ESCHERICHIA COLI AS A MODEL FOR EVALUATING THE PROGRESS OF LEARNING UNDERGRADUATE STUDENTS IN MICROBIOLOGY

ESCHERICHIA COLI COMO MODELO DE AVALIAÇÃO DO PROGRESSO DE APRENDIZAGEM DOS ESTUDANTES DE GRADUAÇÃO EM MICROBIOLOGIA

ESCHERICHIA COLI COMO MODELO PARA EVALUAR EL PROGRESO EN EL APRENDIZAJE DE LOS ESTUDIANTES UNIVERSITARIOS DE MICROBIOLOGIA

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ABSTRACT: To assess the progress of undergraduate students in the Biomedical Sciences course at USP, we implemented the "Adopt a Bacteria" methodology within the bacteriology course. We focused on Escherichia coli as a model to explore fundamental concepts in bacterial genetics, morphology, and pathogenesis. To validate this active learning approach, we collected metrics related to the richness and complexity of the discourse generated by students through their social media posts during 2022 and 2023. We analyzed these metrics using Shannon indices and Python. The results indicated that the posts on the three topics were complex and showed an increase in the richness of the students' discourses. It is concluded that this teaching strategy, based on the methodology, enhanced the repertoire of concepts and the complexity of discourse, reflecting increased student engagement and interest in microbiology.

KEYWORDS: Escherichia coli. EIEC. Pathogenicity. Teaching. Active teaching methodology.

RESUMO: *Com o propósito de avaliar o progresso dos alunos de graduação do curso de Ciências Biomédicas da USP empregamos a metodologia "Adote uma Bactéria", na disciplina de bacteriologia. Com esse objetivo, focamos na espécie Escherichia coli como modelo para explorar conceitos fundamentais na genética, morfologia e patogênese bacteriana. Com intuito de validar a metodologia ativa, buscou-se levantar métricas inerentes a riqueza do discurso e complexidade de informações geradas pelos alunos em troca de informações postadas em rede social ao longo de 2022 e 2023. As análises foram feitas por meio da determinação dos índices de Shannon e linguagem Python. Os resultados demostraram que as postagens sobre as três temáticas foram complexas e demonstram um aumento da riqueza dos discursos empregadas pelos alunos. Conclui-se que a estratégia de ensino calcada na metodologia ampliou o repertório de conceitos e a complexidade de discurso com o engajamento e interesse dos alunos pela microbiologia.*

PALAVRAS-CHAVE: *Escherichia coli. EIEC. Patogenicidade. Ensino. Metodologia ativa de ensino.*

RESUMEN: *Con el objetivo de evaluar el progreso de los estudiantes de pregrado en la carrera de Ciencias Biomédicas de la USP, utilizamos la metodología "Adopta una Bacteria" en la asignatura de Bacteriología. Nos centramos en la especie Escherichia coli como modelo para explorar conceptos fundamentales en genética, morfología y patogénesis bacteriana. Para validar esta metodología activa, recolectamos métricas relacionadas con la riqueza y la complejidad del discurso generado por los estudiantes a través de sus publicaciones en redes sociales durante 2022 y 2023. Los análisis se realizaron mediante la determinación de índices de Shannon y el uso del lenguaje Python. Los resultados mostraron que las publicaciones sobre los tres temas fueron complejas y evidenciaron un aumento en la riqueza de los discursos de los estudiantes. Se concluye que la estrategia de enseñanza basada en esta metodología amplió el repertorio de conceptos y la complejidad del discurso, promoviendo un mayor compromiso e interés de los estudiantes por la microbiología.*

PALABRAS CLAVE: *Escherichia coli. EIEC. Patogenicidad. Docencia. Metodología de enseñanza activa.*

Introduction

The microbiology curriculum is integrated into secondary and higher education, typically segmented into courses dedicated to viruses, bacteria, and fungi (Armellini, 2021). In higher education, students delve deeper into the pathogenicity, survival mechanisms, and cellular structures of microorganisms. However, the complexity of these concepts can present challenges, complicating the learning process. In light of these obstacles, it is imperative for microbiology educators to develop and employ methods that facilitate content assimilation, utilizing engaging and innovative approaches (Da Silva; Colombo, 2019).

Nevertheless, many instructors in this field often persist with traditional memorization techniques, which hinder the development of student's creative and critical thinking skills, which are essential for scientific investigation (Merkel, 2016; Piantola *et al*., 2018). Additionally, reliance on conventional teaching methods, coupled with dense scientific terminology, frequently results in student disengagement (Freeman *et al*., 2014; Piantola *et al*., 2018), leading to a lack of motivation to understand basic microbiological concepts and difficulty retaining information (Piantola *et al*., 2018). Although hybrid teaching methodologies are occasionally incorporated into microbiology to provide more immersive learning experiences (Torrissi-Steele; Drew, 2013), the widespread adoption of these approaches remains limited.

In response to these challenges, the #Adopt project was conceived in 2013 as an innovative teaching approach aimed at enhancing microbiology learning for secondary and higher education students, facilitating the sharing of information through digital platforms. This initiative expands students' communication interfaces, promoting practices that spark interest in the subject and cultivate a "learning to learn" mindset (Armellini 2021; Moreno *et al*., 2023; Piantola *et al*., 2018; Taschner *et al*., 2020). Furthermore, the project's effectiveness in promoting student learning has remained evident, especially during the COVID-19 pandemic and the subsequent shifts in educational paradigms (Armellini ,2021).

The "Adopt a Bacteria" project utilizes digital platforms such as Instagram® to empower students as active participants in learning about bacteria, facilitating their engagement and aiding in the retention of fundamental bacteriology concepts (Piantola *et al*., 2018). Among these concepts is pathogenicity, which is integral to microbiology and explores pathogenic microorganisms capable of causing diseases and their virulence mechanisms (Levinson, 2016).

Escherichia coli, a Gram-negative bacterium, encompasses various strains characterized by distinct biological and serological traits, virulence factors, and clinical manifestations. Each group of strains exhibits different modes of action and virulence-associated characteristics. Some strains possess specific virulence factors that enable the onset of enteric diseases, contributing to the classification of pathotypes within this species (De Souza Moreira; Azola; Gouvêa, 2018). Notably, E. coli demonstrates virulence factors such as capsules, endotoxins, pili, and exotoxins, capable of inducing conditions such as bloody or watery diarrhea, hemolytic-uremic syndrome, and other systemic disease spectrums (Levinson, 2016).

Based on this foundation, the present study aims to analyze the outcomes observed in the 2022 and 2023 cohorts of the "Adopt a Bacteria" project, with a particular focus on the adoption of Escherichia coli and its correlation with the topics of bacterial morphology, genetics, and pathogenicity. This investigation highlights the integration of research and teaching methodologies in microbiology, utilizing Python programming to validate the learning outcomes within the Biomedical Sciences undergraduate program at the University of São Paulo (USP).

Methodology

The "Adopt a Bacterium" project: Analysis of word clouds referring to E. coli posts

The methodology employed in the "Adopt a Bacteria" project involves the use of social media platforms, such as Facebook® and Instagram®, as integrated tools for teaching and research, complemented by practical sessions conducted in teaching laboratories. For each session, a private group is created on one of the platforms, ensuring restricted access exclusively to enrolled members and the content they post (Botte *et al*., 2014; Legaree, 2014). These sessions are organized into groups corresponding to the bacterial genera under study, with each group supervised by postgraduate and/or undergraduate students who act as mediators (Piantola *et al*., 2018).

The mediators play a crucial role in facilitating the student's learning journey, guiding them through the process of constructing new concepts and directing the nature of their posts. Their responsibilities include diagnosing conceptual errors, clarifying doubts, and suggesting relevant topics related to the general themes. Additionally, the mediators provide prompt feedback on the students' posts and assist the professor in addressing any concerns raised. As

the posting period concludes, the mediators help students synthesize the main information shared, which becomes an integral part of the final presentation (Taschner *et al*., 2020). This methodology was implemented during the 2022 and 2023 sessions of the Bacteriology course, offered to 90 undergraduate students in the Biomedical Sciences program at the Institute of Biomedical Sciences, University of São Paulo (USP). It enabled the evaluation of student posts and the analysis of content related to Escherichia coli pathogenicity, among other topics covered in the course. Enrolled students participated in generating posts on this subject.

The analysis of the keywords in the selected posts was used to construct word clouds, followed by data evaluation using the Shannon Diversity Index. This assessment aimed to determine the diversity and richness of the discourse in posts discussing pathogenicity during the specified years. To examine the posts shared within the "Adopt a Bacteria" initiative, the Word Cloud Generator website (jasondavies.com/wordcloud/).

The Shannon Diversity Index

The Shannon Diversity Index (H') (Shannon, 1948) is not commonly applied in educational settings; however, it allows for the assessment of the average diversity of discourse (Armellini, 2021). To evaluate this diversity, posts from each year of the "Adopt a Bacteria" project, focusing on *Escherichia coli*, were analyzed. Specific words related to morphology, genetics, and pathogenicity were selected for this evaluation. Terms related to morphology included: plasma membrane, outer membrane, peptidoglycan, cell shape, capsule, fimbria, pili, flagella, Gram-negative, arrangement, cell wall, and lipopolysaccharides. Genetic-related terms covered: DNA, recombination, lateral transfer, plasmid, genome, gene, conjugation, transduction, transformation, phage, operon, duplication, mutation, genotype, transcription, and base pairs. Pathogenicity-related terms encompassed: *E. coli* pathotypes, virulence factors, biofilm, toxin, serotyping, capsule, fimbria, pili, outer membrane, evasion, and secretion system.

The absolute frequency of each word's appearance in the posts for each year was then tallied. Finally, the Shannon Index formula was applied to determine the richness of discourse for each year, using the formula below, where pk represents the frequency of appearance of each word listed above in the total set of posts, and K represents the total number of words used per year in the total set of posts.

$$H = -\sum_{1}^{k} p_k ln(p_k)$$

To apply the Shannon Index in an educational context, the collected data must be categorized into knowledge domains, which allows for the measurement of frequencies in students' written or spoken discourse. This approach is feasible due to the establishment of an equivalence between the selected words and the concept of "species equivalence," whereby the frequencies of these words correspond to the occurrences of "species appearances" in a given context (Armellini, 2021).

Word Count Graphs

Over two consecutive years, the content of the posts was inspected, and the presence of words related to bacteriology concepts was recorded. The frequency of these words was used to quantify learning about the following aspects of the target microorganism's biology: genetics, morphology, pathotype, and pathogenicity. These four categories were referred to as "types" in the main data table. The genetic type refers to how these microorganisms reproduce and are subdivided into 16 categories. Morphology addresses the presence or absence of structures found in bacteria, corresponding to 12 different categories. Pathotypes refer to pathogenic variations, that is, varieties of bacterial species with similar characteristics, and were divided into 11 categories. Finally, the pathogenicity type refers to how pathogenic microorganisms infect other organisms, with this class divided into 12 categories.

The frequency of categories in each type was visualized using stacked bar charts, constructed using Python and the data manipulation and visualization libraries Pandas, Matplotlib, NetworkX, and Seaborn. The direct comparison of graphs from consecutive years allowed for the observation of variations in word frequency within each category, except for the pathotype type.

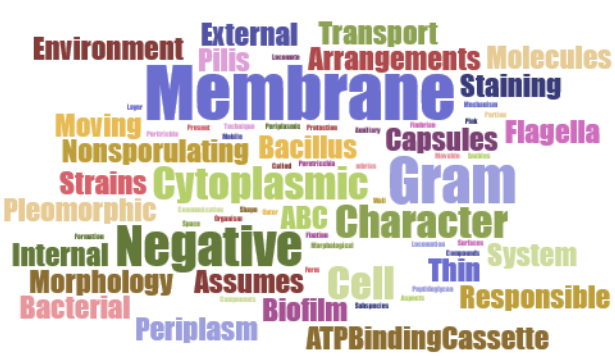
Results

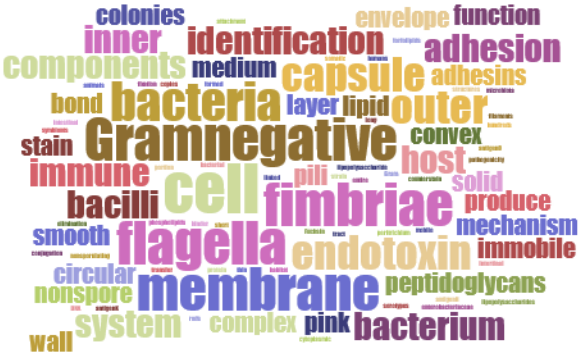
Content analysis of E. coli posts in the "Adopt a Bacterium"

In 2022 and 2023, the "Adopt a Bacteria" project was carried out through the Instagram® platform, where students created posts on various topics under the supervision and guidance of mediators. During these years, students were organized into groups and tasked with producing three posts covering a range of topics, including morphology and structure, genetics, growth and metabolism, pathogenicity, and mechanisms of antibiotic action and resistance. This assignment required the integration of multiple themes in each post.

The analysis of discourse richness, based on the word clouds generated by students who selected *E. coli* as their study subject, revealed satisfactory results regarding bacterial morphology and structure in 2022 and 2023. Terms such as "membrane" and "Gram-negative" were highlighted (Figure 1). In 2022, the focus was on the morphology and structures of *E. coli*, including terms like "lipid A," "polysaccharide" "O antigen," "fimbriae," and "arrangements." In 2023, terms related to additional essential structures of *E. coli* were predominantly highlighted, such as "capsule," "pili," "fimbriae," "flagellum," and "peptidoglycan."

Figure 1 – Word clouds related to the morphology and cellular structure of *E. coli* in posts during the "Adopt a Bacteria" project in 2022 and 2023. Image (A) represents the word cloud for 2022, while image (B) represents the word cloud for 2023. The size of the words is proportional to the number of times they were mentioned by students in Instagram® posts



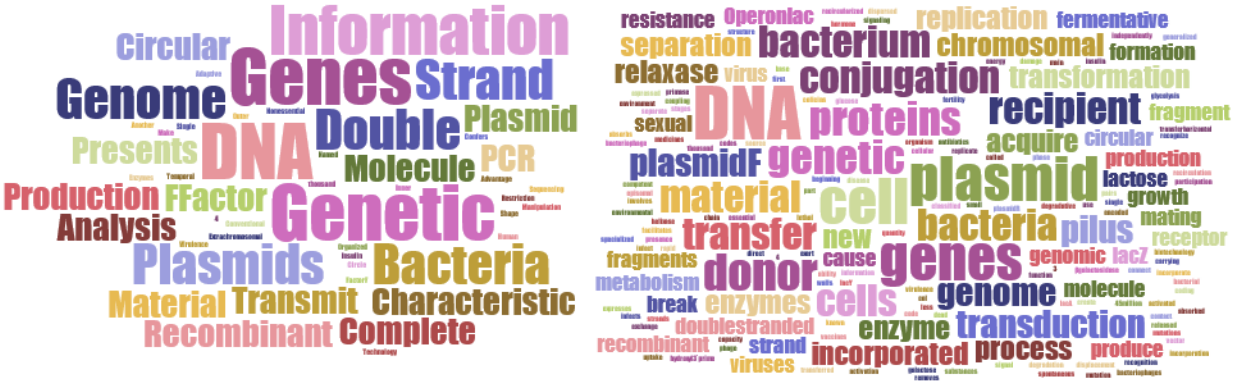


Source: Authors' elaboration (2023)

Morphology and Structure 2022 with 67 words **Morphology and Structure 2023 with 89**

The results related to the genetics theme were also considered positive. In both 2022 and 2023, there was a notable emphasis on terms such as "gene," "genome," "DNA," "genetic material," and "plasmid" (Figure 2). In 2023, there was an observed increase in terms related to genetic recombination, exemplified by the terms "conjugation" and "transformation."

Figure 2 – Word clouds about *E. coli* genetics in posts during the "Adopt a Bacteria" project in 2022 and 2023. Image (A) represents the word cloud for 2022, while image (B) represents the word cloud for 2023. The size of the words is proportional to the number of times they were mentioned by students in Instagram® posts



Genetics 2022 with

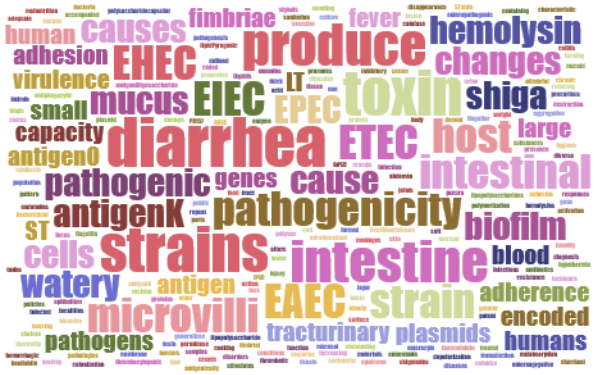
Genetics 2023 with 171 words

Source**:** Authors' elaboration (2023)

The posts focused on pathogenicity topics were considered satisfactory, based on the number of terms used (183 in 2022 and 240 in 2023) during the analyzed period, as indicated by the students' discussions each year. The increase in complexity and diversity of words in this domain suggests greater student engagement with these topics. In the word clouds representing pathogenicity, it can be observed that the students referred to various *E. coli* virulence factors and pathotypes, as highlighted in Figure 3.

Figure 3- Word clouds about *E. coli* pathogenicity in posts during the "Adopt a Bacteria" project in 2022 and 2023. Image (A) represents the word cloud for 2022, while image (B) represents the word cloud for 2023. The size of the words is proportional to the number of times they were mentioned by students in Instagram® posts

Pathogenicity 2022 with



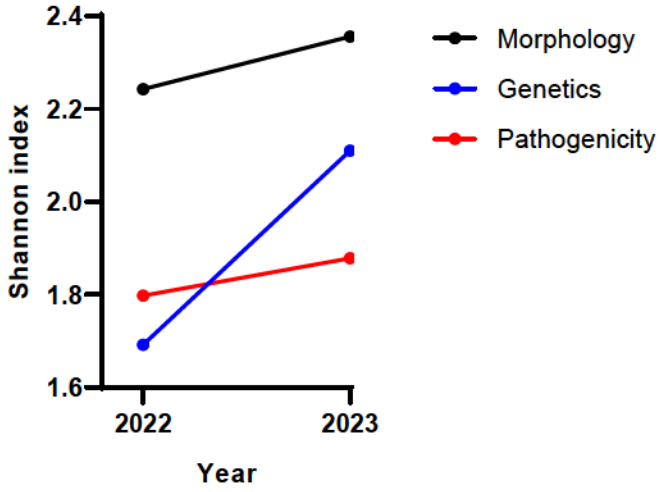
Pathogenicity 2023 with

Source: Authors' elaboration (2023)

After analyzing the word clouds, the Shannon index was calculated to compare the significant terms describing the morphology, genetics, and pathogenicity of E. coli, with the aim of determining which year demonstrated more extraordinary discourse richness. In the morphology analysis, a growth trend was observed between 2022 and 2023. Specifically, the Shannon index increased from 2.24 in 2022 to 2.35 in 2023 (Chart 1).

Similarly, there was a slight increase in the genetics category between the two years, with the Shannon index rising from 1.7 in 2022 to 2.1 in 2023 (Chart 1). A comparable pattern of discourse growth was also observed in the pathogenicity category, with the Shannon index increasing from 1.79 in 2022 to 1.88 in 2023 (Chart 1). This phenomenon can be attributed to the resumption of in-person classes in 2022, after two years of online teaching due to the COVID-19 pandemic, which disrupted established learning routines. The subsequent recovery in 2023 reflects the students' adaptation to in-person instruction.

Graphic 1 – Values of the Shannon index related to morphology, genetics, and pathogenicity of *E. coli* for the years 2022 and 2023. The values computed the absolute frequency of terms which is the sum of the frequencies of the eleven individual words, and, after that, the Shannon index was calculated per year



Source: Authors' elaboration (2023)

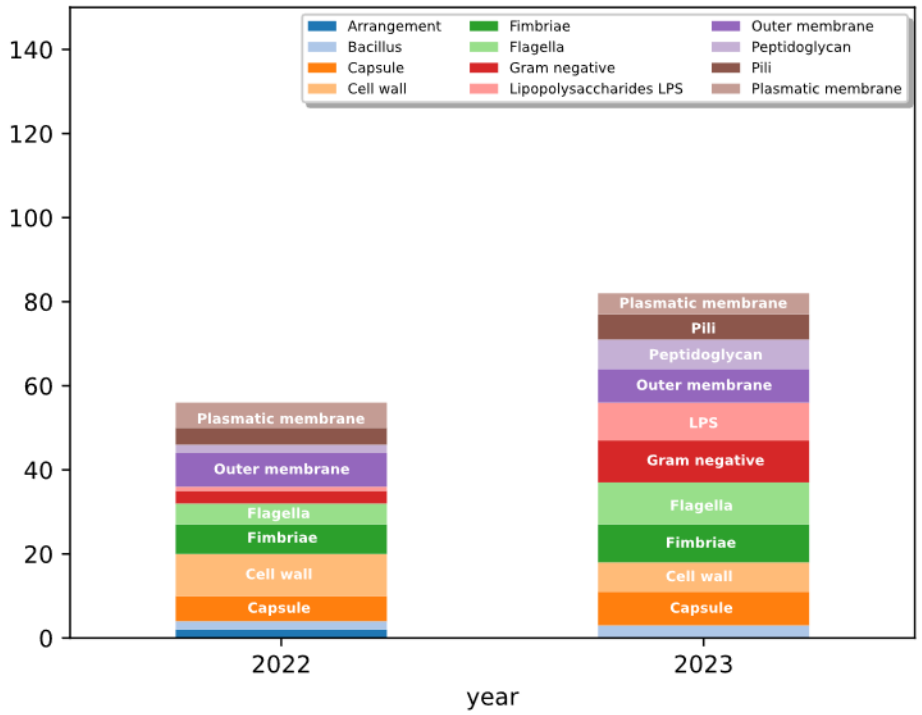
To complement the analysis of discourse richness for the years 2022 and 2023, using the Shannon index on the topics of morphology, genetics, and pathogenicity, a direct visualization of changes in word frequency was generated through data visualization libraries available in the Python programming language. A custom Python script was developed internally to generate the charts presented in Figures 2, 3, and 4, which highlight the most frequently used words for each topic in each of the years analyzed.

The analysis regarding the morphology topic revealed that certain words maintained similar frequencies across both years, such as "outer membrane" and "plasma membrane." In 2022, the most frequent word was "cell wall," whereas in 2023, terms such as "fimbriae," "flagellum," "LPS," "Gram-negative," and "pili" emerged as the most frequently used (Chart 2).

In the field of genetics, it was observed that in 2022, three words stood out: "DNA," "plasmid," and "gene," although these words exhibited higher frequencies in 2023. Furthermore, in 2023, six additional words gained prominence in the chart: "conjugation," "mutation," "lateral transfer," "genome," "operon," and "transduction," with "conjugation," "mutation," and "transduction" being particularly prominent (Chart 3). This result aligns with the findings obtained from the Shannon diversity index, highlighting an increase in the

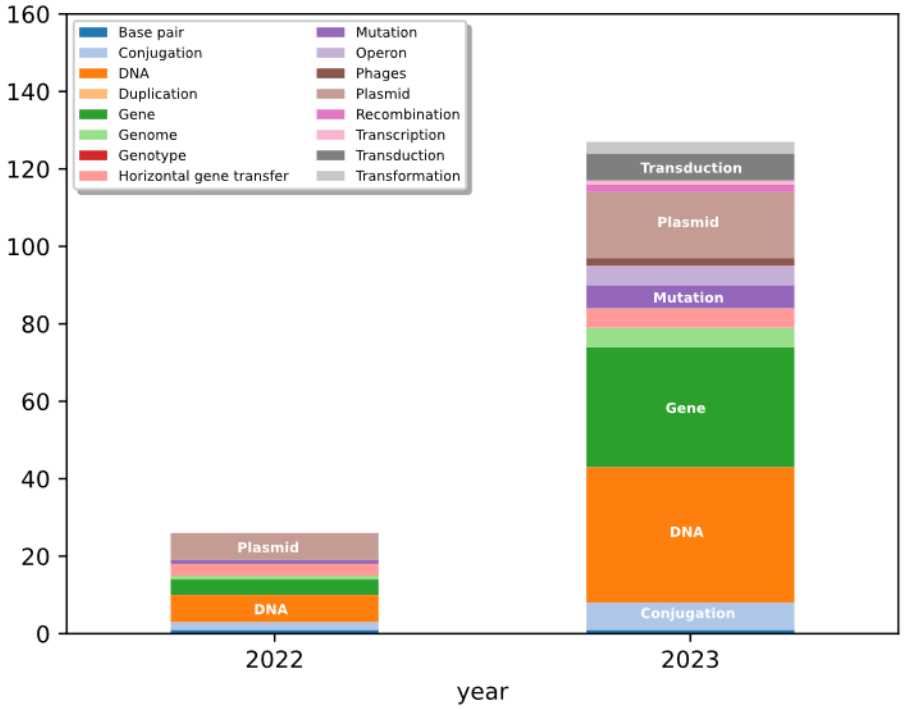
number of words used in 2023 compared to 2022. Although three words stood out in 2022, they displayed lower frequencies compared to 2023.

Graphic 2 – Stacked bar charts representing the frequency of morphology-related terms used by the *E. coli* group in 2022 and 2023. In each year, the frequencies of the words belonging to twelve categories were recorded and illustrated by the height of each colored layer in the bars



Source: Authors' elaboration (2024)

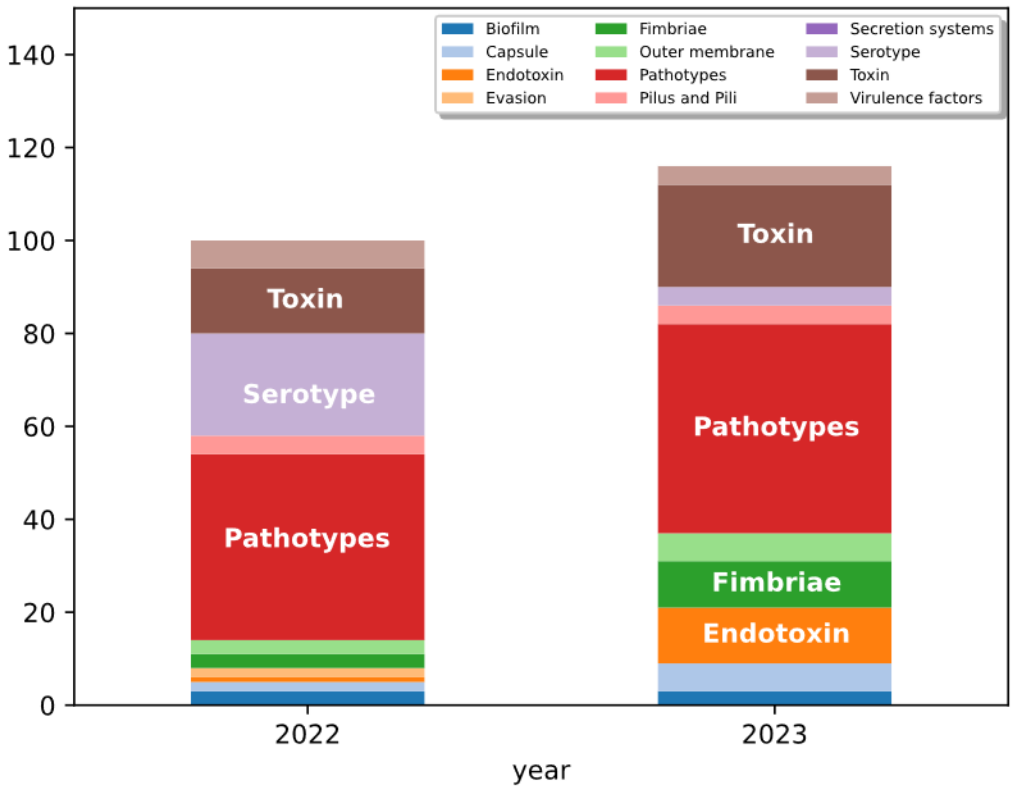
Graphic 3 – Stacked-bar graph depicting the use of genetics related terms by the *E. coli* group in the years 2022 and 2023. The height of each of the sixteen coloured layers is equal to the frequency of the words recorded for each category in the legend (upper right box)



Source: Authors' elaboration (2024)

Regarding the topic of pathogenicity, it was observed that the most frequently used term in both 2022 and 2023 was "pathotype." In 2022, the predominant term was "serotype," whereas in 2023, the words "fimbriae," "endotoxin," and "toxin" emerged as the most frequently used (Chart 4). As illustrated in the Shannon index chart (Chart 1), the discourse richness related to pathogenicity was significantly higher in 2023, with an increase of 0.08. This increase is further corroborated by another chart showing the number of highlighted words each year. This chart demonstrates that 2023 featured three words of greater relevance compared to 2022, emphasizing the importance of using this methodology and its validation through the Shannon index.

Graphic 4 – Stacked-bar graph depicting the use of words of the category "pathogenicity" by the *E.coli* group in 2022 and 2023



Source: Authors' elaboration (2024)

Discussion

The Shannon Index was calculated using word clouds shared on social media to assess the richness of discourse on pathogenicity among students in 2022 and 2023. The analysis revealed a progressive increase in discourse richness related to morphology over these years, peaking in 2023. A similar upward trend was observed in genetics and pathogenicity, with 2023 demonstrating greater discourse diversity compared to 2022. This trend may be attributed to the disruption in study routines caused by the transition back to in-person classes following the COVID-19 pandemic.

These results underscore the relevance of the Shannon Index as a valuable tool for evaluating learning consolidation. Although traditionally used in studies focused on determining diversity within microbiome composition, its application in assessing learning outcomes proves equally effective (Rodrigues Hoffmann *et al.*, 2014). The study demonstrates that applying the Shannon Index to posts created by undergraduate students is

an effective method for quantifying discourse richness and, consequently, enhancing learning consolidation in microbiology, as previously published by the research group (Armellini 2021). It is important to note that, in 2021, the COVID-19 pandemic significantly impacted the educational landscape at USP, leading to a shift to remote learning across all courses, including Bacteriology. The comparatively lower discourse richness among students in 2022 may reflect the disruption in established study routines characterized by remote teaching models.

In addition to the Shannon Diversity Index, bar charts showing word frequency were used to visualize the frequency of words employed in expressing concepts in morphology, genetics, and pathogenicity of E. coli in 2022 and 2023. This approach was particularly useful for identifying content within these topics that required more comprehensive classroom discussion. Additionally, the chart generated by Python complements the data obtained from the Shannon Diversity Index concerning the topics mentioned in this study. It was observed that the greater the increase in discourse richness in the analyzed years, the larger the number of highlighted words in the Python-generated charts.

Final considerations

The study advanced the proposal of using social media as an auxiliary tool to enhance the learning process and increase undergraduate students' interest in microbiology. The report demonstrated that the "Adopt a Bacterium" methodology promoted an increase in discourse complexity by using a single bacterial species (E. coli) as a model to study various topics such as pathogenicity, morphology, and genetics. The final results highlight the relevance of applying active methodologies in the learning process and the potential to increase student interest in microbiology, with possibilities for application in different disciplines and fields of knowledge.

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CRediT Author Statement

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Availability of data and material: Data and materials will be made available upon the publication of the article.

Author contributions: 1st Conducted the word cloud and Shannon analysis, and drafted the manuscript, 2nd Created the graphs using Python, 3rd and 4th helped with the Shannon and word cloud analyses, 5th Contributed to the development of graphs using Python, 6th Developed part of the code for generating the Python-based graphs, 7th Assisted in creating the Shannon index and diversity chart, 8th Taught the subjects evaluated in the study, contributed to the preparation and writing of the manuscript, and is the creator of the #Adote Project.

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