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Differential Knowledge of Free and Subscribed Chatbots on Aspergillus fumigatus, a Mold of Global Importance, and Talaromyces marneffei, a Thermally Dimorphic Fungus Associated with Tropical Infections in Southeast Asia

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Abstract: Chatbots have been widely used in clinical problem-solving and research. However, all the chatbots examined were products of the USA, and there has been no study that compared the knowledge of these chatbots on specific pathogens of global vs. regional importance. In this study, we examined the knowledge of five free chatbots (ChatGPT, Perplexity, Claude, Copilot, and Gemini) and the free vs. subscribed versions of ChatGPT, Perplexity, and Claude on Talaromyces marneffei, a thermally dimorphic pathogenic fungus of regional importance in Southeast Asia, and Aspergillus fumigatus, a mold of global importance, using 200 true/false questions on T. marneffei and A. fumigatus set and cross-validated by three full/assistant professors. There was a statistically significant difference among the median scores of the five free chatbots for the eight subsets of T. marneffei and A. fumigatus questions (p = 0.006). Dunn's test showed that the overall score of Claude 3.5 Sonnet was significantly higher than those of Perplexity (p = 0.032) and Gemini (p = 0.008). Further analysis showed that the median score of Claude 3.5 Sonnet was higher than those of Perplexity and Gemini for both the T. marneffei (p = 0.037 and p = 0.027, respectively) and A. fumigatus questions (p = 0.137 and p = 0.058, respectively). The median score obtained by Perplexity Pro was significantly higher than that of Perplexity (p = 0.038). There was no significant difference between the scores for the chatbots in the four subsets of T. marneffei and the four subsets of A. fumigatus questions. Differential performance exists for the different free/subscribed chatbots in answering the T. marneffei and A. fumigatus questions.

Keywords: Aspergillus fumigatus; Talaromyces marneffei; artificial intelligence; chatbot; fungus; global; regional; thermal dimorphic



1. Introduction

Artificial intelligence (AI) has developed beyond the research realm and evolved into practical real-life applications [1]. In the medical field, one of the most rapidly developed areas for AI is image recognition. For example, in radiology, AI-based image recognition has been used for distinguishing various malignancies against other pathologies [2]. In dermatology, it has been applied not only to skin cancers but also to other non-malignant conditions, such as psoriasis and atopic dermatitis [3]. In histopathology, it has been used for diagnosis, grading, staging and prognostic prediction of a variety of cancers [4,5]. As for clinical microbiology, we have recently demonstrated its usefulness in distinguishing different clinically important *Aspergillus* species [6]. Specifically, we demonstrated that the convolutional neural network ResNet-18 outperformed Inception-v3 and DenseNet-121 and possessed the highest testing accuracy of 99.35% for distinguishing among *A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus* [6]. Others have also used AI for the detection and classification of parasites and laboratory diagnosis of bacterial vaginosis [7–10].

ChatGPT is an advanced chatbot solution developed by OpenAI, Inc. and was introduced to the public in November 2022. Using a large language model, it allows users to shape and direct conversations according to their preferences. Users can refine and control the flow, length, format, style, level of detail and language of the conversation, which means they could have a customizable and personalized conversational experience. Through this process, users can steer the responses of ChatGPT towards their desired outcomes, whether for generating new ideas or providing detailed explanations, etc. Since its release, ChatGPT has gained wide popularity and has become one of the fastest-growing consumer software applications in history. Based on its power and innovation, it was highlighted as a member of the 2023 Nature's 10 list [11]. Its success has spurred the development of competing AI products such as Gemini as well as Claude and Copilot. Some of these software applications are available for free, whereas others may offer advanced versions that require subscriptions. All these have led to ongoing rapid and unprecedented developments in the AI field. In the medical field, ChatGPT has been widely used by students, clinicians and scientists in their studies, clinical problem solving and research [12–16]. Recently, we have shown that chatbots were as good as professors, and much better than final-year medical students, in answering questions in clinical microbiology and infectious disease [17]. However, all these chatbots examined, namely ChatGPT, Perplexity, Claude, Copilot and Gemini, were products of the USA, and there has been no study that compared the performance of these chatbots in answering questions on specific pathogens that are prevalent in the western world and those that are more of regional importance outside the western world. In order to fill these important knowledge gaps, in this study, we specifically set 100 true/false (T/F) questions on Talaromyces marneffei, a thermally dimorphic fungus endemic in Southeast Asia and another 100 on Aspergillus fumigatus, a fungus phylogenetically closely related to T. marneffei but is of global importance [18–20], to examine the performance of these five software applications as well as the free and subscribed versions of ChatGPT, Perplexity and Claude.

2. Methods

2.1. Chatbots

Five chatbots, namely ChatGPT (https://chat.openai.com) (OpenAI, San Francisco, CA, USA), Perplexity (https://www.perplexity.ai) (Perplexity AI, San Francisco, CA, USA), Claude (https://claude.ai) (Anthropic, San Francisco, CA, USA), Copilot (https://copilot.microsoft.com) (Microsoft, Redmond, WA, USA) and Gemini (https://gemini.google.com) (Google, Mountain View, CA, USA) were tested in this study (Table 1). For ChatGPT, Perplexity and Claude, both their free [GPT-40 mini (previously called ChatGPT 3.5), Perplexity and Claude 3.5 Sonnet (previously called Claude Sonnet)] and subscribed [GPT-40 (previously called ChatGPT 4.0), Perplexity Pro and Claude 3 Opus (previously called Claude Opus)] versions were examined. The chatbots were evaluated using 200 T/F questions on *T. marneffei* and *A. fumigatus* in October 2024.

Types of Characteristics Characteristics ChatGPT-40 ChatGPT-Claude 3.5 Claude 3 Perplexity Perplexity Pro Copilot Gemini mini 40 Sonnet Opus Developer OpenAI Perplexity AI Anthropic Google Microsoft LLMs Language model Natural language processing Natural language processing Natural language processing Gemini 1.5 Subscription Subscription Subscription Fee Free Free Free Free Free needed needed needed Yes Internet connection Yes Yes No No Supported languages Over 50 Over 40 Over 40 Over 40 41 No Yes Image generation Yes No Yes Exists, 5× usage Exists, but Exists, but no Limitation on the number of No 3 Pro Searches 300+ Pro No compared to free No limitation no limitation searches per day questions per day clear rules limitation service clear rules

Table 1. General characteristics of chatbots examined in the study.

2.2. Questions

The 100 T/F questions on *T. marneffei* (Table S1) and another 100 on *A. fumigatus* (Table S2) were set and cross-validated by two clinical professors of clinical microbiology and infectious disease and a non-clinical assistant professor who specializes in medical mycology. The two sets of questions were each divided into four subsets, which covered four different aspects of knowledge on the two fungi, including (1) taxonomy and basic mycology, (2) epidemiology and clinical disease, (3) laboratory diagnosis and (4) treatment and prevention.

2.3. Scoring

The final answers decided by the three professors/assistant professor were considered as the correct answers. One mark was awarded for every correct answer. No mark was deducted for wrong answers. 0.5 marks were awarded for a pass.

2.4. Runtime Calculation

A specific model of laptop computer, HP ZBook 17 (Hewlett-Packard, Palo Alto, CA USA) equipped with an Intel Core i7-4800MQ processor running at 2.7 GHz, 32 GB of RAM and an NVIDIA Quadro K4100M GPU, was used for runtime calculation. The laptop computer was connected to the internet through an iPhone 12 Pro, which provided up to 300 Mbps on the 5 GHz band. The upload speed was 94.6 Mbps and the download speed 90.3 Mbps, as tested by Speedtest from Ookla (https://www.speedtest.net/zh-Hant, accessed on 20 May 2025). To estimate the speed of performance for each chatbot, the 100 questions on *T. marneffei* and those on *A. fumigatus* were fed to each chatbot. Each experiment was performed 10 times. The runtime was defined as the time between the pressing of the "Enter" key on the chatbot and the appearance of the results on the computer screen.

2.5. Statistical Analysis

Comparison of the scores among the five free chatbots was performed using the Kruskal-Wallis test, with Dunn's test as the post-hoc test. Comparison among the scores between the subscribed and free versions of ChatGPT, Perplexity and Claude was performed using Mann-Whitney U-test. Comparison of the runtime among the chatbots was performed using the Kruskal-Wallis test.

3. Results

3.1. Correct vs. Wrong Answer Distribution

The answers provided by the five chatbots as well as the subscribed versions of ChatGPT, Perplexity and Claude are shown in Tables S1 and S2. All 100 questions on *T. marneffei* and another 100 on *A. fumigatus* were answered by all the chatbots as "True" or "False", without any "Pass". The distribution of the percentage in correct vs. wrong answers were similar for both *T. marneffei* and *A. fumigatus* questions (Figure 1). There is no significant difference between the overall performance of the chatbots for the *T. marneffei* and *A. fumigatus* questions (median = 88% correct for both sets).

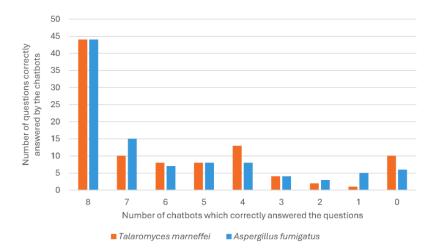
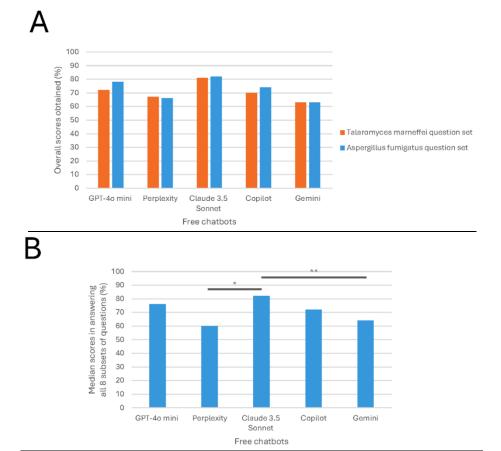


Figure 1. Distribution of correct vs. wrong answers for Talaromyces marneffei and Aspergillus fumigatus questions.

3.2. Performance of Free Chatbots

As a whole, Claude 3.5 Sonnet achieved the highest scores (81% and 82% respectively), and Gemini the lowest scores (63% for both sets), for both the T. marneffei and A. fumigatus question sets (Figure 2A). Statistically, there was significant difference among the median scores of the five free chatbots (GPT-40 mini, Perplexity, Claude 3.5 Sonnet, Copilot and Gemini) in answering the eight subsets of T. marneffei and A. fumigatus questions (p = 0.006 by Kruskal Wallis test). Post-hoc Dunn's test showed that the median overall score of Claude 3.5 Sonnet was significantly higher than those of Perplexity (p = 0.032) and Gemini (p = 0.008), respectively (Figure 2B). Further analysis on the scoring between Claude 3.5 Sonnet and Perplexity and between Claude 3.5 Sonnet and Gemini on the four subsets of T. marneffei questions and the other four of A. fumigatus questions showed that the median score of Claude 3.5 Sonnet was higher than those of Perplexity and Gemini for both the T. marneffei (p = 0.037 and p = 0.027 respectively by Mann-Whitney U-test) and A. fumigatus questions (p = 0.137 and p = 0.058 respectively by Mann-Whitney U-test) (Figure 2C).



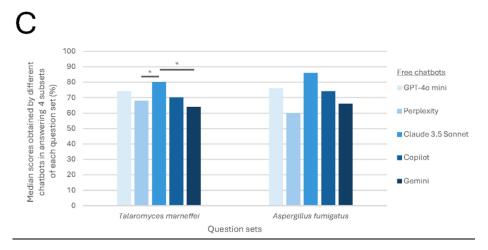


Figure 2. Comparison of the free chatbots in answering the *Talaromyces marneffei* and *Aspergillus fumigatus* questions. (A) Overall scores for the *T. marneffei* and *A. fumigatus* question sets. (B) Median scores for the eight subsets of questions. (C) Median scores for the four subsets of *T. marneffei* and four subsets of *A. fumigatus* questions. * $p \le 0.05$; ** $p \le 0.01$.

3.3. Performance of Free vs. Subscribed Versions

Three chatbots (ChatGPT, Perplexity and Claude) are available in both free (GPT-40 mini, Perplexity and Claude 3.5 Sonnet) and subscribed (GPT-40, Perplexity Pro and Claude 3 Opus) versions. The median score in answering the eight subsets of T. marneffei and A. fumigatus questions obtained by Perplexity Pro was significantly higher than that of Perplexity (p = 0.038 by Mann-Whitney U-test) (Figure 3), but there was no significant difference between the median scores obtained by GPT-40 and GPT-40 mini, and between those obtained by Claude 3 Opus and Claude 3.5 Sonnet (Figure 3).

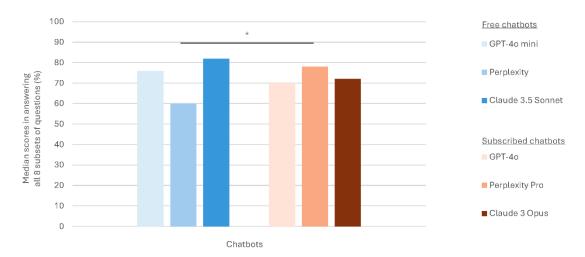


Figure 3. Comparison between free and subscribed chatbots in answering the eight subsets of questions. * $p \le 0.05$.

3.4. Performance of Chatbots on Questions for T. marneffei vs. A. fumigatus

Overall, there was no statistically significant difference between the median score for all the chatbots in answering the four subsets of *T. marneffei* questions and the four subsets of *A. fumigatus* questions. Further analysis also showed no statistically significant difference between the median score of any of the chatbots, the free chatbots, or the subscribed chatbots in answering the four subsets of *T. marneffei* questions and the four subsets of *A. fumigatus* questions.

3.5. Runtime Calculation

As a whole, the median runtimes for Perplexity Pro, GPT-40 and Copilot were the longest and those for Perplexity, GPT-40 mini and Claude 3 Opus were the shortest. Statistically, there was significant difference among the median runtimes for the eight free and subscribed chatbots in answering the four subsets of *T. marneffei*

questions and those for A. fumigatus (p = 0.0001 by Kruskal Wallis test for both analysis). For both the T. marneffei and A. fumigatus questions, post-hoc Dunn's test showed that the runtimes for Perplexity, GPT-40 mini and Claude 3 Opus were significantly shorter than those for Perplexity Pro, GPT-40 and Copilot (Figure 4). In addition, for the T. marneffei questions, Dunn's test showed that the runtime for Perplexity was significantly shorter than that for Gemini; and for the A. fumigatus questions, Dunn's test showed that the runtime for Claude 3 Opus was significantly shorter than that for Gemini whereas the runtime for Claude 3.5 Sonnet was significantly shorter than that for Copilot (Figure 4).

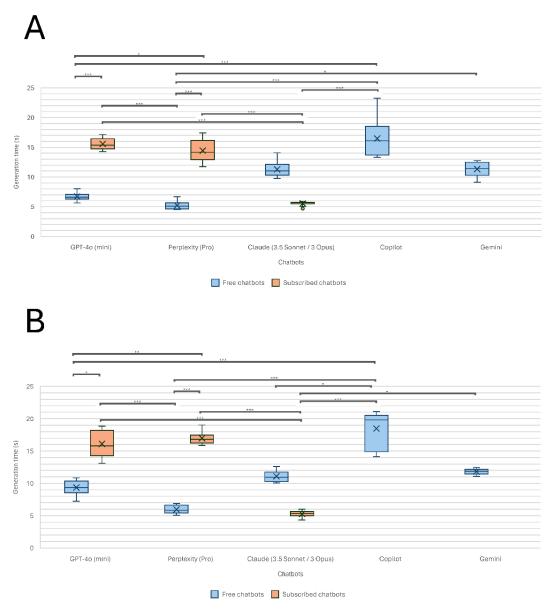


Figure 4. Answer generating time for the chatbots. (**A**) *Talaromyces marneffei* question subset. (**B**) *Aspergillus fumigatus* question subset. * $p \le 0.05$; ** $p \le 0.01$; *** $p \le 0.001$.

4. Discussion

In this study, we specifically examined the knowledge of a number of chatbots in two distinct but phylogenetically related fungal pathogens. Among the known *Aspergillus* species, *A. fumigatus* is the most common species causing human infections on a global scale. It is associated with the highly fatal invasive aspergillosis in patients with hematological malignancies undergoing chemotherapy and transplant recipients, as well as other chronic conditions with high morbidity, such as aspergilloma and chronic pulmonary aspergillosis [21,22]. On the other hand, *T. marneffei* is of regional importance in Southeast Asia; and is classically associated with infections in HIV-positive patients, although in the last decade infections due to this fungus have emerged in HIV-negative patients [23,24]. Clinically, *T. marneffei* infections often present with non-specific symptoms, sometimes mimicking other chronic infections; and therefore, have given rise to a lot of diagnostic difficulties. In

the present study, 100 questions on *T. marneffei* and another 100 on *A. fumigatus* were set and used to examine the knowledge of the chatbots. These questions were set and cross-validated by two clinical professors of clinical microbiology and infectious disease and a non-clinical assistant professor who specializes in medical mycology. The two professors are both fellows of the Royal College of Pathologists and the Royal College of Physicians and one of them is also a fellow of the European Confederation of Medical Mycology. The two sets of questions covered a wide range of knowledge on the two fungal pathogens, including classification, genomics, basic mycology, clinical microbiology, laboratory diagnostics, antifungal resistance, clinical disease, treatment and prevention. Using these questions, we showed that there was a difference in knowledge among the free chatbots, as well as between the subscribed and free versions of Perplexity, on *A. fumigatus* and *T. marneffei*. For the free chatbots, it was observed that Claude 3.5 Sonnet performed significantly better than Perplexity and Gemini (Figure 2B,C); and for the subscribed and free versions, it was found that Perplexity Pro performed significantly better than Perplexity (Figure 3). On the other hand, it is very interesting to note that the free version of ChatGPT (GPT-40 mini) was able to achieve a higher score than its subscribed counterpart (GPT-40), although the difference had not reached a statistically significant level (Figure 3). We speculate that this could be due to a larger database of GPT-40, and some of the extra information might have confused the chatbot which resulted in some wrong answers.

Despite the difference in importance of the two fungal pathogens, the chatbots have similar performance in answering questions on T. marneffei and A. fumigatus. Since T. marneffei is not as globally important, the total number of publications that were found in PubMed search was only around 1000 by the time of writing, in contrast to more than 14,000 for A. fumigatus. In view of this, T. marneffei infection is considered as a "neglected tropical disease" [25]. In fact, this phenomenon was in line with our experience of studying the usefulness of matrixassisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) for identification of T. marneffei [26]. In that study, we showed that using the Bruker original database combined with BDAL v4.0.0.1 and Filamentous Fungi Library 1.0, MALDI-TOF MS failed to identify the 60 T. marneffei strains grown in mold and yeast phases [26]. However, when the combined database was expanded with inclusion of spectra from 21 T. marneffei strains in mold and/or yeast phases, all the remaining 39 T. marneffei strains grown in mold or yeast phases were correctly identified to the species level with scores ≥2.0. This problem of overlooking pathogens that were important regionally was not only limited to T. marneffei, but also some bacteria such as Burkholderia pseudomallei and Laribacter hongkongensis [27,28]. In the present study, in contrast to our experience on MALDI-TOF MS, the knowledge of the various free and subscribed chatbots on A. fumigatus and T. marneffei was similar. There was also no weakness or strength shown by the chatbots on any particular subset of questions for A. fumigatus or T. marneffei. This showed that although our knowledge on T. marneffei is incomparable to that of A. fumigatus due to its markedly smaller number of studies, the relative amount of knowledge acquired by the chatbots on T. marneffei was not inferior to that for A. fumigatus, indicating that there was no discrimination of knowledge input to the chatbots with respect to the geographical distribution of the microorganisms. Further studies on other closely related pathogens that are global and regional distribution (e.g., Burkholderia cenocepacia vs. Burkholderia pseudomallei) could be performed.

Marked difference among the runtimes for the free and subscribed chatbots was observed. In this study, we showed that for Perplexity and ChatGPT, the median runtimes for their free versions (Perplexity and GPT-40 mini) were significantly shorter than their subscribed versions (Perplexity Pro and GPT-40) (Figure 4). For example, the runtime for Perplexity Pro was almost triple that for Perplexity (Figure 4). On the other hand, for Claude, the median runtime for its free version (Claude 3.5 Sonnet) was significantly longer than its subscribed version (Claude 3 Opus) (Figure 4). In fact, the runtime for Claude 3 Opus was only around half of that for Claude 3.5 Sonnet (Figure 4). We speculate that the longer runtime for Perplexity Pro and GPT-40 could be due to their more extensive databases when compared with their free counterparts, whereas the shorter runtime for Claude 3 Opus may be because of its more streamlined algorithm than Claude 3.5 Sonnet. In addition to the runtime, it is also extremely interesting to note that Claude 3.5 Sonnet, despite its short runtime, was actually the free chatbot that has achieved the highest score in answering the *T. marneffei* and *A. fumigatus* questions (Figure 2). This showed that the relationship between runtime and score did not have to be linear. In order to improve the usefulness of the chatbots, further development and refinement should aim at maximizing the knowledge base and reducing the runtime.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2507210937470433/eMicrobe-1235-Supplementary-Materials.pdf. Table S1: True/false questions on *Talaromyces marneffei* and answers provided by chatbots. Table S2. True/false questions on *Aspergillus fumigatus* and answers provided by chatbots.

Author Contributions

Z.-J.L.: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing—original draft, Writing—review & editing. C.-C.T.: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing. C.-S.W.: Writing—original draft, Writing—review & editing. S.K.P.L.: Data curation, Investigation, Methodology, Writing—review & editing. P.C.Y.W.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing—original draft, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Raw data will be made available upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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