Concept Inventory Development Reveals Common Student Misconceptions about Microbiology†

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Misconceptions, or alternative conceptions, are incorrect understandings that students have incorporated into their prior knowledge. The goal of this study was the identification of misconceptions in microbiology held by undergraduate students upon entry into an introductory, general microbiology course. This work was the first step in developing a microbiology concept inventory based on the American Society for Microbiology’s Recommended Curriculum Guidelines for Undergraduate Microbiology. Responses to true/false (T/F) questions accompanied by written explanations by undergraduate students at a diverse set of institutions were used to reveal misconceptions for fundamental microbiology concepts. These data were analyzed to identify the most difficult core concepts, misalignment between explanations and answer choices, and the most common misconceptions for each core concept. From across the core concepts, nineteen misconception themes found in at least 5% of the coded answers for a given question were identified. The top five misconceptions, with coded responses ranging from 19% to 43% of the explanations, are described, along with suggested classroom interventions. Identification of student misconceptions in microbiology provides a foundation upon which to understand students’ prior knowledge and to design appropriate tools for improving instruction in microbiology.

INTRODUCTION

With the publication of the American Society for Microbiology’s (ASM) Curriculum Guidelines for Undergraduate Microbiology (1), microbiology educators and education researchers are well-positioned to examine how those learning and skill goals match up with classroom outcomes. Decades of cognitive science and psychology research on how learning works support the Constructivist Learning Theory (CLT) (2). This theory states that, when faced with new information, students do not absorb that information like a blank slate, but rather incorporate the new information into preexisting knowledge frameworks developed from prior experiences. When building on incorrect understandings, misconceptions, also known as alternative conceptions, result (3, 4). Misconceptions may be artifacts of prior learning (5, 6), and many studies have established how difficult it is to dislodge these misconceptions (7). Education research has documented many misconceptions about biology, such as those in evolution, respiration, genetics, and host-pathogen interactions.

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These misconceptions originate early and stick throughout K–12 (12) and college education (8).

Many recent calls for biology education reforms (13–15), such as the American Association for the Advancement of Science’s Vision and Change (16), have highlighted the need for active learning in the classroom and for students to think conceptually rather than just memorize facts. These recommendations were recently used as a framework for the ASM’s Curriculum Guidelines for Undergraduate Microbiology, which were established to provide educators across the country with a common set of shared values, principles, and skills for facilitating learning in the undergraduate microbiology classroom. These curriculum guidelines include a set of six Core Concepts: evolution, cell structure and function, metabolic pathways, information flow and genetics, microbial systems, and impact of microorganisms (Fig. 1). Each core concept is broken down into a set of fundamental statements, with 27 fundamental statements in total encompassing core knowledge central to understanding microbiology (1). The ASM education community also translated these fundamental statements into example learning outcomes (17) for the students (that is, what will students who master microbiology know and be able to do?).

It is important for instructors to have reliable methods for evaluating student learning gains and for identifying common misconceptions held by their students. Instructors often use the pre-/posttest model as a quantitative assessment of student learning gains to detect such misconceptions. Validated assessments, known as concept inventories, are available for quantifying learning gains in many content areas of biology (https://saber-biologyeducationresearch.wikispaces.com/DBER-Concept+Inventories). By identifying which aspects of students’ knowledge are built on misconceptions, educators can better target their curricula to address these sticking points and provide a framework for targeted interventions to improve student learning gains related to enduring principles (18). Some examples of concept inventories that have been developed for measuring student learning gains include host-pathogen interactions (9), genetics (19, 20), organic chemistry (21), and general biology (22). Based on this previous work, a team set about developing a Microbiology Concept Inventory (MCI) based upon ASM’s Curriculum Guidelines.

In this study, we used the results of an initial true/false (T/F) pretest qualitative analysis of students’ open-ended explanations for their answers to determine what misconceptions about microbiology are most common among undergraduate students from a variety of majors and institution types. Microbiology as a discipline builds upon the foundational concepts of biology such as evolution and structure/function, for which there is substantial research on persistent misconceptions (23, 24). Previous work on misconceptions related to microbiology has focused on specific topics such as host-pathogen interactions (25) and antibiotic resistance (18). There has not previously been a study focused on identifying sustained misconceptions that manifest in students who have finished introductory biology and are taking a general microbiology course. The results of this study provide insight into how students consider the broad range of concepts represented in the ASM fundamental statements and add new and important information about the misconceptions that students hold as they enter a microbiology course. Our hope is that educators can target these misconceptions in their classrooms, and we provide recommendations for doing so.

METHODS

Subjects

Data collection occurred at eight different institutions located in four different states ranging from small liberal arts colleges to PhD-granting institutions (Table 1). Responses containing T/F and answer explanations were obtained from 743 students. Participants were recruited from introductory microbiology courses at each institution. These courses contained primarily biology or microbiology majors, but students from other majors were also included. One course section where data collection occurred enrolled primarily students majoring in allied health.

Informed consent and data handling

All participant data were collected and analyzed with protocols approved by the Institutional Review Boards at each participating institution (Beloit College IRB board, #704284-2 Concordia University Wisconsin, #6663 Rogers State University/University of Oklahoma, #19512 Sam Houston State University, #14194 University of Central Oklahoma, #14-515 University of North Texas, #2014-1466 University of Wisconsin-Madison, #11-017 Virginia Tech). Identifying information (such as student ID numbers, names, institutions) were stripped from the data and replaced with random numbers before analysis.
The first step of the MCI development was to create a set of true/false questions aligned with the learning outcomes for the ASM Curriculum Guidelines' fundamental concepts to elicit student misconceptions. Our concept inventory development was informed by the work on the Host-Pathogen Interactions Concept Inventory (HPICI) (25). Four teams of three researchers each were assigned specific learning outcomes. Individuals within each team wrote questions, which were first reviewed by the team members and then reviewed by the entire development group. After final reviews and revisions, a total of 21 questions, some covering more than one fundamental statement, were developed to address the fundamental concepts found in the ASM Curriculum Guidelines (1) (Appendix 1). Each question was accompanied by the prompt “Please explain your response.” Student responses to the prompt were later used to identify the most common misconceptions for the fundamental concepts. The administration of the inventory for this study occurred in the first two weeks of the semester and prior to the students receiving instruction on the topics.

Analysis of student responses

Qualitative analysis. Using an approach informed by the development of the HPICI (24), student responses from all institutions were pooled, assigned a number, and placed in a random order. Teams consisting of three members of the study authors were each assigned a subset of the questions to analyze. Each coder independently read a random sample of 100 student responses for each question to identify common themes, and the coding team used these themes to develop a common codebook. For example, student responses to the T/F question “Bacteria and Archaea will be equally susceptible to antibiotics such as penicillin” had common themes such as 1) Bacteria and Archaea are the same, 2) confusing innate and acquired antibiotic resistance, and 3) antibiotics are effective because they affect prokaryotes. These common coding themes were used to identify the misconceptions noted in Tables 2 to 5. In addition to the coding themes identified during review of student explanations, codebooks for all questions also included “correct,” “guess,” and “nonsense.” Using the codebook, team members then independently coded at least 300 responses per question. Codes for each response were compared across all coders, and a final code was assigned in cases where at least two of the three coders agreed. One question (number 7) was not included in the final analysis due to problems with the question wording that were identified during coding. For the 20 questions analyzed, the average inter-rater reliability was 81%, with an average number of categories per question of 12.9.

Quantitative analysis. From the consensus codes generated through our qualitative analysis, the number of each of the most common wrong explanation types (codes) was counted for student responses in the set of coded responses. For each question, the proportion of correct and incorrect explanations was calculated, as was the correct/incorrect ratio for groups of questions falling within each core concept category. For each question’s set of incorrect explanations, the most common misconceptions were defined as those incorrect written explanations made in at least 5% of the coded responses. Question difficulty was calculated as the proportion of correct answers (true/false) provided by the entire set of 743 respondents. The ratio of correct explanations given to correct answers given was also calculated.

The T/F questions were grouped according to the core concepts in the ASM Curriculum Guidelines, based on the fundamental statements they were created to address. In addition to individual question analysis, these categories were used in subsequent group analysis of questions related to a particular core concept area. Questions were categorized as follows: Evolution (Questions 1, 2, 3, 14), Cell Structure/Function (Questions 4, 5, 6, 8, 9, 10, 19),

### TABLE 1.
Participating institutions.

| Name                        | Location         | Carnegie Classification                                                                 | # student responses |
|-----------------------------|------------------|----------------------------------------------------------------------------------------|---------------------|
| Beloit College              | Beloit, WI       | Baccalaureate colleges: arts & sciences focus                                          | 10                  |
| Concordia University Wisconsin | Mequon, WI | Master's colleges & universities: larger programs                                        | 81                  |
| Rogers State University     | Claremore, OK    | Baccalaureate colleges: diverse fields                                                  | 37                  |
| Sam Houston State University | Huntsville, TX  | Doctoral universities: moderate research activity                                      | 64                  |
| University of Central Oklahoma | Edmond, OK | Master's colleges & universities: larger programs                                        | 11                  |
| University of North Texas   | Denton, TX       | Doctoral Universities: Highest Research Activity                                        | 35                  |
| University of Wisconsin — Madison | Madison, WI | Doctoral universities: highest research activity                                         | 246                 |
| Virginia Tech               | Blacksburg, VA   | Doctoral universities: highest research activity                                         | 259                 |
Metabolism (Questions 9, 11, 12, 13, 20), Genetics (Questions 4, 14, 15, 16, 17), and Systems/Impact (Questions 18, 20, 21). Note that four questions covered information related to fundamental statements found in two core concept areas (Questions 4, 9, 14, 20).

**RESULTS**

**Most difficult core concepts**

In reviewing the T/F responses to the questions, it was found that questions 5, 6, 8, 9, 12, and 15 all had fewer than 50% correct answers chosen from all collected responses (Fig. 2). Three of these six questions were in the cell structure/function category only, one was in cell structure/function and metabolism, one was in only metabolism and one in genetics. In addition, and independent of whether or not students chose the correct T/F answer, 18 out of the 20 questions on the T/F instrument had fewer than 50% correct explanations for their answers. Questions 15 (genetics) and 20 (metabolism and systems/impact) were the only questions with correct explanation percentages above 50%. So, while students could often identify whether a statement was true, they were almost completely unable to describe why in a manner that is consistent with the scientific explanation. This phenomenon is especially apparent with the questions addressing the evolution core concept (questions 1, 2, 3, and 14). Questions 5, 8, and 15 were the only questions that had similar rates of correct answers and correct explanations.

Students had a great deal of difficulty providing clear explanations for their answers on several questions, as seen in the number of “nonsense” coded answers. The highest was question 21 (systems/impact) with 68% of the answers coded as nonsense, followed by question 10 (cell structure/function) at 40% and question 9 (cell structure/function and metabolism) at 32%. Three other questions had between 20 and 25% nonsense answers: questions 4 (genetics), 11 (metabolism), and 17 (genetics).

**TABLE 2.**

Evolution misconceptions and student examples.

| Theme                  | Misconception                                                                 | Student Example                                                                                                                                 |
|------------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| T/F: “A bacterium can acquire resistance to an antibiotic to which it has not been exposed.” |                                                                                                                                             |                                                                                                                                             |
| Genetic change         | Change is intentional and in response to external conditions                   | “A bacterium must be first exposed to the antibiotic in order to be able to mutate and become resistant to the antibiotic.”                   |
| T/F: “If you cultured two bacteria together, where one is resistant to penicillin, it is possible for the other to acquire the resistance.” |                                                                                                                                             |                                                                                                                                             |
| Genetic change/gene transfer | Change only occurs through mutation, not gene transfer                        | “It could be possible, but it wouldn’t be from the first bacteria, it would have to be through mutation.”                                      |
| Genetic change/gene transfer | Change/transfer occurs through hybridization/crossbreeding in bacteria         | “If the bacteria could reproduce together then this might happen.”                                                                        |
| Immunity               | Confusing immunity with drug resistance                                        | “A bacterium cannot acquire resistance to an antibiotic to which it has not been exposed because we have two types of immunity innate and acquired. An acquired immunity allows immunity to things that we have already been exposed to and if it has not been exposed then we do not have an immunity to it. So therefore it cannot acquire resistance because the acquired immunity has never been exposed to that certain antibiotic.” |
| T/F: “Genome sequencing of a microbial species might demonstrate genes originating from a variety of other species.” |                                                                                                                                             |                                                                                                                                             |
| Gene transfer          | Transfer is only vertical (horizontal not a major factor)                      | “Everything came from one common ancestor, and then branched off into several subspecies.”                                                |
| T/F: “Evidence of a common ancestor for all life on earth is revealed by the presence of the same enzymatic pathways in organisms as diverse as humans and microorganisms.” |                                                                                                                                             |                                                                                                                                             |
| Common ancestry        | Genetics is the only type of evidence of common ancestry (not proteins or pathways, don’t see link)                                   | “Evidence for a common ancestor is revealed by the DNA sequencing of organisms.”                                                           |

T/F = true/false.
Most common misconceptions for each core concept

Analysis of the coding of incorrect explanations revealed several core concepts for which respondents provided interesting misconceptions. We considered misconceptions associated with 5% or more of the responses to be common and grouped these according to themes. These misconceptions are organized by core concept in Tables 2 to 5 and are shown with an example of a student response for each. The misconceptions given most often by student respondents include the following: “In bacteria, prior exposure is required to acquire antibiotic resistance” (43% of coded responses to question 1, core concept = evolution); “Vaccines must cause disease in order to work” (21.7% of coded responses to question 19, core concepts = cell structure/function and metabolism); “Oxygen is required for bacterial growth and/or speeds up growth” (19.5% of coded responses to question 11, core concept = metabolism); “The only way gene
sequences can be similar in different organisms is via vertical transmission from a common ancestor” (19.4% of coded responses to question 3, core concept = evolution); and “Confusion about the role of RNA, RNA polymerase, and RNA primase in DNA replication and transcription” (19% of coded responses to question 17, core concept = genetics).

Misalignment between answer choices and explanations of those choices

By core concept, students struggled the most in explaining their thinking about evolution, and best articulated their answers about cell structure/function (Fig. 3). By fundamental statement (FS), students had the most difficulty in explaining their answers to FS 16 (“Although the central dogma is universal in all cells, the processes of replication, transcription, and translation differ in bacteria, Archaea, and eukaryotes”) and FS 21 (“Most bacteria in nature live in biofilm communities”). While students overall struggled explaining evolution, they were adept at explaining their answers to FS 5 (“evolutionary relatedness of organisms

FIGURE 3. Ratio of correct explanations to correct answers for each microbiology core concept. Subjects with lower bars indicate answering correctly, but not being able to explain why. Evo = evolution; Cell = cell structure and function; Met = metabolic pathways; Gen = information flow and genetics; Sys = microbial systems; Imp = impact of microorganisms.

FIGURE 2. Concept inventory question difficulty, as measured by proportion of correct answers (true or false choice) (black bars) and correct explanations (free response to “Please explain your response”) (white bars), grouped by microbiology core concept.
is best reflected in phylogenetic trees”). They also did well explaining FS 6 (“The structure and function of microorganisms have been revealed by the use of microscopy”), FS 8 (“Bacteria and Archaea have specialized structures that often confer critical capabilities”), and FS 15 (“Genetic variation can impact microbial functions”) (Fig. 4).

**DISCUSSION**

**Study summary**

In this study, student answers and associated explanations to a T/F survey were used to reveal their thinking about six core areas of microbiology identified in the ASM Curriculum Guidelines: evolution, cell structure and function, metabolic pathways, information flow and genetics, microbial systems, and impact of microorganisms. The most informative parts of the survey were student explanations to their answers, exposing the most frequent misconceptions that students have when coming into their first microbiology course.

Our data show that students arriving for their first microbiology course come into class with a significant number of misconceptions, the most striking being their misunderstanding of evolution and an inability to correctly explain its concepts. Many students have absorbed the basic idea of evolution, but have difficulty with its workings. Assignment of determinism in evolution and being unaware of the influence of horizontal gene transfer were the two major flaws in their thinking. The former is likely a consequence of prior instruction in which the examples in biology they are given indicate that the organism will respond after exposure to a stimulus, thus it may seem counter-intuitive to them that the organism responds before being exposed to the selective pressure. They also may not be thinking about a population of organisms, but instead, the response of a single microbe. In the latter case, horizontal gene transfer is likely an unfamiliar concept, since much of their previous introductory biology courses traditionally focus more on eukaryotic organisms where such processes are less common.

Given the broad nature of each of the core concept areas as well as the fundamental statements, it is unlikely that this work has elicited all possible misconceptions, but a number of important misconceptions in microbiology have been identified. The limitations of this study include that it focused only on the fundamental statements framed by the ASM Curriculum Guidelines and that it only elicited misconceptions triggered by the questions we wrote. As well, even though a diverse set of institutions was used for recruiting subjects, two-year institutions are not represented in the dataset, and there is a potential for an over-representation of a few institutions in the data collection due to variations in response rates and institution sizes. Additionally, student explanations varied greatly in amount of detail, so they were not always informative. This could have limited identification of some misconceptions due to unclear or partial explanations. In particular, student answers in the impact of microorganisms/microbial systems core concept areas were difficult to interpret, with many incorrect explanations appearing to stem from not knowing about the specific scenarios/relationships rather than not understanding underlying core concepts or principles.

**Top misconceptions and suggested interventions**

Our analysis identified five top misconceptions held by undergraduate students at the beginning of their general microbiology courses, listed in order below beginning with the most commonly held as shown in this data. We propose possible articulated learning outcomes and classroom activities/interventions that could be used to address these misconceptions.

**Misconception #1: In bacteria, prior exposure is required to acquire antibiotic resistance (core concept = evolution).** Learning outcome that could address this misconception: After performing a Luria-Delbrück experiment (26), students will discover that mutations occur randomly before being put under selective pressure. Students can inoculate similar cultures of *E. coli* onto LB +

![FIGURE 4. Ratio of correct explanations to correct true/false answers for each concept inventory question, grouped by microbiology core concept. Horizontal bars indicate a ratio of 1:1, which would indicate an equal proportion of students providing correct answers and correct explanations (ratios < 1 indicate fewer students provided correct explanations than correct answers, and ratios > 1 indicate fewer students provided correct answers than correct explanations.](image-url)
streptomycin agar plates. After incubation, a random distribution of resistant mutants will arise, dependent upon the time of mutation during incubation. This could be a virtual or in-class experiment.

**Misconception #2: Vaccines must cause disease in order to work** (core concept = cell structure function and metabolism). Learning outcome that could address this misconception: Students will categorize vaccines by the four vaccine types. In this activity, students will research common vaccines in order to categorize them into the types based on how they are made. This activity could include students developing an explanation for how each vaccine type can stimulate an immune response and could explore how subunit or toxoid vaccines serve as antigens without causing disease.

**Misconception #3: Oxygen is required for bacterial growth and/or speeds up growth** (core concept = metabolism). Learning outcome that could address this misconception: Students will analyze experimental data to determine whether oxygen affects the growth of a lactic acid bacterium. Comparison of the growth rates of Lactococcus lactis (or another lactic acid bacterium) demonstrates similar growth rates under aerobic and anaerobic conditions. Students will realize that L. lactis metabolism (homolactic) does not improve in the presence of oxygen. Students can generate two growth curves for L. lactis grown in the presence and absence of oxygen, and have them propose hypotheses as to why the resulting growth curves are the same (27).

**Misconception #4: The only way gene sequences can be similar in different organisms is via vertical transmission from a common ancestor** (core concept = evolution). Learning outcome that could address this misconception: After performing a conjugation experiment, students will be able to explain that bacteria can acquire drug resistance by horizontal gene transfer and by vertical transmission. Students could be given data or generate data in laboratory that show a high rate of transfer of genes from one microbe to another. Conjugation is a great way to do this, because it is of a high frequency. Bioinformatics-based analysis of bacterial genome structure would be another approach to show students the mosaic nature of genome architecture, including prophages, transposons, gene islands, etc.

**Misconception #5: Confusion about the role of RNA, RNA polymerase, and RNA primase in DNA replication and transcription** (core concept = genetics). Learning outcome that could address this misconception: Students will be able to match the function of RNA, RNA polymerase, and RNA primase. A simple matching question that requires students to describe various proteins’ roles could provide practice in connecting each protein with its function. For example, students could be asked to match the correct protein (RNA polymerase, RNA primase, DnaA, etc.) to the appropriate functional description and the role of the protein in DNA replication or transcription. This exercise could be connected with classroom activities such as animations that show the process of DNA replication along with other activities on central dogma.

**CONCLUSION**

The data collected during the initial stages of the development of a Microbiology Concept Inventory provide valuable insight into undergraduate students’ misconceptions upon entry into introductory microbiology courses. This study has identified several common misconceptions that students hold and creates an opportunity for microbiology instructors to better prepare instruction that addresses these misconceptions. This first step in identifying students’ misconceptions in microbiology provides a foundation upon which additional studies can be conducted to further expand our knowledge of what students understand when they enter our classrooms. The information provided here, in combination with the forthcoming Microbiology Concept Inventory, provides a valuable tool for improving instruction in microbiology.

**SUPPLEMENTAL MATERIALS**

Appendix I: MCI T/F questions

**ACKNOWLEDGMENTS**

The authors would like to acknowledge assistance from the American Society for Microbiology for this work and, in particular, support from Amy Chang, ASM Director of Education. The authors declare that there are no conflicts of interest.

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