Metacognitive Argument-Driven Inquiry in Teaching Antimicrobial Resistance: Effects on Students’ Conceptual Understanding and Argumentation Skills

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ABSTRACT
Developing and communicating evidence-based explanations are regarded as essential skills in 21st-century learning. These skills are central to the process of scientific argumentation. Hence, teachers must adhere to a student-centered pedagogy that cultivates students’ understanding and argumentation skills. This study investigated the effects of the Metacognitive Argument-Driven Inquiry (MADI) approach in promoting students’ conceptual understanding of Antimicrobial Resistance and scientific argumentation skills. The study employed a mixed-method approach, which involved both quantitative and qualitative data. The participants were third-year Biological Science Education students (n=23) in a public university in Central Luzon, the Philippines. Quantitative data were obtained from the validated 30-item conceptual understanding test and six-point teacher-made written argumentation skills test, administered before and after students’ four-week exposure to the MADI approach. Qualitative data from video-recorded sessions and focus group discussions were used to substantiate the quantitative findings. Descriptive and inferential statistics were utilized to determine if there were significant improvements at the end of the study. Results showed that students’ conceptual understanding and argumentation skills significantly improved after exposure to the MADI approach with large effect sizes. Students’ development of argumentation skills was evident during the implementation of the study, as seen in their increasing mean scores in each activity. Moreover, students signified the efficacy of the MADI approach in facilitating substantial improvements in their conceptual understanding and argumentation skills. Thus, it is suggested for Biology teachers to integrate the MADI approach in delivering their lessons and designing inquiry-based activities to support students’ development of understanding and argumentation skills.

Introduction

The development of students’ scientific literacy has become the primary aim of science education (American Association for the Advancement of Science, 1993; Department of Education, 2016). Students are expected to demonstrate scientific knowledge, inquiry skills, and attitudes to become informed and participative citizens in society. Scientific literacy also necessitates students to tackle relevant socio-scientific issues, entailing the skills in explaining phenomena, evaluating, and interpreting data and evidence (Organisation for Economic Co-operation and Development, 2013),
which are central to the process of scientific argumentation. Hence, meaningful opportunities for the cultivation of scientific understanding and argumentation skills have to be afforded to the students. It is a significant task for teachers to make scientific concepts more authentic and reflective of the actual practices of science that may lead to students’ development of conceptual understanding and argumentation skills.

**Scientific Argumentation in Science Teaching**

Argumentation is described as a rational and logical discursive process that aims at establishing relationships between ideas and evidence (Duschl, Scweingruber, & Shouse, 2007). Essential to this process, students generate evidence-based explanations, critique each other’s explanations, and improve their explanations (Sengul, 2019). It offers avenues to view science as a dynamic process in which ideas are investigated, questioned, and often changed or revised (Diehl, 2000). It affords students with opportunities to integrate their existing scientific knowledge and develop a novel understanding based on the ideas of others (Brown & Campione, 1998, as cited in Cross, Taasoobshirazi, Hendricks, & Hickey, 2008). It is a critical thinking skill and a key component of scientific literacy that enables students to make informed decisions about personal and relevant issues (Cavagnetto, 2010; Llewellyn, 2013).

Numerous studies have established the significance of scientific argumentation in promoting scientific literacy and improving the teaching and learning of science. It was found vital in increasing students’ engagement (Sengul, 2009), developing students’ critical thinking skills, promoting the spirit of inquiry, inducing conceptual change, and enhancing academic performance (Driver, Newton, & Osborne, 2000; Foutz, 2018). For example, in the context of a socio-scientific issue, Dawson and Carson (2018) investigated the effects of argumentation on students’ understanding of climate change and their ability to construct arguments. The results of the study showed improvements in students’ understanding and argumentation skills. Students also became aware of the importance of justifying claims with scientific evidence. In Chemistry learning, on the other hand, Cetin (2014) found out that argumentation assisted students towards the significantly better acquisition of scientific reaction rate-related concepts, which also led to the improvements in the structure and complexity of their arguments. Similarly, Kaya (2013) reported the efficacy of argumentation practices on students’ understanding of chemical equilibrium and argumentation ability. The results further revealed that students exposed to argumentative practices could develop more quality arguments than those who received conventional instruction. Correspondingly, Songsil, Pongsophon, Boonsoong, & Clarke (2019) concluded that students exposed to the adapted ADI approach had significantly higher scientific argumentation skills than students in the traditional group.

Considering the positive impacts of incorporating scientific argumentation in teaching and learning, designing an effective learning environment that promotes a culture of scientific argumentation, however, remains a challenge for science teachers. This is because scientific argumentation is often neglected and not usually integrated into the delivery of science instruction (Driver et al., 2000; Erduran, Simon & Osborne, 2004), which may be due to teachers’ inadequate pedagogical skills in implementing argumentation practices within the classroom (Driver, Newton, and Osborne, as cited by Hsu, Mukhopadhyay & Al-Ararah, 2020). Hence, most science classrooms become teacher-directed, which hinders the development of students’ argumentation skills (Songsil et al., 2019; Zohar & Nemet, 2002). This is true to the researchers’ observations in which most science classrooms, especially in tertiary classes, mainly rely on the transmission of a set of known facts or theories, hampering students’ critical thinking skills (Cross et al., 2008).

The Programme for International Student Assessment (PISA) results in 2018 revealed the unsatisfactory performance of the Philippines in science. The majority of Filipino students had performed below par in the science literacy test and below the levels of most international students (OECD, 2019). Taking this into account, science teachers ought to revamp their instructional practices by implementing student-centered pedagogies anchored on scientific argumentation. This is hoped to simultaneously improve the teaching and learning of science and create opportunities for students to develop their understanding and argumentation skills. In this present study, an innovative pedagogical
approach-- driven by the synergy of scientific argumentation and inquiry-based learning within a metacognitive learning environment, is adapted.

Argument-Driven Inquiry

Scientific inquiry is a way of asking questions and investigating natural phenomena. This necessitates the essential skills of observing, measuring, designing and conducting investigations through experimentations, employing different strategies to obtain information, and communicating results (Trautmann, MaKinster, & Avery, 2004). Inspired by inquiry-based learning, the Argument-Driven Inquiry (ADI) approach, developed by Sampson, Grooms, and Walker (2009), aims to provide students with learning experiences that mimic the actual practices of science through scientific argumentation and inquiry-based learning. The original iteration of this ADI pedagogical approach provides students with opportunities to carry out investigations, collect and analyze data, communicate ideas with others through argumentation sessions, write investigation reports, and engage in peer review during a laboratory investigation. It is theoretically based on the social constructivist theory of learning (Sampson & Walker, 2012), highlighting that learning stems from students' social interactions and between students and knowledgeable adults (Vygotsky, 1978).

In the literature, the potential use of ADI was found to improve students' conceptual understanding (Celep, 2015; Celik & Kilic, 2014; Enderle, Grooms, & Sampson, 2013; Ping, Halim, & Osman, 2020; Myers, 2015) and argumentation skills (Cetin & Eymur, 2017; Fadillah & Deta, 2020; Kadayifci & Celik, 2016; Myers, 2015; Ping et al., 2020). In addition, some studies reported the effectiveness of ADI in developing students’ academic achievement (Demircioglu & Ucar, 2015) and scientific process skills (Eymur, 2018; Kadayifci & Celik, 2016; Ping et al., 2020). Studies also explored its positive effects on students’ perceptions of their own inquiry skills or self-efficacy (Eymur, 2018), attitudes towards science (Celik & Kilic, 2014; Walker, Sampson, Grooms, Anderson, & Zimmerman, 2012), and engagement in science (Myers, 2015). For instance, in the study of Sampson et al. (2013), the impact of ADI on students’ science proficiency over traditional laboratory instruction was investigated. Although both groups of students exposed to ADI and traditional laboratory instruction made substantial gains concerning their content knowledge, only the students exposed to ADI made significant gains with respect to their scientific writing abilities and understanding of the development and nature of scientific knowledge. Further, larger effect sizes are reported in science proficiency aspects compared to students exposed to traditional laboratory instruction.

Metacognition and Argument-Driven Inquiry

While implementing an inquiry-based pedagogy like ADI may promote students’ understanding and argumentation skills, metacognitive opportunities have to be provided to students to ensure effective and successful learning transfer (Seraphin, Philippoff, Kaupp & Vallin, 2012). Metacognition is defined as one’s awareness of knowledge and thought processes regarding learning (Kaberman & Dori, 2009). It is seen as a significant element of any inquiry process, for it leads students to more fully functional processes that assist them in their learning (van Opstal & Daubenmire, 2017).

In performing ADI-based activities, where the teacher provides students with an open-ended problem, students need to be metacognitive to actively control cognitive processes (Kaberman & Dori, 2009). This can be achieved by utilizing metacognitive regulation strategies such as planning, monitoring, and evaluating (Schraw, 1998). Students also need to be supported in defining and setting their goals, monitoring, and evaluating their progress in achieving them. Moreover, they must be able to activate their prior knowledge, examine their current thinking, identify their confusion, recognize conceptual change, and reflect on their learning and experience, which are deemed vital in assisting them toward meaningful learning (Tanner, 2012). In an inquiry-based learning environment like ADI, the teacher-researchers believe that a metacognitive environment can help students to take control of
their own learning by defining learning goals and monitoring their progress in achieving them (Buonicristiani & Buonicristiani, 2012).

**Antimicrobial Resistance**

One of the relevant topics nowadays in the study of Biology is Antimicrobial Resistance. The emergence and spread of Antimicrobial Resistance among pathogenic bacteria have been a growing problem for public health in recent decades. In fact, the World Health Organization [WHO]'s Report in 2007 highlighted the issue of Antimicrobial Resistance as one of the major threats to public health in this century. This phenomenon happens when microorganisms, mainly bacteria, resist the actions of antibiotics. Consequently, medicines become ineffective, and infections persist in the body.

According to the WHO's Global Action Plan towards combating Antimicrobial Resistance, it is encouraged that school curricula should include the use of antimicrobial agents and resistance to promote a better understanding and awareness through effective communication, education, and training (WHO, 2015). In response to this, there are some studies (Fonseca, Santos, Costa, Lencastre, & Tavares, 2012; Friedrichsen, Sadler, Graham, & Brown, 2016; Valente et al., 2009) that tackled about Antimicrobial Resistance. For instance, Friedrichsen et al. (2016) developed a socio-scientific issue-based unit in high school Biology about Antimicrobial Resistance. Additionally, Fonseca et al. (2012) conducted an interventional program to promote awareness about Antimicrobial Resistance at the high school level, while Valente et al. (2009) developed a game that integrates basic bacteriology and the skills concerning antimicrobial agents.

Despite these efforts, there is still a dearth in the literature concerning the integration of this socio-scientific issue and its underlying biological concepts in teaching and learning towards students' improved conceptual understanding. Meanwhile, the potential of ADI has not yet been explored in much detail in higher education, particularly in Biology. Moreover, to the best of teacher-researchers' knowledge, no study has attempted to explore ADI within a metacognitive environment.

Further, the abstract nature of biological concepts, students' misconceptions about microorganisms (Jones & Rua, 2008; Milandri, 2004; Fonseca et al. 2012), and their difficulties in transitioning between micro-and macro-levels of conceptualization (Tibell & Rundgen, 2010) have been the challenges in teaching biological concepts, in which the topic of Antimicrobial Resistance is no exception. Considering this, teachers must be able to enhance instructional delivery that will facilitate and support students' learning. It is for this contention that the teacher-researchers believe that the teaching and learning of Antimicrobial Resistance is a good context to integrate metacognition and ADI. Hence, this study investigates the effectiveness of the Metacognitive Argument-Driven Inquiry (MADI) approach in enhancing students' conceptual understanding of Antimicrobial Resistance and argumentation skills. Specifically, this study sought to answer the following research questions:

1. Is there a significant change in students' conceptual understanding before and after exposure to the MADI approach?
2. Is there a significant change in students' argumentation skills before and after exposure to the MADI approach?
3. What are the changes in students' argumentation skills before, during, and after exposure to the MADI approach?

**Methodology**

**Research Design**

The study employed a mixed-method design, where both quantitative and qualitative data were collected and analyzed to determine the efficacy of the MADI approach in developing students' conceptual understanding and argumentation skills. The Conceptual Understanding Test on Microbial Genetics with a content focus on Antimicrobial Resistance (CTAMR) and Argumentation Skills Test (AST) were applied as pretest and posttest to examine the changes in students' conceptual
understanding and argumentation skills. Students' scientific arguments in each MADI-based activity were also examined. Additionally, students’ perceptions on the effects of the MADI approach through focus group discussions were collected and analyzed to support the quantitative findings.

Research Context and Participants

The study involved a group of third-year Biological Science Education students (n=23) enrolled in a public university in the province of Bulacan, in Central Luzon, the Philippines. These participants were enrolled in the General Microbiology course during the second semester of the academic year 2018-2019. The majority of these students were female with a frequency of 17 (73.91%). In terms of age, the majority of the students are within the age range of 21-22 (30.43%), followed by 19-20 (21.74%), 23-24 (17.39%), 25-26, and 29-30 (13.04%), and 27-28 (4.38%).

Research Instrument

MADI-based Unit Plan on Microbial Genetics

The teacher-researchers developed a MADI-based unit plan on Microbial Genetics with a content focus on Antimicrobial Resistance. The contents of the lesson plans included in the unit were based on the learning competencies of the course syllabus as stipulated by the Commission on Higher Education in General Microbiology, particularly in the Microbial Genetics unit. Hence, the following topics were the scope of the study: 1) Structure and Function of the Genetic Material, 2) DNA Replication, 3) Protein Synthesis, 4) Gene Regulation, 5) Mutation, and 6) Antimicrobial Resistance. The lessons on these topics were contextualized towards developing students’ understanding of Antimicrobial Resistance.

The six lesson plans included in the unit were structured using the 7E instructional model (Eisenkraft, 2003). Specifically, each lesson plan was designed within a metacognitive environment that consisted of an argument-driven inquiry-based activity, which required students to conduct an investigation to generate a scientific argument that addresses the guiding question posed in the activity. An example of the MADI-based activity is given in Appendix A.

Three expert-evaluators in Biology Education and one expert on metacognition were requested to examine the content validity of the unit plan. The comments and feedback of the expert-evaluators were incorporated towards the refinement of the unit plan.

Conceptual Understanding Test on Antimicrobial Resistance (CTAMR)

The teacher-researchers developed a Conceptual Understanding Test on Microbial Genetics with a content focus on Antimicrobial Resistance (CTAMR). It was administered to the students before and after their exposure to the MADI approach. Specifically, the CTAMR was a 30-item multiple-choice test consisting of questions with four options that were constructed based on the topics included in the unit. The items were classified under Bloom’s Taxonomy of Cognitive Domain as shown in Appendix B. In terms of scoring, one point was given for each question answered correctly and zero points for each question answered incorrectly. The maximum score for the test is 30 and the minimum score is 0.

Three expert-evaluators in Biology Education were requested to evaluate the face and content validity of the test using the evaluation checklist of Morales (2003). The checklist used a 5-point Likert evaluation scale consisting of 20 statements that reflected the characteristics of a good and valid test. The teacher-researchers then incorporated the comments and feedback of the expert-evaluators in improving the CTAMR. An acceptable Cronbach’s alpha value of 0.939 was calculated in terms of the reliability of the instrument. Table 1 shows the sample multiple-choice questions in the CTAMR.
### Table 1

**Sample Multiple-Choice Questions in the CTAMR**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Item Placement</th>
<th>Number of Items</th>
<th>Sample Question</th>
</tr>
</thead>
</table>
| Structure and Function of the Genetic Material | 1, 2, 3, 4     | 4               | 1. Decapribonucleic acid (DNA) is a type of nucleic acid that contains the genetic information for the development and function of living things. In the case of bacteria, one of these genetically encoded information may be genes that confer resistance to antibiotics. Which of the following figures below is a CORRECT representation of a DNA molecule of bacteria? (a) ![Image](a.png)  
   (b) ![Image](b.png)  
   (c) ![Image](c.png)  
   (d) ![Image](d.png) |
| DNA Replication                      | 5, 6, 7, 8     | 4               | 5. Which of the following base pair sequences could be produced during DNA replication?  
   (a) 5' AGCTGT 3'  
        3' TCGTGA 5'  
   (b) 5' AGCTTG 3'  
        3' TCAGCT 5'  
   (c) 5' ACGTGT 3'  
        3' GCATGA 5'  
   (d) 5' AGCTGA 3'  
        3' UCAGUA 5' |
| Protein Synthesis                    | 9, 10, 11, 12, 13, 14, 15 | 8               | 12. Macrolide antibiotics are clinically important antibiotics for they are effective inhibitors of bacteria by means of binding to the S5S subunit in bacterial ribosomes. In which specific cellular process do macrolide antibiotics interfere with?  
   (a) DNA replication  
   (b) Translation  
   (c) Transcription  
   (d) Gene expression |
| Gene Regulation                      | 16, 17, 18, 19 | 3               | 19. Suppose you inoculate three flasks of minimal salt broth with E. coli. Flask A contains glucose. Flask B contains glucose and lactose. Flask C contains lactose. After a few hours of incubation, you test the flasks for the presence of β-galactosidase, a bacterial enzyme that splits lactose into glucose and galactose. Which flask(s) do you predict will have this enzyme?  
   (a) Flask A  
   (b) Flask B  
   (c) Flask C  
   (d) Both flasks B and C |
| Mutation                             | 20, 21, 22     | 3               | 22. In your study, you have found out that there is a mutation in the rpoB gene of *Mycobacterium tuberculosis* which resulted in an alteration of the structure of the binding site for antibiotic rifampin. Which of the following would happen as an effect of this mutation?  
   (a) Bacterium will be susceptible to antibiotic rifampin.  
   (b) Bacterium will be resistant to antibiotic rifampin.  
   (c) Replication will occur via RNA polymerase alone.  
   (d) Replication will require a DNA template from another source. |
| Antimicrobial Resistance             | 23, 24, 25, 26, 27, 28, 29, 30 | 8               | 26. How does natural selection affect the frequency of traits for antibiotic resistance in a bacterial population?  
   (a) Resistant bacteria survive antibiotic treatment and can increase in numbers  
   (b) Nonresistant bacteria survive antibiotic treatment and can increase in numbers  
   (c) Both resistant and nonresistant bacteria survive antibiotic treatment and can increase in numbers  
   (d) Natural selection does not have an effect on the frequency of traits for antibiotic resistance |

### Argumentation Skills Test (AST)

The teacher-researchers developed an Argumentation Skills Test (AST) to determine students’ argumentation skills prior to and after exposure to the MADI approach. The AST was adapted from nextgenscience.org (2014). The written test focused on the topic of Antimicrobial Resistance, which asked students to generate a scientific argument addressing the guiding question: “Does antibiotic
streptomycin affect the frequency of traits in a bacterial population over a span of time?” Three (3) expert-evaluators in Biology Education were requested to assess the face and content validity of the test. The teacher-researchers then considered the comments and suggestions of the expert-evaluators in improving the test.

In assessing the students’ arguments in the AST as well as in each MADI-based activity, the argumentation skills scoring rubric of McNeill and Krajcik (2011) was used. As shown in Table 2, the scoring rubric consisted of the three (3) components of a scientific argument: claim, evidence, and reasoning. Each component used a three-point range in which scores ranged from 0 to 2. The maximum score that can be obtained in each component is 2, while the minimum is 0. Overall, the highest score is a total of six (6) points.

Table 2

<table>
<thead>
<tr>
<th>Components of Scientific Argument</th>
<th>Score 0 point</th>
<th>Score 1 point</th>
<th>Score 2 points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claim</td>
<td>Does not make a claim, or makes an inaccurate claim</td>
<td>Makes an accurate but vague or incomplete claim</td>
<td>Makes an accurate and complete claim</td>
</tr>
<tr>
<td>Evidence</td>
<td>Does not provide evidence, or only provides inappropriate evidence (evidence does not support the claim)</td>
<td>Provides appropriate but insufficient evidence to support the claim. May include some inappropriate evidence</td>
<td>Provides appropriate and sufficient evidence to support the claim</td>
</tr>
<tr>
<td>Reasoning</td>
<td>Does not provide reasoning, or only provides reasoning that does not link evidence to claim</td>
<td>Repeats evidence and links it to some scientific principles, but not sufficient</td>
<td>Provides accurate and complete reasoning that links evidence to claim. Includes appropriate and sufficient scientific principles</td>
</tr>
<tr>
<td>Total Score</td>
<td>/6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. (McNeill & Krajcik, 2011)

**Focused Group Discussion**

After the implementation of the study, two (2) focus group discussions were conducted involving 2-3 representatives of each group in the class. These were done to collect qualitative data on their perceptions of the effects of the MADI approach on their conceptual understanding and argumentation skills. Semi-structured interview questions were prepared and asked to the students. The generated interview transcripts were analyzed using thematic analysis.

**Research Procedure**

Prior to the implementation of the study, permission was sought from the administration of the university where the study was conducted. Informed consent was also obtained from the students. The CTAMR and AST pretests on students’ conceptual understanding and argumentation skills were then administered. After this, an orientation session on the MADI approach was carried out using the argument-driven inquiry-based activity titled “Are viruses living or non-living?” of Sampson and Schleigh (2013). This was done to introduce and familiarize students with how an argument-driven inquiry-based activity is being performed.
In the implementation of the study, the following stages of the MADI approach were adapted. Table 3 summarizes a detailed description of each stage in the MADI approach.

Table 3
The Metacognitive Argument-Driven Inquiry (MADI) Approach

<table>
<thead>
<tr>
<th>7E Instructional Model (Eisenkraft, 2003)</th>
<th>Metacognitive Argument-Driven Inquiry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elicitation</td>
<td>The teacher provided students with metacognitive opportunities (Tanner, 2012) to examine their current thinking and existing knowledge through pre-assessments (e.g. KWL and IRF charts).</td>
</tr>
<tr>
<td>Engagement</td>
<td>The teacher captured students’ attention and stimulated students’ thinking by introducing the guiding question that they have to answer and investigate through the activity.</td>
</tr>
<tr>
<td>Exploration</td>
<td>The teacher guided students in performing their activity (e.g. web-based simulation) with the goal of generating a scientific argument that addresses the guiding question. In the conduct of the activity, the teacher asked students to plan their strategies and monitor their progress by answering the metacognitive prompts for planning and monitoring (Schraw, 1998), such as: “What is our goal?”, “What are the information and materials/resources we need to successfully complete this task?”, and “Are the strategies working well to complete the task within the given time frame? If not, what should we do?”.</td>
</tr>
<tr>
<td>Explanation</td>
<td>The teacher facilitated the argumentation sessions, where groups of students shared their initial arguments with other groups and critique others’ arguments using their argument boards. This was done using a round-robin format, wherein a member of the group stayed at their lab station to share their group’s initial argument while the other members of the group went to the other lab stations one at a time to listen to and critique the arguments developed by their classmates.</td>
</tr>
<tr>
<td>Elaboration</td>
<td>The teacher facilitated a reflective post-discussion of the topic or lesson covered in the activity. The teacher also clarified students’ understanding during the discussion.</td>
</tr>
<tr>
<td>Evaluation</td>
<td>The teacher guided students to write an argumentation report that articulated their final scientific arguments. Students were asked to reflect through metacognitive prompts for evaluation (Schraw, 1998) and retrospective post-assessment as to what they thought about a topic or concept before the session and what they think about it now. Examples of metacognitive prompts for evaluation include: “Have we reached our goal?”, “What worked?”, “What didn’t work?”, and “How should we do things differently next time?”.</td>
</tr>
<tr>
<td>Extension</td>
<td>The teacher tasked students to describe what they didn’t understand during class and what they think might help them through reflective writing. Students were also encouraged to connect what they are learning and how they are integrating the content into their current learning structures (Tanner, 2012).</td>
</tr>
</tbody>
</table>

The implementation of the MADI approach was carried out for 4-weeks, wherein two 3-hour class meetings per week were conducted. In each MADI lesson, students’ argumentation sessions were video-recorded. Sample students’ activities during the argumentation sessions are presented in Figure 1. After a four-week exposure to the pedagogical approach, the CTAMR and AST posttests were
administered to examine the changes in students’ conceptual understanding and argumentation skills. Moreover, students’ written arguments in each MADI-based activity were assessed. Two (2) focus group discussions were conducted involving 2-3 representatives of each group in the class. This was done to collect qualitative data on students’ perceptions of the effects of the MADI approach on the development of their conceptual understanding and argumentation skills.

**Figure 1**

(a) Students Performing an Inquiry-Based Investigation Through a Web-Based Simulation  
(b-c) Students Presenting Their Initial Arguments During the Argumentation Sessions  
(d) A sample of the Initial Argument Board During the Argumentation Session

Data Analysis

Appropriate descriptive and inferential statistical analyses were utilized to determine if there were significant changes in students’ conceptual understanding and argumentation skills at the end of the study. Since the study involved a small number of participants (n=23), the non-parametric Wilcoxon Signed Ranks Test was utilized to determine if the implementation of the MADI approach produced a significant difference in students' conceptual understanding and argumentation skills. Specifically, in the Wilcoxon Signed Rank Tests analysis, the z statistic results were converted into r effect size, which was obtained by dividing the z values by the square root of N or the total number of observations (Allen & Bennett, 2008; Clark-Carter, 2004).

In determining the magnitude of the effectiveness of the MADI approach, the result was then interpreted according to Cohen’s (1988) criteria: \( d = 0.10 \) as small effect, \( d = 0.30 \) as medium effect, and \( d = 0.50 \) as large effect size. Quantitative data were analyzed using the SPSS 23.0 software.
Students’ responses in the focus group discussions were transcribed and analyzed using thematic analysis. These qualitative findings were then corroborated to the quantitative data.

Results and Discussion

Change in Students’ Conceptual Understanding

Table 4

<table>
<thead>
<tr>
<th>Topics</th>
<th>Pretest</th>
<th>Posttest</th>
<th>z</th>
<th>Asymp. Sig.</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure and Function of the Genetic Material</td>
<td>2.304</td>
<td>2.913</td>
<td>-2.499</td>
<td>.013*</td>
<td>0.368</td>
</tr>
<tr>
<td>DNA Replication</td>
<td>2.391</td>
<td>2.739</td>
<td>-1.660</td>
<td>.097</td>
<td>0.245</td>
</tr>
<tr>
<td>Protein Synthesis</td>
<td>3.261</td>
<td>4.174</td>
<td>-2.348</td>
<td>.019*</td>
<td>0.346</td>
</tr>
<tr>
<td>Gene Regulation</td>
<td>0.652</td>
<td>2.130</td>
<td>-4.102</td>
<td>.000*</td>
<td>0.605</td>
</tr>
<tr>
<td>Mutation</td>
<td>1.435</td>
<td>1.348</td>
<td>-3.714</td>
<td>.000*</td>
<td>0.555</td>
</tr>
<tr>
<td>Antimicrobial Resistance</td>
<td>3.087</td>
<td>5.130</td>
<td>-3.673</td>
<td>.000*</td>
<td>0.542</td>
</tr>
<tr>
<td>Overall</td>
<td>13.130</td>
<td>18.348</td>
<td>-4.063</td>
<td>.000*</td>
<td>0.600</td>
</tr>
</tbody>
</table>

Note: No. of items = 30; *significant at α = 0.05; effect size (Cohen’s d) value 0.10 (small effect), 0.30 (medium effect), and 0.50 (large effect); a. Wilcoxon Signed Ranks Test; b. Based on negative ranks; c. Based on positive ranks.

The Wilcoxon Signed Ranks Test was utilized to determine if there is a significant difference in students’ conceptual understanding at the end of the study. As can be gleaned in Table 4, results revealed that there was a significant difference (z = -4.063, p<0.001) between the mean scores in the pretest and posttest. The magnitude of the impact of the MADI approach on students’ conceptual understanding resulted in an effect of size of r = 0.600, which indicated that the MADI approach had a large and positive effect on students’ conceptual understanding.

The result suggests that students’ improved understanding of the concepts of Antimicrobial Resistance was facilitated by the MADI-based activities, which highlighted the integration of scientific argumentation and inquiry-based learning within a metacognitive environment. These findings of the present study were consistent with the previous research (Celep, 2015; Celik & Kilic, 2014; Sampson et al., 2013; Ping et al., 2020; Myers, 2015), which supported the effectiveness of ADI in improving students’ understanding.

Table 5

<table>
<thead>
<tr>
<th>Main Theme</th>
<th>Sub-theme</th>
<th>Codes</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>REAT uwineoe</td>
<td>features of MADI approach</td>
<td>diversity of ideas/explanations</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>collaboration of ideas</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>peer tutoring</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mentoring</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>inquiry-based learning</td>
<td>discovery learning</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>student-centered</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>learning by oneself</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>comprehension</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>develops understanding</td>
<td>helpful in the preparation of exams</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>self-explain</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>knowledge retention</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>easy to recall and remember</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>requires one’s thinking</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>helpful in writing scientific</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>explanations</td>
<td></td>
</tr>
</tbody>
</table>
To substantiate the quantitative findings, the thematic analysis of the focus group discussion is presented in Table 5. The generated themes provide further evidence that the MADI approach facilitates the development of students’ conceptual understanding. Students signified that the use of the MADI approach in teaching and learning led to their improved conceptual understanding. The following are some verbatim responses of the students:

Student 3: “…the information/ideas we are getting from other groups during the argumentation session are really helpful. For example, in the claim… then, you are going to discover something different from the other groups. Our knowledge improved because we are linked to new ideas from other groups.”

Student 5: “The varied ideas during the argumentation session helped us a lot. Just like when you got the idea of others, you will get to know many things that you don’t already know.”

Student 13: “We constructed our own understanding using the MADI approach. On our other activities, we didn’t have any prior knowledge but because of the simulation, we were able to follow what was happening in the process.”

Student 14: “…[MADI] made it easy for me to remember all the concepts in case we will have an exam about it… that’s why if ever you didn’t review, just one keyword can help you remember the answer.”

In a four-week exposure to the MADI approach, students accomplished several MADI-based learning activities, which led to the conceptual gains of the students. These activities required them to make sense of the data/information from their inquiry-based investigation to generate an argument that addresses the guiding question posed in the activity. Through the argumentation sessions in the MADI approach, students had the opportunity to express their initial understanding of the topic and share it with their classmates. The collaborative sharing of ideas or dialogic interaction in the argumentation sessions helped them understand the concepts better (Asterhan & Schwarz, 2007; Venville & Dawson, 2010).

Apart from this, the significant change in students’ conceptual understanding can be associated with the metacognitive environment designed in the MADI approach. It helped them become actively engaged in the inquiry-based process using both metacognition and content knowledge (Seraphin et al., 2012). In the MADI approach, several metacognitive strategies were explicitly embedded in the lessons. These included metacognitive prompts for planning, monitoring, and evaluating. Students were also prompted to activate their prior knowledge, examine their current thinking, identify their confusion, recognize conceptual change, and reflect on their learning and experience through reflective writing.

### Change in Students’ Argumentation Skills

**Table 6**

<table>
<thead>
<tr>
<th>Components</th>
<th>Pretest</th>
<th>SD</th>
<th>Posttest</th>
<th>SD</th>
<th>Mean Difference</th>
<th>z</th>
<th>Asymp. Sig. (2-tailed)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claim</td>
<td>1.348</td>
<td>.885</td>
<td>2.000</td>
<td>.000</td>
<td>.652</td>
<td>-2.762b</td>
<td>.006*</td>
<td>.407</td>
</tr>
<tr>
<td>Evidence</td>
<td>1.304</td>
<td>.765</td>
<td>1.782</td>
<td>.421</td>
<td>.478</td>
<td>-2.517b</td>
<td>.012*</td>
<td>.371</td>
</tr>
<tr>
<td>Reasoning</td>
<td>0.435</td>
<td>.662</td>
<td>1.696</td>
<td>.559</td>
<td>1.261</td>
<td>-3.938b</td>
<td>.000*</td>
<td>.581</td>
</tr>
<tr>
<td>Overall</td>
<td>3.087</td>
<td>1.649</td>
<td>5.479</td>
<td>.790</td>
<td>2.391</td>
<td>-3.844b</td>
<td>.000*</td>
<td>.567</td>
</tr>
</tbody>
</table>

*Note: No. of items = 6; *significant at α = 0.05; effect size (Cohen’s d) value 0.10 (small effect), 0.30 (medium effect), and 0.50 (large effect); a. Wilcoxon Signed Ranks Test; b. Based on negative ranks.

The Wilcoxon Signed Ranks Test was utilized to determine if there is a significant improvement in students’ argumentation skills after exposure to the MADI approach. As seen in Table 6, the overall computed z value of -3.844 confirmed that the students did show significant improvement in their argumentation skills at the end of the study. The difference in pretest and posttest mean scores was highly significant at 0.05 level of significance such that the value 0.000 is less than 0.05. Also, it can be
noted that the MADI approach resulted in a large and positive effect size of $r = 0.567$. These results are suggestive of the efficacy of the MADI approach in improving students’ argumentation skills.

To support these findings, Figures 2 and 3 show the change in students’ argumentation skills before and after exposure to the MADI approach based on their written arguments. Figure 2a displays a pretest response of the student, in which she was able to make an accurate and complete claim in answering the guiding question. Although she supported her claim by providing appropriate and sufficient evidence, her argument lacked reasoning in which she might have included scientific concepts that would link her presented evidence to the claim. This pretest written argument of the student was rated with a total score of four (4) points.

**Figure 2**

(a-b) Sample Student’s Responses in the Argumentation Skills Test (Pretest)

---

**Figure 2a**

**Guiding Question:** Does antibiotic streptomycin affect the frequency of traits in a bacterial population over a span of time?

*Obviously, antibiotic streptomycin affects the frequency of traits in a bacterial population over a span of time. As the table shows, the different effect of antibiotic streptomycin in the frequency of variant X and variant Y in the population. We can notice that the frequency of variant X increased from generation to generation, while the frequency of variant Y decreased. From this data, we can say that the changes in frequency of both variant X and Y in the population shows that the exposure to antibiotic streptomycin do have affect the frequency of traits in bacterial population over a span of time.*

---

**Figure 2b**

**Guiding Question:** Does antibiotic streptomycin affect the frequency of traits in a bacterial population over a span of time?

*From the data that were collected from a study, it shows the comparison of the frequency of injection traits in bacteria over a period of time. From one more strain that generates different variation from a single strain, variant X and variant Y. It show that variant X is more dominant than variant Y. From the data on the frequency of variant X in generation Y, we see increase the population from the antibiotic streptomycin. Therefore, when it comes to the population of variant Y, it is not much affected from the antibiotic streptomycin or while the variant X has strong dominant traits.*
On the other hand, it can be noticed in Figure 2b that the student did not include a clear answer to the guiding question, hence lacking a claim in her argument. The expected claim in the guiding question was: Antibiotic streptomycin does affect the frequency of traits in a bacterial population over a span of time. Additionally, the evidence she presented were insufficient. She failed to account for the growth of Variant Y which decreased in the bacterial population. Also, she did not include any reasoning in her argument in which she might have supported her claim and evidence with underlying scientific concepts, particularly the susceptibility and resistance of bacteria to antibiotics. This written argument was rated with a total score of one (1) point.

Figure 3

Sample Student’s Response in the Argumentation Skills Test (Posttest)
Figures 3a and 3b capture substantial improvements in students’ argumentation skills based on their posttest responses. After exposure to the MADI approach, both students made high-quality arguments that consisted of an accurate and complete claim, appropriate and sufficient evidence, and appropriate and sufficient underlying scientific concepts or reasoning that link evidence to the claim. Both of these arguments obtained a score of 6 points.

Although the study did only focus on students’ generation of scientific arguments consisting of claim, evidence, and reasoning, the results of the present study corroborate the findings of a great deal of the previous works that have examined the effect of ADI on students’ argumentation skills (Çetin & Eymur, 2017; Fadillah & Deta, 2020; Kadayifci & Celik, 2016; Myers, 2015; Ping et al., 2020). These studies noted that students’ argumentation skills could be developed when they are exposed to a teaching and learning environment infused with scientific argumentation.

Aside from this, the improvement of students’ argumentation skills could be linked to the inquiry-based nature of ADI. Thoron and Myers (2012) argued that inquiry–based instruction can help promote students’ argumentation skills, mainly scientific reasoning. In the present study, students performed inquiry-based activities, which allowed them to craft scientific arguments that addressed the guiding questions posed in the activities. Besides, it can be seen that students’ ability to write scientific arguments seems to be linked to their understanding of the concepts. This supports the claim of Rudsberg, Ohman, and Ostman (2013) that improvements in argumentation skills and understanding occur simultaneously. This coincides with Demiral and Çepni (2018) who found out that students’ content knowledge could influence their argumentation skills.

Furthermore, the teacher-researchers monitored the development of argumentation skills of the students through the six (6) activities in the unit. Table 7 presents the mean scores in the written scientific arguments of the students in each activity. Similar to the AST, students’ written arguments in each activity were assessed according to the adapted scoring rubrics of McNeill and Krajcik (2011).

Table 7

<table>
<thead>
<tr>
<th>Topic</th>
<th>Guiding Question in the Activity</th>
<th>Description of the Task</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Genetic Material</td>
<td>What is the structure of the DNA?</td>
<td>Students analyzed the existing data in the DNA Fact Sheet that helped them develop a three-dimensional model of the structure of DNA (Sampson, 2014).</td>
<td>4.087</td>
<td>1.345</td>
</tr>
<tr>
<td>DNA Replication</td>
<td>Does DNA follow the conservative, semi-conservative, or dispersive model of replication?</td>
<td>Students analyzed and interpreted the data from the pulse-chase experiment of Meselson and Stahl to ascertain which model describes the process of DNA replication.</td>
<td>4.130</td>
<td>2.380</td>
</tr>
<tr>
<td>Protein Synthesis</td>
<td>How does the genetic information flow from DNA to proteins?</td>
<td>Students explored and investigated the processes of transcription and translation using a web-based simulation (<a href="http://lab.concord.org">http://lab.concord.org</a>).</td>
<td>4.609</td>
<td>2.271</td>
</tr>
<tr>
<td>Gene Regulation</td>
<td>How does <em>Escherichia coli</em> regulate the production of β-Galactosidase?</td>
<td>Students conducted an investigation using a web-based simulation to explore how gene expression is regulated at the levels of transcription and translation in bacteria (<a href="https://phet.colorado.edu/">https://phet.colorado.edu/</a>).</td>
<td>5.045</td>
<td>1.09</td>
</tr>
</tbody>
</table>
Mutation Does a variant rpoB gene sequence result in resistance to the antibiotic rifampin? Students used online bioinformatics tools to compare gene sequences and analyze if the mutation will affect the structure and function of the protein (Taylor, Davidson, & Strong, 2014).

Antimicrobial Resistance Are all bacteria susceptible to antibiotics? Students performed a Kirby-Bauer disk diffusion lab susceptibility test to investigate the susceptibility or resistance of the test bacteria to antibiotics.

5.045 1.463

5.227 1.412

Note: Highest score = 6 points

In Table 7, it is interesting to note that there was a clear trend of increasing mean scores at the end of the unit. This reflects that students’ argumentation skills developed as they progressed through the sequence of activities, gaining experience in developing scientific arguments with each successive activity. This gradual improvement could be associated with the argumentation session in the MADI approach. According to Mastro (2017), argumentation sessions specifically support students’ ability to utilize the components of scientific arguments, which are the claim, evidence, and reasoning.

Figure 4
Kirby-Bauer Disk Diffusion Susceptibility Test

In addition, it can be seen that the culminating activity on Antimicrobial Resistance had the highest mean score of 5.227 (SD=1.412). In this activity, students conducted a hands-on investigation, particularly the Kirby-Bauer disk diffusion susceptibility test, to determine the susceptibility or resistance of the test bacteria to antibiotics (Figure 4). However, the lowest mean score of 4.087 (SD=1.345) was recorded in the first activity on the Structure and Function of Genetic Material. This implies that students were not yet aware of articulating their arguments at the beginning of the unit.

Concerning the components of a scientific argument, the mean score distributions as seen in Figure 5 revealed that the highest mean scores across the activities mostly occurred in the claim component, while the lowest mean scores occurred in the reasoning component. These findings indicate that students found it easier in writing their answers to the guiding question (claim) but more difficult in providing appropriate and/or sufficient underlying scientific concepts (reasoning) to link evidence to their claims. This is consistent with the previous research of Hsu et al. (2020) which reported students’ difficulty in providing evidence to support reasons and establishing the connections between the two.

Moreover, it can be gleaned in Figure 5 that students’ mean scores on each component of scientific argument varied across the MADI-based activities included in the unit. Such a variation...
depends on the nature and context of the activity that contributed to the fluctuations in students' scores (Lee, Schultheis, Kjelvik, Mead, & Stuhlsatz, 2019).

Figure 5

Mean Scores in Each Component of Scientific Argument Across the Six (6) MADI-Based Activities

Argumentation skills can be expressed and assessed through writing and oral discussion. In this study, the argumentation sessions were video-recorded to capture students’ dialogic interactions. Below is a sample transcript in the argumentation session on the topic of Protein Synthesis:

Student 19: So, the guiding question is: How does the genetic information flow from DNA to proteins? Our claim for that is: The genetic information flows from DNA — transcription — translation — protein. Our evidence is based on the computer simulation of protein synthesis. Here, we have seen that the DNA divides in half. The enzymes help the DNA divide in half. The next step is transcription where we have seen that there is an enzyme that sticks into the other… that produces RNA… The bases of the DNA are complementary to the bases of the RNA. But, thymine is changed into uracil. So that’s not thymine but uracil in the mRNA...

Student 10: Question! In your claim, you have written there that the genetic information flows from DNA — transcription — translation — protein. I think you have missed writing the RNA.

Student 19: No, it’s included in our evidence. Transcription converts DNA into mRNA. It is present there in the process of transcription.

Student 10: I think, in order to further justify your claim and evidence, you need to include RNA in your claim, which is DNA-RNA-Proteins, which is all about the central dogma in molecular biology that states that DNA becomes RNA, and RNA becomes proteins.

Student 10: Question! In your claim, you have written there that the genetic information flows from DNA — transcription — translation — protein. I think you have missed writing the RNA.

Student 19: No, it’s included in our evidence. Transcription converts DNA into mRNA. It is present there in the process of transcription.

Student 10: I think, in order to further justify your claim and evidence, you need to include RNA in your claim, which is DNA-RNA-Proteins, which is all about the central dogma in molecular biology that states that DNA becomes RNA, and RNA becomes proteins.

The above sample excerpt in the argumentation session exemplifies how students constructed and developed their understanding of Protein Synthesis concepts. In their argumentative discourse, Student 10 clarified that there had to be “RNA” in the claim of the argument of the other group to further justify their evidence. The students then suggested that their claim may be transformed into: “Genetic information flows from DNA → RNA → Proteins”, to clearly show the process of protein synthesis. This excerpt reflects a crucial characteristic of argumentation, which happens when students provide reasons to support or challenge a claim, conclusion, or explanation (Sampson et al., 2012).
Table 8

<table>
<thead>
<tr>
<th>Main Theme</th>
<th>Sub-theme</th>
<th>Codes</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MADI approach facilitates the development of students’ argumentation skills</td>
<td>Effects of MADI approach on students’ argumentation skills</td>
<td>enhancing argumentation skills</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>improving reasoning skills</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>developing speaking skills</td>
<td>1</td>
</tr>
</tbody>
</table>

Furthermore, the focus group discussion results further confirmed that the MADI approach supports the development of students’ argumentation skills. To elaborate on this theme, the following are sample verbatim responses of the students:

Student 22: “Sir, our inductive and deductive reasoning skills worked even better. Because what you gave us, Sir… from general, we will do it specifically in our claims. Then, from claim to evidence to reasoning. So inductive, from specific… we are going to make it general. So, “How are we going to explain it to others?” through reasoning. Our reasoning skills improved better. Also, it helped us enhance our skills in defending our arguments.”

Student 17: To me, Sir, I developed the skills of making a firm argument. Because of that approach, you need to have a claim, evidence, and reasoning in which you need to be evident and reasonable.

Student 10: “…other groups are being afraid when it is my turn to go to their group because I keep on asking questions. That is my way to know why it is their justification so that I can get something to put on my notes to know why it became their answer.”

The resounding findings obtained from the focus group discussions reflect that the MADI approach promoted students’ argumentation skills. The dialogic interactions during argumentation sessions facilitate students’ understanding (Memis & Cevik, 2018; Venville & Dawson, 2010) and assist them to propose, support, critique, refine, justify, and defend their positions (Llewellyn, 2013), hence developing students’ argumentation skills. Through argumentation, students are found collaboratively communicating and exchanging their ideas, where they considered giving counter-arguments before agreeing on a joint conclusion (Songsil et al., 2019). This provided a learning atmosphere where a social learning process is evident (Sampson et al., 2012) between and among students. Students were able to test and examine their understanding and of others as a mechanism for enriching, interweaving, and expanding their knowledge of particular issues or phenomena (Savery & Duffy, 2001).

Conclusion and Recommendations

The study investigated the effectiveness of the MADI approach in developing students’ conceptual understanding of Antimicrobial Resistance and argumentation skills. From the analysis of the data gathered, the following major findings were drawn: (1) Students’ conceptual understanding of Antimicrobial Resistance significantly changed after exposure to the MADI approach. (2) Students’ argumentation skills significantly changed after exposure to the MADI approach. (3) Students’ development of argumentation skills is evident during the implementation of the study.

Considering the positive impacts of the MADI approach on students’ development of conceptual understanding and argumentation skills, teachers might adapt this pedagogical approach to teaching other topics in Biology. Additionally, further studies on the use of the MADI approach might be conducted in different disciplines of science and its usefulness in improving 21st-century skills or those critical skills in learning Biology.

Since the generalizability of the findings of the present study is limited due to the small sample size, future studies might involve a larger sample to further examine the effects of the MADI approach in facilitating the development of students’ conceptual understanding and argumentation skills. Future
studies might also employ a quasi-experimental design that includes both the control and experimental group to generate substantial information on the effectiveness of the MADI approach in science teaching and learning.

References


APPENDIX

Appendix 1

Sample Activity

Activity 5

**DOES A VARIANT RPOB GENE SEQUENCE RESULT IN RESISTANCE TO THE ANTIBIOTIC RIFAMPIN?**

---

**Background**

Tuberculosis (TB) is a deadly infectious disease, primarily affecting the lungs. It is caused by the pathogenic bacterium *Mycobacterium tuberculosis* (MTB). Transmission occurs when a person with active TB disease coughs and aerosolizes the bacteria, which then spread to other individuals. Symptoms of active TB include chest pain, prolonged cough, and blood in the sputum. Because TB is caused by a bacterium, it can often be treated using antibiotics, although the course of treatment typically extends 6 months or longer. Alarmingly, there is an increasing prevalence of TB strains that are resistant to the antibiotics typically prescribed, including multi-drug resistant (MDR) and extensively drug resistant (XDR) strains. MDR-TB infections are resistant to two of the first line TB antibiotics, isoniazid and rifampin, and XDR-TB infections are resistant to four types of antibiotics.

![Image of bacteria](https://microbiologyonline.org/file/418e66e6b0b6e7eb65b59b71cc91f1pdf)

*Figure 1. Extensively drug-resistant tuberculosis (XDR-TB)*

Source: https://microbiologyonline.org/file/418e66e6b0b6e7eb65b59b71cc91f1pdf
MICROBIAL GENETICS: THE RISE OF ANTIMICROBIAL RESISTANCE

A wild-type strain of *M. tuberculosis* does not have any gene mutations that confer antibiotic resistance, making it susceptible to all standard classes of TB antibiotics. Other strains of MTB, including MDR and XDR TB strains, have mutations in the form of single-nucleotide polymorphisms (SNPs) which lead to resistance to antibiotics.

### An Example of a single-nucleotide polymorphisms

<table>
<thead>
<tr>
<th>Allele 1</th>
<th>A G G T G A T T</th>
<th>A G G T G A T T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele 2</td>
<td>A G G T G A T T</td>
<td>A G G T G A T T</td>
</tr>
</tbody>
</table>

### Possible consequences of SNPs

- **Wild-type allele**: A G G T G A T T
- **mRNA transcript**: U C C A G A U A A
- **Original amino acid sequence**: Serine-Arginine-STOP
  - **ORIGINAL**

- **Synonymous polymorphism**: A G G T C C A T T
- **mRNA Transcript**: U C C A G G U A A
- **Alternative amino acid sequence**: Serine-Arginine-STOP
  - **SENSE**

- **Nonsynonymous polymorphism**: A G G T G A T T
- **mRNA transcript**: U C C A G A U A A
- **Alternative amino acid sequence**: Serine-Threonine-STOP
  - **MISSENSE**

- **Nonsynonymous polymorphism**: A G G A C T A T T
- **mRNA transcript**: U C C U G A U A A
- **Alternative amino acid sequence**: Serine-STOP
  - **NONSENSE**

A SNP is a single base pair substitution that can be observed when comparing similar DNA sequences of the same gene—either between organisms, strains, or homologous chromosomes. A SNP can lead to a synonymous polymorphism or a nonsynonymous polymorphism. Synonymous polymorphisms (also known as ‘silent mutations’) do not lead to an amino acid change in the translated protein sequence, since multiple codons can code for the same amino acid. Nonsynonymous polymorphisms, however, lead to a change in the protein sequence and can either cause
MICROBIAL GENETICS: THE RISE OF ANTIMICROBIAL RESISTANCE

A missense mutation, which results in a different amino acid in the protein sequence or a nonsense mutation that results in an early stop codon.

Rifampin, an antibiotic against TB, binds to and inhibits a subunit of MTB’s RNA polymerase. If the cell’s RNA polymerase doesn’t work, genes can no longer be transcribed into proteins, and no proteins means no functional cellular machinery. Bacteria that are resistant to rifampin can have a mutation in their rpoB gene which alters the site of where rifampin binds to RNA polymerase. Therefore, rifampin isn’t able to bind to the polymerase, the polymerase continues to work, and the bacteria continue to exist.

Your Task

Using the DNA sequences of the rpoB gene from a wild-type and a variant strain of MTB, identify any SNPs in the variant gene sequence, and tell whether the SNP is a synonymous or nonsynonymous polymorphism. You will then need to critically evaluate what effect the SNP may have on conferring antibiotic resistance to the variant strain of MTB.

Materials

You will use two (2) bioinformatics tools for your investigations. To identify single-nucleotide polymorphisms (SNP), go to http://www.genome.jp/tools/clustalw, and to translate each gene sequence to an amino acid sequence, visit http://exon.gatech.edu/genemarks.cci

Getting Started

As mentioned in the Background section, an SNP in the rpoB gene region that changes the structure of the binding site in the polymerase for rifampin could cause rifampin to no longer work. If that happens, the bacterium with that mutation is considered to be resistant to rifampin. If there is no change to the rifampin binding site in RNA polymerase, then the bacterium is considered to be susceptible to rifampin.

Your task now is to investigate whether or not the bacteria from which your variant TB allele came is resistant or susceptible to rifampin. Take note that the binding site for rifampin is located between amino acids 36 and 67.
MICROBIAL GENETICS: THE RISE OF ANTIMICROBIAL RESISTANCE

Investigation Proposal Required?  ○ Yes  ● No

Argumentation Session

Once your group has finished analyzing your data, prepare a whiteboard that you can use to share your initial argument. Your argument must consist of a **claim** (a clear statement that answers the guiding question), **evidence** (data from the investigation that supports your claim), and **reasoning** (explains clearly why the data you presented supports your claim and consists of underlying scientific principles that link evidence to your claim). Your whiteboard should include all the information shown in the figure below.

To share your argument with others, we will be using a round-robin format. This means that one member of your group will stay at your lab station to share your group’s argument while the other members of your group go to the other lab stations one at a time to listen to and critique the arguments developed by your classmates.

The goal of the argumentation session is not to convince others that your argument is the best one; rather, the goal is to identify errors or instances of faulty reasoning in the arguments, so these mistakes can be fixed. You will therefore need to...
## Appendix 2

*Conceptual Understanding Test in Antimicrobial Resistance (CTAMR)*

### Table of Specifications

<table>
<thead>
<tr>
<th>Topics</th>
<th>Learning Objectives</th>
<th>Remembering</th>
<th>Understanding</th>
<th>Applying</th>
<th>Analyzing</th>
<th>Evaluating</th>
<th>Creating</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure and Function of the Genetic Material</strong></td>
<td>Explain how DNA serves as genetic information; Construct a three-dimensional model of DNA including all important chemical groups; Describe the structure of nucleic acids and the types of molecules that contain them; and Recognize the importance of DNA as a vessel of genetic information.</td>
<td>#1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><strong>DNA Replication</strong></td>
<td>Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information; Cite evidence from Meselson and Stahl's experiment that enabled scientists to differentiate between semiconservative replication of DNA and alternative models; and Recognize the role of DNA replication in the purpose of regrowth, regeneration and development among organisms.</td>
<td>#5</td>
<td>#6</td>
<td>#8</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><strong>Protein Synthesis</strong></td>
<td>Explain the basic processes of transcription and translation; Differentiate eukaryotic and prokaryotic gene expression; Construct a graphic organizer that summarizes protein synthesis; and Recognize the importance of proteins in cells.</td>
<td>#9</td>
<td>#11</td>
<td>#12</td>
<td>#13</td>
<td>#14</td>
<td>#15</td>
<td>8</td>
</tr>
<tr>
<td><strong>Gene regulation</strong></td>
<td>Define operon and explain the functions of the operator and promoter regions; Describe how lac operon works; and Recognize the advantage it provides to a bacterial cell.</td>
<td>#17</td>
<td></td>
<td>#19</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><strong>Mutation</strong></td>
<td>Describe what mutation is; Discuss how different types of mutations in the DNA sequence may or may not result in phenotypic change; and Explain how genetic mutations can give rise to antibiotic resistance that can be inherited.</td>
<td>#20</td>
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<tr>
<td><strong>Antimicrobial resistance</strong></td>
<td>Describe the horizontal gene transfer mechanisms that allow antibiotic resistance to be transferred between bacteria; Explain the main mechanisms of resistance that bacteria have developed to counteract the action of antibiotics; Discuss how evolution and natural selection maintain antibiotic resistance in bacteria; Conduct a Kirby-Bauer disk diffusion susceptibility test; Explain how the overuse and misuse of antibiotics contribute to antibiotic resistance; and Recognize the impact of antimicrobial resistance to human health, and cite ways to reduce antimicrobial resistance.</td>
<td>#23</td>
<td>#24</td>
<td>#25</td>
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<td>#26</td>
<td>#27 #29 #30</td>
<td>8</td>
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Total: 30