a role in tumour suppression. They have eight residues known as

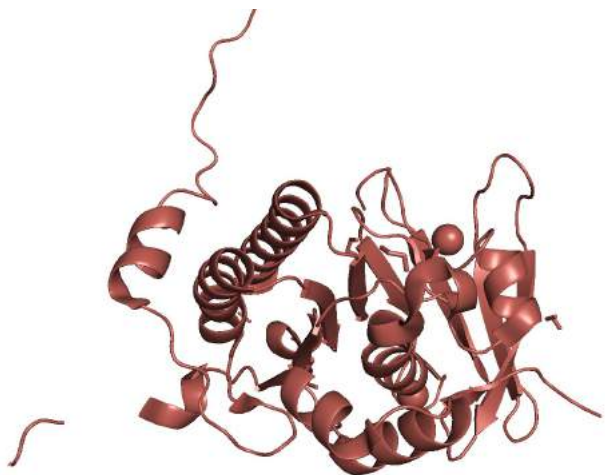
BRC repeats that assist in the binding of the protein RAD51 and BRCA2, as shown in Figure 4.<sup>21</sup> The binding of BRCA2 to the protein allows for the repair of DNA via homologous recombination.<sup>23</sup>

When these particular genes are mutated, the risk of developing cancer increases significantly. Research has shown that tumours originating from carriers of the mutated gene have undergone a deletion at the BRCA1/2 locus, which results in the disappearance of the wild-type allele leading to BRCA1/2 deficiency.<sup>23</sup> This deficiency makes cells more susceptible to cross-linking agents,<sup>23</sup> which is the typical mode of action of some chemotherapeutics, including cisplatin.<sup>13</sup> This means that patients who have BRCA1/2 deficiency usually have a more promising initial response to platinum agents since their mutated cells are more sensitive to these drugs than patients who possess the healthy genotype.<sup>14</sup>



**Figure 3.** BRCA1 RING domain that forms a heterodimer with BARD1.<sup>22</sup>

A study by Alsop et al. (2012) found that for patients with BRCA mutations who relapse soon after the first round of treatment, platinum therapy has a better prognosis than in those without the mutation. However, while the original treatment for those with mutations in BRCA1/2 typically has a promising response, they may also develop resistance to platinum-based chemotherapeutic agents.<sup>23</sup> It has been suggested in the literature that secondary mutations could be the reason for this resistance.<sup>14,17,23</sup> This likely occurs by altering the proteins involved in DNA repair mechanisms, which increases the amount of DNA repair that occurs. An increased amount of DNA repair can cause an increase in homologous recombination, among other repair mechanisms,<sup>14</sup> which is the method by which BRCA1/2 engages in DNA repair.<sup>20,23</sup> Homologous recombination can lead to secondary mutations that revert the mutated BRCA gene back to the wild-type gene and restore the typical BRCA phenotype.<sup>14,23</sup> In order to revert to typical function in BRCA2, one study identified that the reading frame that was altered by a frameshift mutation of 6174delT must be restored.<sup>23</sup> In this study, all samples that were resistant to cisplatin



**Figure 4.** RAD51-BRCA2 BRC repeat complex.<sup>24</sup>

had mutations that corrected errors caused by the original frameshift.<sup>23</sup> Furthermore, it is reported that 28.3% of ovarian tumours that result from relapse have secondary mutations.<sup>17</sup> In addition, while 5.3% of carcinomas that are sensitive to platinum drugs have these mutations, the number is much higher for platinum-resistant tumours.<sup>17</sup> Secondary mutations were found to be present in 46.2% of platinum-resistant ovarian tumours, which builds upon the evidence of secondary mutations being responsible for the acquired resistance.<sup>17</sup>

### TP53 AND p53 MUTATIONS RELATING TO PLATINUM RESISTANCE

Along with BRCA1/2, the TP53 gene also has a significant impact on the development and drug resistance of HGSOV. TP53 is located on the short arm of chromosome 17 at position 13.1.<sup>25</sup> It is 19,198 nucleotides long and contains seven exons, with the sequence beginning in exon two.<sup>25</sup> The gene is responsible for encoding p53, a tumour suppressor protein that regulates transcription through binding DNA at various genomic segments.<sup>26</sup> P53 is able to regulate cell cycle growth, control gene expression, and damage repair mechanisms.<sup>26</sup> Most of the mutations in the gene occur in the DNA binding domain, but it is also possible to detect mutations elsewhere within the sequence.<sup>25</sup>

There are a variety of mutations that occur within the gene, including base substitutions, deletions, and insertions.<sup>25</sup> To date, 2,329 different p53 mutations are known to affect human ovarian cancers, most of which are missense mutations, or mutations of a single nucleotide.<sup>26</sup> p53 can undergo a gain of function, loss of function, or dominant-negative mutation<sup>26</sup>, however, the type of mutation is dependent upon where the mutation has occurred<sup>25</sup>. It is predicted that the mutated protein, mutp53, affects the body because it loses its ability to bind to necessary elements of DNA, altering its efficacy as a transcription factor.<sup>25</sup> Mutations of

p.Arg175His, p.Gly245Ser, p.Arg248Trp, p.Arg248Gln, and p.Arg273His are all linked to promoting tumour growth, while mutations in p.Arg175His and p.Arg273His are connected to the development of platinum resistance in tumours.<sup>25</sup> A study by Brachova et al. (2014) determined that mutations where tumour growth is promoted have increased levels of platinum resistance and significantly worse survival rates compared to other alterations such as loss-of-function mutations.<sup>27</sup> Another study on platinum resistance suggests that activated p53 upregulates proteins that promote apoptosis and downregulates proteins that prevent cell death. This leads to the conclusion that inactive p53 may result in chemotherapeutic resistance.<sup>28</sup>

### APPLICATIONS TO TREATMENT IN HIGH GRADE SEROUS OVARIAN CANCER

Evidently, platinum resistance is a significant problem in the treatment of ovarian cancer. Genetic analysis and a better understanding of the underlying molecular mechanisms could give medical professionals new strategies for approaching patient care. A review of the literature leads to some potential recommendations in treating HGSOV. Although platinum resistance is common, original treatment has a success rate of 60-80%.<sup>12</sup> These statistics indicate that administering a round of platinum-based chemotherapy could be the most effective and feasible option. However, it is also recommended that a more individualized approach be taken in order to improve prognosis in patients with HGSOV.

A good first step would be to begin screening for BRCA mutations following diagnosis. The results of this test can determine how well patients will react to platinum-based therapies. Those with BRCA mutations should be monitored for any potential secondary mutations, as this is an indicator that platinum resistance is more likely and other courses of treatment should be considered. Although TP53 mutations are common in ovarian cancer, it may improve treatment if medical professionals can narrow down the type and location of the mutation that affected the gene, as certain mutations are correlated with increased resistance. Knowing whether or not a patient has the propensity to develop platinum resistance can aid physicians in outlining treatment plans. If it is determined that a platinum treatment will prove less effective, it is possible to turn to options such as rational drug design, enrolling patients in clinical trials for novel chemotherapeutics that are currently being developed, or employing strategies such as maintenance chemotherapy in order to delay the inevitable resistance.<sup>28</sup>

In theory, genomic screening can provide information to medical professionals to guide HGSOV treatment. However, the question of feasibility still remains as it

would be necessary to perform molecular analysis on thousands of affected patients in order to take an individualized approach to their care.

## ACKNOWLEDGEMENTS

We would like to thank Dr. Rosa da Silva for her guidance and advice throughout this project. Both authors contributed equally and no funding was received for this work.

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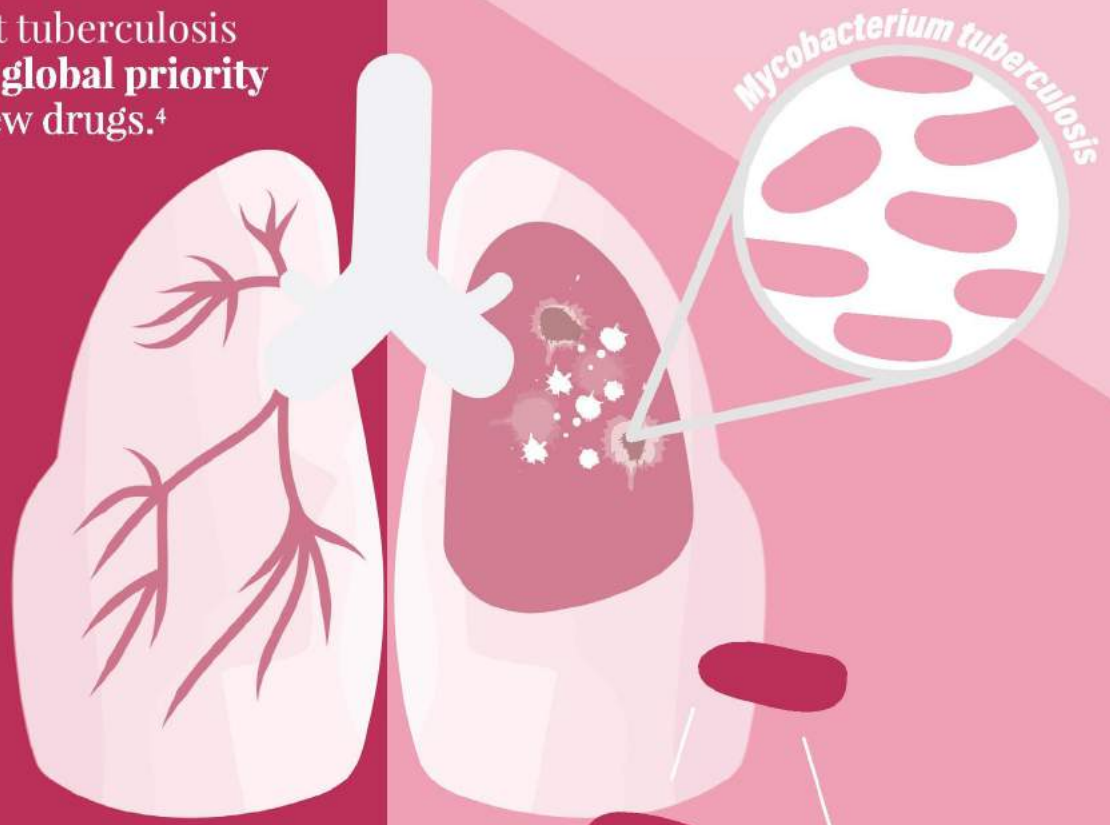
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# ANTIMICROBIAL RESISTANCE & TB

Multi-drug resistant tuberculosis (TB) is considered a **global priority** for investment in new drugs.<sup>4</sup>

TB is one of the **top 10 causes** of death globally.<sup>5</sup>

In 2017, a study found **600,000 cases** worldwide were **resistant** to the most effective first-line drug, **Rifampicin**.<sup>5</sup>



## TUBERCULOSIS

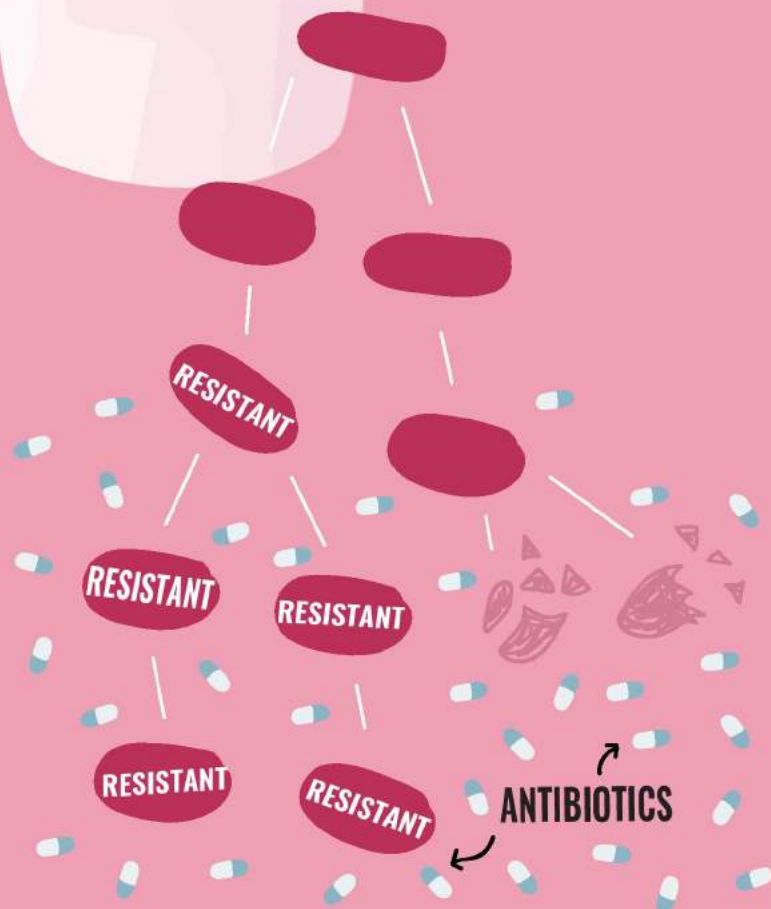
Infectious, airborne disease.<sup>8</sup>

Results in necrosis in lungs.<sup>8</sup>

Only active TB can spread to other areas.<sup>8</sup>

Primarily caused by a bacteria called *Mycobacterium tuberculosis*.<sup>8</sup>

Acknowledgements: This infographic has been submitted on behalf of the Demystifying Medicine club.



## ARTICLE INFORMATION

Received: 16 October 2019  
Accepted: 15 November 2019  
Published: 29 November 2019

**Senior Editor**  
Ishita Paliwal

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## CAUSES OF RESISTANCE



**Overuse** of drugs on illnesses that cannot be treated with antibiotics.<sup>1</sup>



Extensive **agricultural use** for supplementation of livestock.<sup>2</sup>



**Lack of economic incentives** has led to fewer studies and advances in drug development.<sup>3</sup>

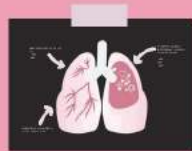
## ANTIMICROBIAL RESISTANCE

- Bacterial cells acquire advantageous **mutations** that increase survival through **natural selection**.<sup>6</sup>
- These mutations can arise from **random genetic changes** and can be shared with neighbouring bacteria through horizontal gene transfer or passed down during budding.<sup>6</sup>
- When antibiotics are introduced, bacterial cells without this advantage die, while remaining **resistant cells proliferate**.<sup>6</sup>

## ANTIBIOTIC STEWARDSHIP



List antibiotics in use in hospitals.<sup>7</sup>



Develop standard treatment methods.<sup>7</sup>



Monitor antibiotic prescriptions and track use.<sup>7</sup>



Educate the public on antibiotic resistance and proper drug use.<sup>7</sup>

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# INTERVIEW

WITH DR. AYESHA KHAN

## SCIENCE MEETS COMMUNITY: MCMASTER UNIVERSITY AND COMMUNITY-ENGAGED EDUCATION IN SCIENCE

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### ARTICLE INFORMATION

Received: 16 October 2019

Accepted: 18 November 2019

Published: 29 November 2019

#### Senior Editor

Amama Khairzad

#### Section Editors

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Reza Khorvash

#### Layout Editor

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### ABSTRACT

At McMaster University, community-engaged education gives students the opportunity to contemplate the importance of their learning on a broader level. Dr. Ayesha Khan's influence on this type of education in the Faculty of Science is important due to the impact it has made and will continue to make in the future. Community-engaged education in science allows students to approach what they are learning from different perspectives and to understand how it applies to different groups of people. As individuals in the Faculty of Science are trying to make a positive impact on outside communities through what is learned and taught within science courses, community-based education is crucial and fortifying for the parties involved.



Dr. Ayesha Khan, Ph.D.

*In January 2019, Dr. Ayesha Khan became the Associate Director of the School of Interdisciplinary Science at McMaster University. She completed the entirety of her post-secondary education at McMaster University attaining her Ph.D. in Behavioural Neuroendocrinology. In 2013, after teaching at the University of Toronto Mississauga and Ryerson University, Dr. Khan returned to McMaster University to teach in the Life Sciences program and in the Department of Psychology, Neuroscience and Behaviour. Dr. Khan took inspiration from the then President Dr. Patrick Dean's letter, Forward with Integrity, and implemented the principles she cultivated from this letter into her teaching approach. This occurred through the integration of community-engaged education projects in some of the courses she taught and currently teaches. These projects are known as MacEngaged and involve the translation of relevant scientific information to benefit the community by fulfilling a civic duty. Dr. Khan is empowered by the interactions she has with her students as well as their enriching and mutual learning experiences.*

► **You have recently been appointed Associate Director of the School of Interdisciplinary Science (SIS), how have your role and your day-to-day experiences changed since beginning this role?**

On a day-to-day basis, I think about trying to answer

the following questions: How can we innovate so that our students are getting the highest quality education? How can we have more connection with our students so that we feel they are interacting with faculty members in a way that is meaningful to them? How can we recruit more instructors who are disruptors and innovators in their approach to delivering high-quality education? I'm in a wonderful position to not only think about curriculum design and student experience from the perspective of the classroom that I teach in, but also at the program level. It is very gratifying to be able to think about the student experience on a broader scale. No day is the same, and I love that!

► **What are some of the challenges for students when implementing this community-engaged education?**

One of my personal philosophies in life is that there has to be some amount of challenge for growth to occur. For example, take the analogy of exercise. If a student goes to the gym and experiences an easy and effortless workout, will that produce a change in their endurance and result in muscle growth? Probably not. At the same time, one is not going to challenge themselves with the aim of lifting a 150lb dumbbell because that is not realistic either. My goal is to challenge my students with a reasonable amount of difficulty in figuring out solutions to real-world problems which at the start don't seem very clear. I aim to do this because that is what will be expected of them in the workforce regardless of the careers they pursue.

“There has to be some amount of challenge for growth to occur”

► **Where do you feel community-engaged education stands in the science community?**

There are many definitions and interpretations of community-engaged education. In a nutshell, it is placing the student in an experience that helps them to think about the workings and needs of a particular community in a non-intrusive and non-judgmental way. There should be a reciprocal benefit to both the community and to the learning of the student. At McMaster University, there is a lot of support for community-engaged education. The challenge for us in the Faculty of Science is how to bring authentic experiences to our students. From my perspective, it is important to curate creative experiences so that students learn the benefit of community-engaged education through exercises that are well-crafted with ample support from peer mentors, teaching assistants, and of course the instructor. And at the same time, we must not be a burden to our community partners.

► **Do you have any specific community-engagement projects that you have seen start in the classroom and continue to live on after the class concludes for the semester?**

Students in my neuropsychology course have formed clubs that are raising awareness about Parkinson's Disease. Others have donated many resources from their projects to schools across the world, as far as Korea and Bangladesh. There have been items that are donated to student organizations on campus as well as local community centres. For example, students in my Life Sciences seminar course donate brochures on reproductive health to the *Student Health Education Centre* on campus. I have also partnered with *Bleed Free McMaster* to donate student designed health kits to local shelters. Through my partnership with *Let's Talk Science*, countless teams have had access to schools to share their knowledge about the inner workings of the brain and how to maintain brain health. On an individual level there is the personal benefit of problem-solving, collaboration, innovation, and starting something from scratch; I think of all of this as an invaluable experience.

► **Why do you feel that community-engaged education is beneficial for students?**

Students are introduced to a lot of information in the classroom. It is possible to use community-engaged education as a useful method to assist students in figuring out why their learning matters and to keep the bigger picture in mind. I think community-engaged education brings meaning and value to what we are teaching and what students are learning. The other fantastic element is that students get to collaborate with their peers to hopefully enrich the lives of others! This is the type of educational experience that can have a long-lasting impact on not only the students but also their professors.

“It is important to curate creative experiences”



# Alternative Treatment for Alzheimer's Disease: *Porphyromonas gingivalis* Inhibitors

## ARTICLE INFORMATION

Received: 15 October 2019  
Accepted: 15 November 2019  
Published: 29 November 2019

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## ABSTRACT

*Porphyromonas gingivalis* (*P. gingivalis*), has been identified as a primary pathogen in causing chronic periodontitis, or gum inflammation. *P. gingivalis* was also isolated in brain samples of patients suffering from Alzheimer's disease. A virulence factor of *P. gingivalis* called gingipains, releases proteases responsible for neurodegeneration and has been identified in the brain of patients suffering from Alzheimer's. Studies show that mice infected with *P. gingivalis* demonstrate an increase in amyloid plaque deposition in brain samples. Further investigation identified gingipains as a neurotoxic agent, both *in vivo* and *in vitro*, which impacts the structure of tau protein, responsible for the normal functioning of neurons. Small-molecule inhibitors targeting gingipains are utilized to prevent the neurotoxic effects of gingipains and facilitate neuronal regeneration. Inhibition of this virulence factor reduced the overall bacterial load, blocked amyloid-beta production, prevented neuroinflammation, and allowed for neuronal recovery. These findings provide a new outlook for the onset of Alzheimer's disease and elucidate a much-needed potential treatment for the condition.

**Keywords:** Alzheimer's disease, *Porphyromonas gingivalis*, gingipains, inhibitor, periodontal disease, virulence factor

Alzheimer's disease (AD) is a neurodegenerative disease that induces dementia and is associated with the accumulation of two proteins, amyloid-beta (AB) and tau, in the brain. The aggregation of these proteins in the brain interferes with normal neuronal function and leads to degradation of neurons over time. Based on this, researchers proposed the "Amyloid Hypothesis", which states that the defective regulation of such protein can lead to a toxic plaque formation within the brain, which induces the impairment of cognitive functioning exhibited in AD.<sup>1</sup> This model has been accepted for many years, however, novel research has proposed an alternative hypothesis. These new findings suggest that plaque deposits may be a protective response against bacteria that find their way to the brain. In 2016, Kumar et al. discovered that amyloid seems to function as a sticky defence mechanism against bacteria that cross the blood-brain barrier.<sup>2</sup> They found that this protein can act as an antimicrobial compound that kills bacteria; when bacteria were injected into the brains of mice engineered to make tau and amyloid proteins, plaques developed around

the bacterial cells overnight.<sup>2</sup>

*Porphyromonas gingivalis* is an oral pathogen that primarily inhabits the human oral cavity. It is also the prime agent in causing periodontal diseases, such as gum disease.<sup>3</sup> This pathogen is a non-motile, gram-negative anaerobic bacterium, meaning it has a thin peptidoglycan wall and prefers an environment that lacks oxygen. *P. gingivalis* produces a wide variety of virulence factors, which are molecules produced by bacteria that allow them to invade the host and initiate an immune response. The main virulence factor is *gingipain*, which not only plays a role in gum disease, but has also been linked to AD.<sup>4</sup> Although *P. gingivalis* is mainly found in individuals with gingival and periodontal diseases, in twenty-five percent of the cases, individuals without oral disease test positive for the bacteria.<sup>5</sup> Further research into *P. gingivalis* also demonstrated that it may be a risk factor for AD as it has been found to invade the brain and cause inflammation.<sup>6</sup>

Novel research conducted by Dominy et al. (2019) has validated the previous findings for the association between AD and *P. gingivalis*.<sup>7</sup> However, the most significant finding of this research is that inhibiting gingipains leads to therapeutic effects in preventing neuronal degradation and facilitating recovery.<sup>7</sup> This finding may revolutionize AD in the medical community due to countless potential future applications for the prognosis and treatment of this disease.

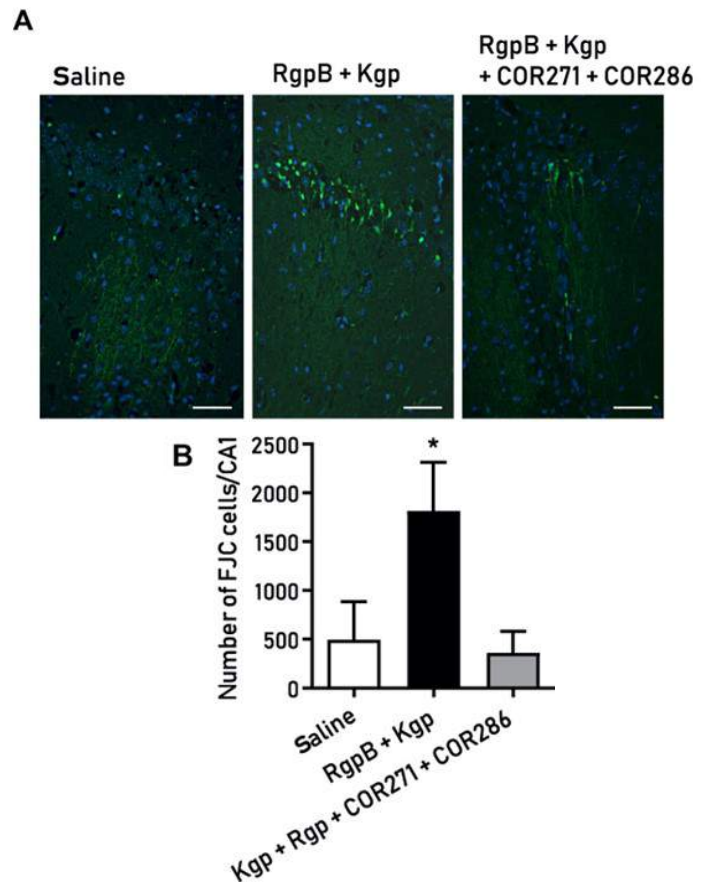
### Comparative Assessment of Bacterial Load in AD and Control Brain Samples

Assessment of brain samples of Alzheimer's-affected patients was matched based on sex and age. This comparative analysis of the brain tissue samples demonstrated a significantly higher level of gingipain antigens compared to control brain core samples of healthy patients. Additionally, as Figure 1 illustrates, *P. gingivalis* bacteria were also found in the cerebrospinal fluid of 70 percent of affected patients. This helps validate the role of *P. gingivalis* in infecting the central nervous system and contributing to neuronal degradation. Overall, these findings help link gingipains to AD pathogenesis, even in cases with optimal dental care. Interestingly, gingipain antigens were also present in patients with AD pathology in the absence of dementia. This finding helps establish the presence of gingipains as an early occurrence in AD progression for middle-aged individuals in the absence of cognitive decline. The significance of this is the potential use of gingipains as a marker for identifying the onset of AD.

### Role of *P. gingivalis* and Gingipains Inhibition in AD Pathogenesis in Mice

Dominy et al. (2019) demonstrated evidence that *P. gingivalis* and the virulence factor gingipains play a crucial role in AD pathogenesis. In mice, the researchers illustrated reduced host AB response following the administration of small-molecule gingipain inhibitors. The inhibitors facilitated a decrease in the overall gingipain load in the brain of mice and impacted the progression of AD by blocking the gingipain-induced neurodegeneration. Figure 1 illustrates the findings when two different strains of *P. gingivalis* (RgpB and Kgp) were used to mimic *P. gingivalis* infection in neuroblastoma cells in mice through intravenous injection of gingipains. The neuroblastoma samples were stained with Fluoro-Jade C (FJC), a fluorescent stain used to visualize degenerating neurons. The researchers found that not only does gingipain induce neurodegeneration, but also the small inhibitory molecules of gingipains (COR271 and COR286) can facilitate neuronal recovery. This was further demonstrated by the significantly higher number of FJC-positive cells after exposure to gingipains, in comparison to much lower neurodegeneration with low numbers of FJC-

positive cells in saline control and gingipain-inhibited trials. These results are significant as they demonstrate the efficacy of gingipain inhibitors as a treatment for AD in mice, a commonly used model organism for humans. As a result, further investigation in the field of pharmacology may allow for the treatment against gingipain-induced Alzheimer's in humans.



**Figure 1. Neuronal protection of small-molecule gingipain inhibitors.** A) Fluoro-Jade C (FJC) staining (green) of hippocampal neurons in mice following injection with saline (control), RgpB+Kgp (gingipains), and RgpB+Kgp+COR271+COR286 (gingipains and small-molecule inhibitors). Scale bars, 50  $\mu$ m. B) Total number of FJC-positive cells in an entire hippocampus. There is significant reduction in FJC-positive cells following injection of gingipains and inhibitors (\* $P < 0.05$ ,  $n = 14$ ). Graph with SEM error bars. Reprinted from "Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors", by Dominy, SS., 2019, Sci Adv, 5, 1–22. Copyright 2019 by American Association for the Advancement of Science (CC by 4.0).

## CONCLUSION

This study has also demonstrated the neuroprotective mechanism behind the use of small-molecule inhibitors against gingipains. This provides a novel method for preventing AD pathogenesis and slowing disease

progression prior to the onset of dementia. Results have established gingipains as an early event in AD pathogenesis. This may allow for the utilization of gingipains as a marker for AD progression. For these concepts to be applied for detecting and treating AD, supplemental research is required. Currently, the research in this field has focused on model organisms, with mice being the main source for ample literature. Additional research is required in humans in order to truly identify the efficacy of using inhibitors against gingipains for treating AD in the future. There are also several limitations for this paper that are worth noting. There are various strains of *P. gingivalis* and this paper was not able to identify specifically which strain plays a prominent role in AD progression. This research also overlooked other potential sources of gingipain transmission in the absence of oral infection. However, the novel findings help support an alternative hypothesis for AD involving the role of AB plaques as antimicrobial peptides. The neuroprotective role of gingipain inhibition provides potential treatment for AD in the future.

## ACKNOWLEDGEMENTS

This work did not receive funding. There are no conflicts of interest.

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# Induced Pluripotent Stem Cells—Bringing Humanity One Step Closer to Curing Cancer

## ARTICLE INFORMATION

Received: 15 October 2019  
Accepted: 10 November 2019  
Published: 29 November 2019

**Senior Editor**  
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## ABSTRACT

Cancer develops when healthy cells experience a mutation, allowing for rapid and abnormal growth. Mutagens, such as radiation and carcinogens, allow fast-growth variant cells to be positively selected and thus propagate the development of cancer. Radiation and chemotherapy are prevailing, but non-ideal forms of cancer treatment as they can harm healthy cells in the body. Stem cells can be used to replace the healthy cells that were lost, but there are ethical concerns regarding the acquisition of embryonic stem cells (ESCs), or technicalities in obtainment and usage of adult stem cells (ASCs). Thus, the discovery of induced pluripotent stem cells (iPSCs) allows for the use of ASCs that are given the pluripotent characteristics of ESCs. In 2018, Kooreman and his colleagues from Stanford University coaxed iPSCs to display the epitopes of breast cancer. After exposing mice with breast cancer to iPSCs, 70% of the mice had a decreased tumour size compared to control mice. Thus, iPSCs may work as a vaccine for cancer and potentially treat and cure the disease. Further research is required to study the feasibility of the use of iPSCs for human breast cancer.

**Keywords:** iPSCs, breast cancer, vaccine, adaptive immunity

When one thinks of a cure for cancer, one tends to imagine a miraculous medication discovered in an exotic rainforest or a unique concoction invented by a genius. What if a part of the answer was not as far as one tends to perceive it to be; what if the path towards a cure was right under one's nose in the form of stem cells? In order to harness the full potential of these cells, it is beneficial to first understand how cancer cells develop into a tumour.

Tumours are initiated by a genetic mutation that allows for the development of abnormal cells and rapid rates of cell division.<sup>1</sup> A mutation can occur randomly or can be induced by radiation or carcinogens that damage DNA.<sup>1</sup> Other carcinogens can be tumour promoters, like phorbol esters, which increase the rate of cell proliferation by activating proteins such as protein kinase C.<sup>1</sup> A tumour progresses as more mutations accumulate in the population of tumour cells. Some of these mutations may provide the selective advantage of faster growth to the cell and a possibility to invade nearby tissues.<sup>1</sup> From this, variants of a tumour are

grown which result in a transition from a benign to a malignant tumour, as the cells confined to its original location begin to invade surrounding tissue, representative of cancer.<sup>1</sup>

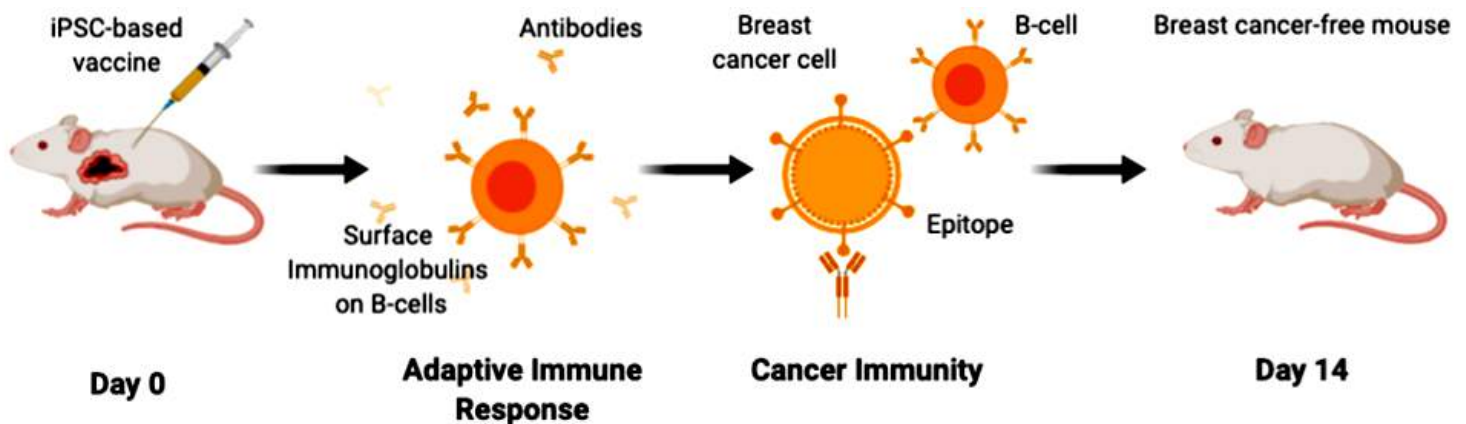
Currently, chemotherapy and high doses of radiation therapy are used to target and eliminate cancerous cells.<sup>2</sup> A drawback of this treatment method is that some of the non-cancerous but rapidly dividing healthy cells are also eradicated in the treatment process. As such, both of these therapeutic options seem to be less desirable, especially chemotherapy, as the medicine may be ingested or administered intravenously and thus, has the potential to affect any cell in the body. For recovery from the loss of healthy cells in the body due to aforementioned treatments, stem cells are injected into a vein, allowing them to implant in the bone marrow to form blood cells and platelets.<sup>2</sup> Most stem cells are taken from the patient's bone marrow as these cells have a lower likelihood of being rejected by the body after transplantation.<sup>3</sup> Unlike embryonic stem cells (ESCs), adult stem cells (ASCs) are

harder to isolate due to their scarcity and poor differentiation capacity.<sup>3</sup> The lack of pluripotency of ASCs means that they cannot differentiate into multiple cell types. Although ESCs have more technical advantages than ASCs, they are not used due to the ethical concerns involved, such as the destruction of a potential human life and the infliction of pain to the embryo during this process. As such, ASCs are usually the default cells of choice.

To continue using ASCs while harvesting the advantages of ESCs, induced pluripotent stem cells (iPSCs) can be used for cancer treatment. iPSCs are formed through somatic cell nuclear transfer, in which three transcription factors that allow ESCs to be pluripotent are introduced to the nucleus of ASCs.<sup>4</sup> iPSCs are useful as they can be used to battle cancer.

control group. Furthermore, two of the treated mice completely rejected breast cancer cells.<sup>5</sup> Hence, it appears that iPSCs have a substantial likelihood of being able to prevent breast cancer in humans.

Overall, a cure for cancer may be closer than anticipated, as treatment techniques are currently exploring the use of induced pluripotent stem cells, reaping the benefits of both adult and embryonic stem cell characteristics. If so far, the use of these cells can evolve from controlling the repercussion of cancer to reducing its growth, it is only natural to wonder whether future developments in stem cell research hold the key to a cure for cancer. Future research should be directed towards determining whether it is feasible to use iPSCs for treating human breast cancer through the possibility of eventually administering clinical trials.



**Figure 1. Overview of iPSC-mediated cancer treatment.** The mice were injected with an iPSC-based vaccine. T-cell-activated B-cells from the immune system were exposed to the epitopes on iPSCs. This allowed them to recognize the same protein on cancer cells and reduce cancer growth over 14 days via memory-based mechanisms of adaptive immunity.<sup>5</sup> Figure created using BioRender.

Using appropriate laboratory techniques, iPSCs can form a teratoma, which is a tumour composed of different cell types.<sup>5</sup> As such, both iPSCs and cancer cells have the same protein on their surface, termed epitopes.<sup>5</sup> These two characteristics of iPSCs can be used to develop a vaccine that will train the immune system to fight against cancerous cells.

Using this information, Kooreman and his colleagues from Stanford University used iPSCs to train the immune system of mice to attack tumours. This was done by introducing breast cancer cells in mice after the injection of iPSCs and an immune-stimulating agent called adjuvants (refer to Figure 1).<sup>5</sup> iPSCs work as vaccines by exposing the adaptive immune system to antigens so that the body can prepare antibodies without the concomitant effect of the cancer cells. This leads to memory formation through effector B-cell and T-cell responses, which allows for the recognition of the antigen almost immediately upon exposure to a true cancer cell.<sup>5</sup> After experimenting on mice, it was found that cancer in seven of the ten mice in the treatment group decreased in size when compared to the

## ACKNOWLEDGEMENTS

This work did not receive funding. There are no conflicts of interests.

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# Prevention of Dengue Virus through Citizen Science

## ARTICLE INFORMATION

Received: 16 October 2019  
Accepted: 15 November 2019  
Published: 29 November 2019

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## ABSTRACT

Dengue virus, a mosquito-borne viral infection native to many subtropical regions, is transmitted from *Aedes aegypti* and *Aedes albopictus* mosquitoes to human hosts, resulting in debilitating symptoms for the affected persons.<sup>1</sup> Common vector grounds for these female mosquitoes include uncovered water storage containers and garbage disposal units that are commonly observed amongst endemic regions.<sup>1,2,5</sup> The virus symptomatology resembles the flu. However, unlike the flu, there is little knowledge about the infectious mechanism utilized by the virus. As a result, opportunities for drug discovery, effective prevention, and management strategies to tackle the infection are hindered. This paper aims to embark on a discussion regarding the lack of awareness and prevention of the dissemination of this virus. Furthermore, it will discuss a citizen science approach to address the public health burden imposed by the Dengue virus and outline suggested improvements in disease control strategies.

**Keywords:** Vector transmittance, Dengue, epidemiology, citizen science

Despite western medical breakthroughs, many third world countries are still victim to evolving superbugs and lack access to medical treatment. Thus, plagues such as Dengue fever continue to tyrannize various countries. Dengue is a mosquito-borne viral infection responsible for many historic and current epidemics, such as those in South East Asia, and more specifically India, Pakistan, and Bangladesh.<sup>1</sup> As many as 40% of individuals across 128 countries are at risk for acquiring the infection, with nearly 400 million cases reported each year.<sup>1,2</sup> Dengue thrives in tropical and subtropical regions, particularly in urbanized areas, within these 128 countries.<sup>1</sup>

Dengue virus is a vector-borne disease, in which the *Aedes aegypti* and *Aedes albopictus* female mosquitoes act as vectors, or carriers, of the virus and transmit the virus to animals and humans.<sup>1</sup> Mosquitoes may transmit one of the four strains of the virus: DEN-1, DEN-2, DEN-3, or DEN-4, into the bloodstream of individuals upon feeding.<sup>1</sup> This results in severe flu-like symptoms that often progress to severe Dengue fever and death if left untreated.<sup>1,2</sup> Mortality rates upon contracting the viral infection can be upward of 20%, but upon medical intervention, drop to less than 1%.<sup>2</sup> Current medical interventions include the admin-

istration of the tetravalent Dengue virus vaccine, which was developed in 2015 using the Yellow Fever backbone and recommended by the World Health Organization.<sup>3</sup> However, follow-up studies and surveillance reported an increase in Dengue virus prevalence amongst seronegative individuals—those who received the vaccine and had no previous history of Dengue infection.<sup>3</sup> As a result, the vaccine is only beneficial to those who had the virus in the past, reducing their risk of Dengue by 93%, and omits a large portion of the seronegative population that will remain susceptible to the virus. Thus, future drug development would include designing an ideal tetravalent vaccine with Dengue virus (DENV) wild-type structural and non-structural proteins to build immunity amongst the four variable strains of the virus.<sup>3</sup>

Although current pharmaceutical companies have yet to design an effective antiviral treatment, current options include safe water practices, symptom management through careful observation, and intravenous hydration therapy for those with substantial vascular leakage, the permeability of blood vessels to harmful pathogens.<sup>4,5</sup> There is a lack of knowledge surrounding the disease pathogenesis which hinders drug discovery. However, this inadequacy in understanding the

burden of this disease is due to the lack of education and awareness of the virus.<sup>1</sup> Modern medicine, vaccines, and public awareness campaigns have decreased the total global incidence of Dengue infection by 28%, however, it is still the leading cause of death and illness in numerous Asian and Latin-American countries. Thus, is this decrease in global incidence attributed to a large decrease in western countries?<sup>2,1,2,4</sup>

In 2015, Jeelani et al. discussed the lack of epidemiological knowledge about the DENV amongst various classes and educational levels of residents of Puducherry, South India. Puducherry consists of a large population of individuals who have little knowledge about Dengue pathogenesis.<sup>5</sup> The paper indicates that increased population density, urbanization, lack of vector control, improper water storage, stagnant water bodies, and improper water treatment techniques are major contributing factors to the spread of DENV in subtropical regions. In addition, DENV symptoms of fever and myalgia are often misdiagnosed as symptoms of other common diseases, such as typhoid and influenza.<sup>1,2,5</sup>

It appears that the lack of knowledge in South East Asian countries pertaining to this viral infection is hindering its eradication. Given this, implementing a citizen science monitoring study could increase DENV education amongst citizens, such as those of Puducherry. Citizen science uses a unique approach to data collection where data is analyzed and interpreted by scientists and community volunteers. When the citizen science approach is carried out with rigour and integrity, it has the potential to produce valuable scientific data that can be applied to solve problems.<sup>6</sup>

A Dengue reporting system following the citizen science approach can be beneficial in collecting data to further research and understand the epidemiology of the disease, through creating an effective vector control system. Based on the information presented, the following is an example of a possible effective citizen science approach. Regions within South East Asia that are heavily affected or at risk, such as India, Pakistan, and Bangladesh, will be the targets of this experiment.<sup>1</sup> In this citizen science approach, water will be the sole measure for larvae testing as it is most convenient for citizens to obtain water samples and mosquitoes prefer to breed in stagnant water. Although larvae presence in a water sample may indicate an increased risk of Dengue fever, it may not be the sole contributor to one contracting the virus.<sup>1,2,5</sup> This citizen science experiment will commence with each participating household being provided with a water collection kit consisting of four 50 mL sterile test tubes and latex gloves. Citizens will be asked to submerge each sterile test tube in their household drinking, daily usage and community water supplies, and local sewer, separately. Next, citizens will be asked to deliver all four samples to a designated local community location. Sam-

ples will be collected on a weekly basis at the predetermined location and then delivered to a local Primary Health Centre to test for Dengue larvae. According to Jeelani et al. (2015), Puducherry citizens received information regarding DENV through televised programs, radio shows, and local newspapers.<sup>5</sup> Therefore, results from the science monitoring experiment should be delivered through television, radio, and newspapers to maximize outreach. This particular method promotes a deeper understanding of Dengue epidemiology, transmittance, and prevention of this infection. Subsequently, the citizen science approach allows citizen scientists to make a personal investment in the study and apply the recommendations that may come from this study to their households. As a result, this approach will aim to spread newfound knowledge amongst households, clarify misconceptions, introduce safer household water storage and garbage disposal practices, and decrease DENV rates in targeted geographical locations.

The Dengue virus has the potential to be eradicated, even though current solutions in place to eradicate Dengue are falling short. It is vital to broaden the way in which we think about the problem and how it has been previously addressed. With this in mind, a citizen science approach towards improving education and prevention strategies will be impactful, as it directly incorporates the residents of the high-risk population as a central resource to the solution.

## ACKNOWLEDGEMENTS

The completion of this paper could not have been possible without the assistance of Jawaria Karim and Hamza Furmli. Their knowledge, advice, endless support, and understanding spirit throughout the writing and citizen science project development process were greatly appreciated. Their timely advice and attention to detail played a big role in the completion of the final piece throughout my study period.

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## Is Complementary and Alternative Medicine a Legitimate Treatment for Infertility Today?

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### ARTICLE INFORMATION

Received: 15 October 2019

Accepted: 14 November 2019

Published: 29 November 2019

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Complementary and alternative medicine (CAM) has become more common for the treatment of infertility in recent years. Common alternative methods for treating infertility include the use of acupuncture and herbal supplements.<sup>1</sup> Acupuncture involves the insertion of thin needles into the body, whereas herbal medicine uses medicinal plants as the basis for treatment.<sup>1</sup> It has become common to see references to unconventional reproductive health practices in popular culture. There has also been a rise in the use of websites such as goop.com, which recommend novel methods to boost reproductive health to thousands of people. Goop corporation has been repeatedly criticized for giving advice that is not rooted in science. This leads people to accuse Goop of exploiting vulnerable women by promoting products that claim to improve their reproductive health but may not actually work. The ultimate question is, are these alternative practices evidence-based, or are they simply being used because they are a cheaper alternative to the expensive fertility treatments modern medicine has to offer?

There have been very few studies on the efficacy of CAM and the limited data available is inconclusive. A study by Boivin et al. (2009) investigated the link between CAM and pregnancy outcomes and found that CAM does not mitigate infertility in patients.<sup>1</sup> In this study, the pregnancy rates, as well as various lifestyle factors of CAM users and non-users were recorded during a 12-month period.<sup>1</sup> The use of herbal medicines, acupuncture, and reflexology, or application of pressure to the feet and hands, was associated with a 30% lower pregnancy rate that could not be explained by external factors such as lifestyle or lower probabili-

### ABSTRACT

With a rise in the use of alternative medicine, such as acupuncture, reflexology, and herbal remedies to treat infertility, it is important to consider if such treatment regimens are rooted in evidence. This piece provides a brief overview of the literature on the efficacy of common forms of alternative methods to treat infertility and concludes by suggesting potential reasons for its increased use.

**Keywords:** Alternative medicine, infertility, acupuncture, herbal medicine

ties of conceiving a child due to reproductive health issues.<sup>1</sup> Conversely, the findings of a study by Shahin et al. (2009) lend support to CAM's effectiveness in treating infertility.<sup>2</sup> The researchers investigated the impact of herbal supplements on the menstrual cycle and found better outcomes in individuals using herbs. Healthy menstrual cycle characteristics tend to improve the probability of conceiving a child, therefore, investigation of the link between the use of CAM and the menstrual cycle is of high relevance to the potential of conception. The herbs used in this study were derived from phytoestrogens, which are compounds found in various plants and fungi.<sup>2</sup> In this study, 134 women were randomly assigned to either the herb supplement group or the control group and their menstrual characteristics, such as regularity of cycle, health and thickness of uterine tissues, were measured and analyzed.<sup>2</sup> The results indicated that women in the herbal supplement group experienced significantly improved menstrual cycle characteristics, while those in the control group did not. However, pregnancy rates among these groups were not significantly different.<sup>2</sup> Another study by Rubin et al. (2015) aimed to assess the effect of CAM on outcomes of *in vitro* fertilization (IVF)— a method of assisted reproduction that involves the combining of eggs and sperm in a laboratory to form embryos, which are later implanted into the uterus.<sup>3</sup> In this study, patients were assigned to one of three conditions: a control of IVF with no additional treatment, IVF and acupuncture, or IVF and herbal treatment.<sup>4</sup> Patient records were reviewed to compare the effects of these three conditions on IVF outcomes. It was found that patients who received acupuncture or herbal medicine in conjunction with IVF had significantly greater chances of giving birth to a healthy, liv-



ing baby compared to the control group.<sup>4</sup>

Based on these studies alone, one cannot definitively conclude the impact of complementary and alternative medicine on fertility. Therefore, alternative medicine cannot be considered a legitimate treatment for infertility at this stage. While Shahin et al. and Rubin et al. provide support for the benefits of such therapies, they also conclude that more research is needed in this field. When these methods are promoted in everyday settings, they are typically discussed in the context of anecdotes, rather than rigorous empirical data. Not only is the research in this field severely limited, but a large majority of the studies on alternative medicine are in the form of individual case studies or do not employ the use of standard research practices, such as the use of a control group and randomized assignment of participants. The lack of conclusive research in this field would suggest that couples are not turning to alternative methods because of their scientific legitimacy. Therefore, the question of why these types of treatments are being used more frequently is still unanswered. This presents the need to consider other factors that are driving people towards these methods. One significant factor may be the high cost of modern medical treatments which makes them inaccessible to the working class. For instance, the cost of IVF can range from \$15,000 USD to \$40,000 USD per cycle, and this does not include the cost of vital medications that must be taken in conjunction with this treatment.<sup>5</sup> People who cannot afford these treatments could be turning to alternative methods, such as herbal remedies or acupuncture, as a cheaper alternative. Ultimately, it is more likely that women are using alternative medicine to treat their infertility because of the financial benefit, and not due to the underlying, but limited, scientific evidence.

## ACKNOWLEDGEMENTS

This work did not receive funding. There are no conflicts of interests.

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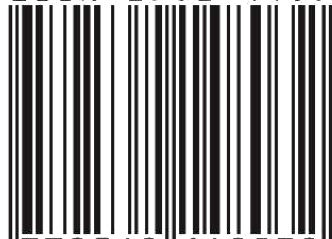
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ISSN 2562-1483



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