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THE MOST GRACIOUS, THE MOST MERCIFUL

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contents

ORIGINAL ARTICLES

CASE REPORT

ORIGINAL ARTICLE

Trends in Utilizing CT Scan in the Emergency Department: Correlation with Significant Imaging Findings and Subsequent Hospital Admissions. A Retrospective Study

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Abstract

Objective: To assess the association between boarding time in the emergency department (ED) and increased patient mortality at the King Abdul-Aziz University Hospital in Jeddah, Saudi Arabia.

Methods: A retrospective study was conducted on 28,066 patients admitted through the ED and discharged from January to December 2019 at the King Abdul-Aziz University Hospital in Jeddah, Saudi Arabia. Data on patients' demographics, age, nationality, arrival method, eligibility, triage level, and outcome (alive or dead) was collected from medical records.

Results: This study found a strong link between patient mortality and boarding time, with patients who died experiencing significantly longer boarding times than those who survived. The average boarding time for those who died was more than 12 hours, compared to only 2.2% of those who did not. Additionally, the death rate was higher among non-eligible and male patients. Patients with injuries or orthopedic diseases had a notably increased likelihood of boarding for more than 12 hours. Both long-term boarding and non-eligibility for management were risk factors for death among the patients studied.

Conclusion: This paper highlights the impact of protracted boarding times on patient outcomes and emphasizes the urgent need to take immediate steps to decrease ED boarding.

Keywords

Association, Boarding, Emergency, Mortality

INTRODUCTION

Emergency department (ED) overcrowding is a significant challenge for hospitals and is considered a hospital-wide problem[1]. It has been described as "the most serious issue confronting EDs in the developed world[2]. It is associated with poor quality of care and unfavorable events following ED assessment[3,4], leading to delays in treatment and high complication rates, which have increased the mortality rate[5-12]. Although unnecessary ED visits are thought to be the cause of ED overcrowding, ED boarding seems to be the leading cause, characterized by patient waiting time in the ED after primary assessment and initial care[7, 13,14]. ED boarding can have serious implications that act as major barriers to advanced medical treatment and can lead to delays in time-sensitive procedures[14,15]. For example, the outcome in sepsis and septic shock patients can demonstrate a substantial change if seen in early target therapy[16,17]. Studies conducted in France, Greece, and Canada found that an increased length of stay and boarding in the ED is associated with increased mortality and comorbidity[17,18,19]. However, a large retrospective study showed prolonged stays in the emergency department may increase the risk of mobility problems for patients but not necessarily increase motility[20].

In the Kingdom of Saudi Arabia (KSA), ED non-emergency cases are considered the leading cause of ED overcrowding, adversely affecting healthcare providers, patients, and the country's economy [21]. In KSA culture, patients prefer to stay in the ED for better care. A 2018 study was conducted to identify the preferences of patients and their attendants regarding staying in the ED or being transferred to inpatient units. The results indicated that 59% of patients preferred staying in the ED due to the facilities and prompt attention provided by the staff. The study emphasized the importance of a timely transfer to inpatient units to reduce medical mistakes and complications, especially in overcrowded EDs. However, until now, there has not been enough data to support the idea that there is a clear relationship between a long stay in the ED and mortality from local hospitals in KSA.

Given the controversy surrounding the issue and the limited number of studies conducted in KSA, this retrospective study aims to examine the association between boarding time in the ED and increased patient mortality at King Abdul-Aziz University Hospital in Jeddah, Saudi Arabia, in 2020–2021.

SUBJECTS AND METHODS

Study design and time frame: This study was a retrospective study conducted from 2020 to 2021.

STUDY SETTING

The study was conducted at the Emergency Department (ED) of the King Abdul-Aziz University Hospital in Jeddah, Saudi Arabia. This tertiary care center serves the middle- and low-income population in Jeddah.

STUDY POPULATION

All patients admitted to the hospital through the ED and discharged from January to December 2019 were included, leaving a sample size of 28,066 patients. Patients with missing data on the system, OB patients, and pediatrics were excluded.

TIMELINE

In this study, we will consider a stay in the ED for more than 12 hours to be a prolonged ED stay.

DATA COLLECTION

Data was collected from patients' medical records, including demographics, age, nationality, arrival method, eligibility, outcome, triage level, and whether they died or were still alive.

STATISTICAL ANALYSIS

Data was coded, tabulated, and analyzed using SPSS version 20 (Armonk, NY: IBM Corp.). Qualitative data was expressed as numbers and percentages, and the Chi-squared test ($\chi 2$) was used to test the relationship between variables. Quantitative data was expressed as mean and standard deviation (Mean ± SD), and the Mann-Whitney test was used for non-parametric variables. Binary logistic regression was used to assess the independent predictors of death in the ED. A p-value of < 0.05 was considered statistically significant.

RESULTS

The total number of patients enrolled in the study was 28,066 patients. Table 1 shows that the age of male participants was 34.17 ± 20.76 years, with 50.4% male and 78.7% Saudi nationals. 52.8% of patients had a triage level of priority 4 (less urgent), 99.2% arrived by

Table 1. Distribution of the studied patients according to their characters, arrival method, eligibility, outcome, triage level, and their fate

Variable	No (%)
Age (mean SD)	34.17±20.76
Boarding time (mean SD)	4.35 ± 3.79
Gender	
Female	13,919 (49.6)
Male	14,147 (50.4)
Nationality	
Saudi	22,095 (78.7)
Non - Saudi	5,971(21.3)
Triage level	
Priority 1. Resuscitation	327 (1.2)
Priority 2. Emergent	4,008 (14.3)
Priority 3. Urgent	8,039 (28.6)
Priority 4. Less Urgent	1,480 (52.8)
Priority 5. Non-Urgent	887 (3.2)
Arrival method	
Ambulance	229 (0.8)
Other (Arrival)	27,837 (99.2)
Eligible	
No	25,543 (91.0)
Yes	2,523 (9.0)
Arrival timing	
am	16,302 (58.1)
pm	11,764 (41.9)
Boarding time (mean ± SD)	4.35 ± 3.79
Fate	
No death	27,655 (99.5)
Death	411 (1.5)
Comorbidities	
Heart disorders	4,582 (16.3)
Hypertension	3,075 (11.0)
Diabetes mellitus	2,902 (10.3)
GIT disorders	2,885 (10.3)
Pulmonary disorders	3,590 (12.8)
Neurologic disorders	2,145 (7.6)
Musculoskeletal disorders	3,376 (12.0)
Skin disorders	969 (3.5)
ENT disorders	777 (2.8)
Injury and orthopedic disorders	637 (2.3)
Urinary disorders	577 (2.1)
Thyroid disorders	653 (2.3)
Blood disorders	587 (2.1)
Fluid and electrolyte disorders	56 (0.9)
Deficiency anemias	414 (1.5)
Psychological disorders	631 (2.2)

a method other than ambulance, 91% were eligible for management in the ED, and 76.9% were discharged. For 77.1% of patients, the main responsible physician (MEP) was an emergency physician. The average boarding time was recorded as 4.35 ± 3.79 hours. Of all patient arrivals, 58.1% occurred during the day shift (7 AM to 7 PM), while 41.9% occurred during the night shift (7 PM to 7 AM). The total mortality rate was found to be 1.5% for all arrivals. The most common comorbidities among patients were heart disorders (16.3%), followed by pulmonary disorders (12.8%), and musculoskeletal disorders (12%).

Figure 1 shows that half of the participants (50.8%) had a boarding time of less than two hours, while 2.9% had a boarding time of more than 12 hours.

Table 2 shows that patients who died had a significantly higher boarding time (11.23 ± 5.56 hours) compared to those who did not die (4.24 ± 3.66 hours), with a p-value of < 0.001. 46.2% of patients who died had a boarding time of more than 12 hours, compared to only 2.2% for those who did not die (Figure 2). Furthermore, there was a significant relationship between patients' deaths and their eligibility status, with a *p*-value of 0.006. However, no significant relationship was found between patients' deaths and their age, nationality, gender, arrival method, arrival timing, outcome, MRP, triage level, or comorbidities with a p-value of > 0.05.

Table 3 shows that male patients had a significantly longer boarding time of more than 12 hours compared to female patients. A non-significant difference was found between boarding time and patients' age, nationality, arrival method, eligibility, outcome, MRP, arrival timing, or triage level ($p = > 0.05$). Patients who had injuries or orthopedic disorders had a significantly higher percentage of those who had boarding time of more than 12 hours (5.2%), followed by those who had neurological disorders (3.8%) and those who had pulmonary disorders (3.5%) ($p = < 0.05$).

Table 4 shows that binary logistic regression analysis found long boarding time and non-eligibility for management as independent predictors of death among studied patients.

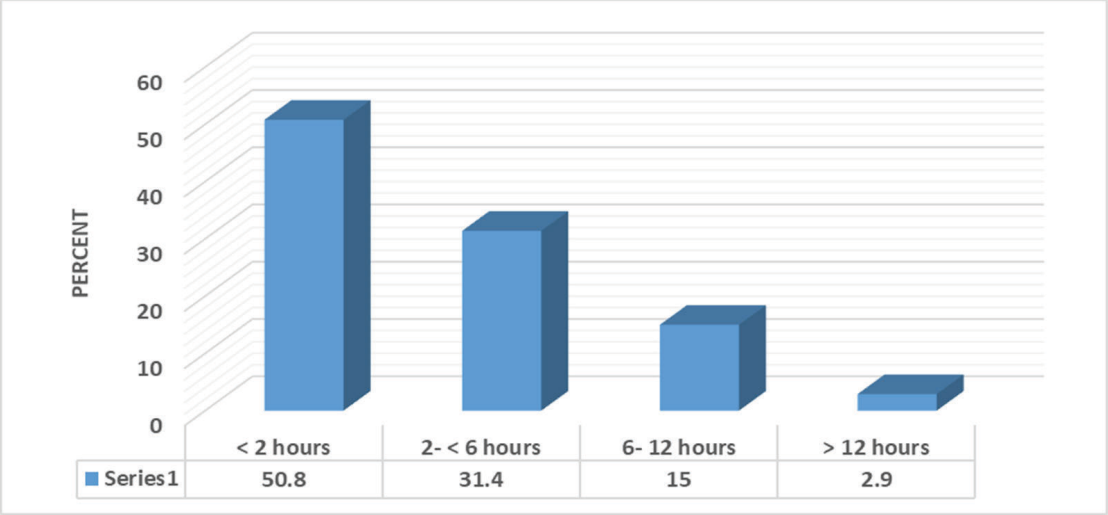


Figure 1. **Distribution of the studied patients according to their boarding time.**

Table 2. **Relationship between patients' death and their characters, arrival method, eligibility, outcome, triage level, and comorbidities**

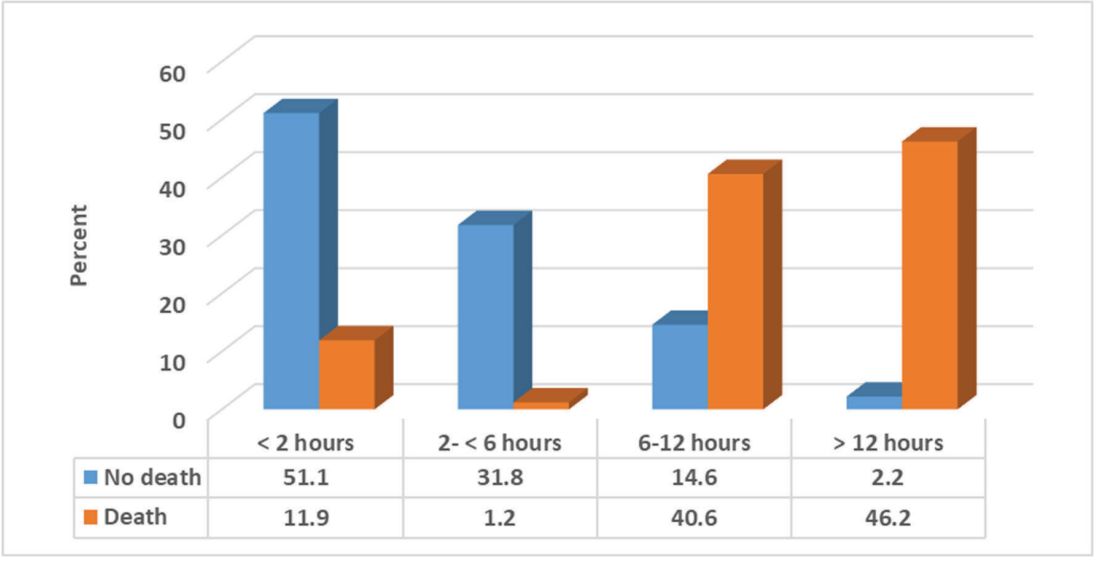
Variable	Death		Test	*p*-value
	Death	No Death		
Age	34.16 ± 20.74	35.11 ± 22.22	0.53*	0.59
Boarding time	11.23 ± 5.56	4.24 ± 3.66	24.4*	< 0.001
Gender				
Female Male	213 (1.5) 198 (1.4)	13706 (98.5) 13949 (98.6)	0.83**	0.36
Triage level				
Priority 1 - Resuscitation Priority 2 - Emergent Priority 3 - Urgent Priority 4 - Less Urgent Priority 5 - Non-Urgent	2 (0.6) 75 (1.9) 102 (1.3) 218 (1.5) 14 (1.6)	325 (99.4) 3933 (98.1) 7937 (98.7) 14587 (98.5) 873 (98.4)	8.46**	0.07
Nationality				
Saudi Non - Saudi	314 (1.4) 97 (1.6)	21781 (98.6) 5874 (98.4)	1.34**	0.24
Arrival method				
Ambulance Other	3 (1.3) 408 (1.5)	226 (98.7) 27429 (98.5)	0.03**	0.84
Eligibility				
No Yes	52 (2.1) 359 (1.4)	25184 (98.6) 2471 (97.9)	6.93**	0.006
Arrival timing				
am pm	16057 (98.5) 11598 (98.6)	245 (1.5) 166 (1.4)	0.39	0.52

*N.B.: *$\chi 2$; **Mann-Whitney test*

Table 3. Relationship between boarding time and patients' characters, arrival method, eligibility, outcome, triage level, fate, and comorbidities

Variable	Boarding Time				Test	*p*-value
	< 2 hours	2- < 6 hours	6-12 hours	> 12 hours		
Age	34.28 ± 10.78	34.07 ± 20.54	34.06 ± 20.79	33.85 ± 22.58	3**	0.47
Gender						
Female Male	7123 (51.2) 7126 (50.4)	4288 (30.8) 4523 (32)	2132 (15.3) 2068 (14.6)	376 (2.7) 430 (3.0)	9.01*	0.02
Nationality						
Saudi Non - Saudi	11244 (50.6) 3005 (50.3)	6894 (31.2) 1917 (32.1)	3327 (15.1) 873 (14.6)	630 (2.9) 176 (2.9)	2.26*	0.51
Triage level						
Priority 1 - Resuscitation Priority 2 - Emergent Priority 3 - Urgent Priority 4 - Less Urgent Priority 5 - Non-Urgent	166 (50.8) 2042 (20.9) 4043 (50.3) 7554 (51.0) 444 (50.1)	99 (30.3) 1216 (30.3) 2570 (32) 4642 (31.4) 284 (32)	48 (14.7) 628 (15.7) 1200 (14.9) 2185 (14.8) 139 (15.7)	14 (4.3) 122 (3.0) 226 (2.8) 424 (2.9) 20 (2.3)	9.2*	0.66
Arrival method						
Ambulance Other	120 (52.4) 14129 (50.8)	62 (27.1) 8749 (31.4)	39 (17.0) 4161 (14.9)	8 (3.5) 798 (2.9)	2.46*	0.48
Eligibility						
No Yes	1299 (51.5) 12950 (50.7)	761 (30.2) 8050 (31.5)	379 (15) 3821 (15)	84 (3.3) 722 (2.8)	2.64*	0.3
Arrival timing						
am pm	8176 (50.2) 6073 (51.6)	5179 (31.8) 3632 (30.9)	2470 (15.2) 1730 (14.7)	477 (2.9) 329 (2.8)	5.95	0.11

*N.B.: *$\chi 2$; **Mann-Whitney test*



*N.B.: *$\chi 2$; **Mann-Whitney test*

Figure 2. Relationship between patients' death and boarding time.

Table 4. Binary logistic regression analysis regarding the risk factors for death

Variable	Depression			*p*-value
	Beta	Wald	Exp. (B)	
Boarding time	1.86	839.82	6.46	< 0.001
Eligibility	0.36	5.08	1.43	0.02

DISCUSSION

The current research aimed to assess the association between boarding time in the ED and increased patient mortality at the King Abdul-Aziz University Hospital. Patients who died had a significantly longer boarding time than those who survived, indicating a highly significant link between death and boarding time. This study found that 46.2% of patients who died had a boarding time of more than 12 hours compared to 2.2% for those who did not die, with a statistically significant difference. Our results are similar to previous studies, which found lower mortality among patients transferred out of the ED in less than 6 hours[22,23,24,25].

About half of the participants (50.8%) had a boarding duration of less than two hours, while just 2.9% had a boarding time of more than 12 hours. Patients who were sicker and required more resource-intensive inpatient care had the greatest ED waiting time, according to a prior study. These excessive wait times are linked to a much longer length of stay in the hospital[19]. Another study found that patients who were in the ED for more than 4 hours before being transferred to an inpatient bed had a higher risk of dying in the hospital[17].

Singer et al. discovered a similar link between patient fatality and boarding time. This study discovered that hospitalized patients who spent more than four hours in the emergency room were at a higher risk of serious health problems and mortality[26]. Intas et al. discovered that ED boarding for longer than 6 hours was linked to a higher risk of death in the hospital[27]. Patients presenting to the emergency department during shifts with longer waiting times were linked to a higher risk of death and hospital admission in the near term, according to a study conducted in the United States[7].

Our study showed that patients with orthopedic or neurological disorders and injuries had a significantly higher percentage of boarding time of more than 12 hours (5.2%). This is because these patients require more time for stabilization and imaging. These findings are consistent with a study by Nori et al. that showed that the mean stay time in the ED for boarded and non-boarded orthopedic patients was 1.07 hours and 10.18 hours, respectively[28].

In the present study, 52.8% of the patients were non-emergency cases, and 99.2% were walk-in patients to the ED, which is considered the primary cause of ED overcrowding. This matter can affect health caregivers, care recipients, health supplies, and eventually, the country's economy[29]. Primary health care, staff clinics, family physicians, urgent care centers, and even home healthcare services can help in most circumstances. The current study found that 52.8% of the patients were non-emergency cases, and 99.2% of them were walk-in patients to the ED, which is the main cause of ED overcrowding. This issue impacts healthcare providers, patients, medical supplies, and the country's economy[30]. However, alternatives such as primary health care, staff clinics, family physicians, urgent care centers, and home healthcare services can help in most situations. In Saudi Arabia, citizens have complete access to KAUH without any restrictions to receive free medical treatment for people of all ages, and medical services are available to everyone equally. The Ministry of Health serves primary health care through a network of regional health centers[31]. For the past 20 years, the government has funded new and innovative programs to ensure health facilities are available to all citizens at all care levels including primary, secondary, and tertiary centers like KAUH ED, which accept referrals from various areas.

Furthermore, non-Saudi tourists accounted for 21% of all ED visits. According to current Saudi legislation, any health insurance must cover all non-Saudis (excluding sponsored individuals) before performing their legal papers. Health insurance in Saudi Arabia requires the prior submission of papers, which includes all nationalities (excluding individual sponsorships)[30, 32]. However, a previous study found that the sickest hospital patients are "frequent flyers." Their admission

rates are the highest, their death rates are the highest, and their resource consumption is disproportionately high[33].

Various strategies can be used to alleviate ED overpopulation. Bedside registration and effective utilization of hospitalists are examples of internal departmental advances. Additional solutions include provider triage and provider scheduling optimization. Adding beds to the emergency room does not always solve the problem of boarding and overcrowding. Externally, smoothing elective (schedulable) admissions is likely the single most important strategy for increasing capacity, reducing boarding, maintaining nurse-to-patient ratios, and improving ICU access. Early morning discharges from inpatient units also substantially decrease ED boarding[34, 35]. Increased weekend discharges and improved weekend services will increase capacity and reduce boarding. When an institution's bed capacity is exceeded, a full-capacity strategy, in which inpatient units go over the census rather than boarding patients in the ED, is safer, preferable to patients, and reduces the duration of stay.

LIMITATIONS

Considering the limitations of the study design, a cause-and-effect relationship is difficult to establish. A retrospective analysis of the data also carries the risk of missing data or inaccurate entries.

CONCLUSION

This study discovered a significant association between patient death and boarding time, with patients who died having a significantly longer boarding time than those who survived. About 46% of patients who died had an average boarding time of more than 12 hours, compared to 2.2 percent of those who did not. The death rate was significantly higher among non-eligible patients and male patients, and those with injuries or orthopedic diseases had a significantly higher percentage of those who had more than 12 hours of boarding time. Long-term boarding and non-eligibility for management were both risk factors for death among the patients investigated. The impact of protracted boarding times on patient outcomes is highlighted in this paper, highlighting the necessity for immediate steps and attempts to decrease ED boarding.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the contents of the manuscript. There are no financial interests to disclose. We certify that the submission is an original work and is not under review at any other publication.

DISCLOSURE

The authors did not receive any form of commercial support, either in the form of compensation or financial assistance, for this case report. The authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

This is a retrospective study for which the consent requirement was waived. The study was conducted in accordance with the Declaration of Helsinki, and the Ethics Committee of King Abdulaziz University approved the protocol (Ethical Approval Reference No 281-18).

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ORIGINAL ARTICLE

Visual Interpretation of Pediatric Panoramic Radiographs: An Eye-Tracking Mixed-Method Observational Study

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Abstract

Background: Recently, the use of eye-tracking technology (ETT) has gained popularity in the medical field for interpreting visual gaze patterns. However, its application in dental research remains in its early stages. We investigated the performance of postgraduate students (PGs) and dental interns in detecting abnormalities in pediatric panoramic radiographs using ETT. We explored the association between different eye-tracking (ET) measurements and students' detection accuracy. Finally, we assessed participants' experiences with ETT as a learning aid.

Methods: This mixed-method observational study involved 30 pediatric PGs and 32 dental interns at King Abdulaziz University Dental Hospital. Nine pediatric panoramic radiographs containing abnormalities were used for the text. A RED-m® SMI ET system tracked participant gaze patterns and BeGaze software identified abnormalities. Parameters extracted included entry time, dwell time, fixation time, fixation count, and revisit count. Univariate, bivariate, and multivariable analyses were conducted. Semi-structured interviews were analyzed and categorized into themes using NVivo 11.

Results: PGs were more successful at identifying abnormal radiographs compared to dental interns (p = 0.003) with PGs revisiting areas of interest (AOIs) significantly more often (p = 0.003). Interestingly, for each additional revisit, the odds of correct detection increased by 1.17 (p = 0.009). The interview data analysis uncovered five themes: ETT experience, challenges encountered, ETT as an educational tool, anticipated improvements, and final recommendations.

Conclusion: This study employed ETT and demonstrated that pediatric PGs have a higher likelihood of correctly identifying abnormalities in radiographs. Additionally, PGs exhibited a significantly higher frequency of revisiting AOIs compared to dental interns.

Clinical significance: This study objectively assessed how dentists at different training levels detect lesions in pediatric dental radiographs. It also explored the potential of ETT as an innovative pedagogical aid in dental education.

Keywords

Eye-tracking technology, Eye gaze, Education, Panoramic radiography, Pediatric dentistry

INTRODUCTION

In pediatric dentistry, panoramic radiography is a preliminary tool for detecting developmental defects, odontogenic cysts, various inflammatory diseases, and systemic metabolic disorders[1]. Pediatric tumors and cysts in the jaws are often asymptomatic, posing challenges for interpretation, especially for undergraduate students[2]. Fortunately, most pediatric oral tumors are benign, with only 3% classified as malignant[3]. However, radiographic misinterpretations can result in inaccurate diagnoses[4], potentially delaying treatment[5].

Eye-tracking technology (ETT) can aid in understanding how clinicians visually identify pathologies in dental radiographs[6]. It provides an objective and reliable way to analyze factors influencing an observer's decision-making and the causes of misdiagnosis[7–9]. By tracking specific visual search parameters, ETT can shed light on the reasons behind false positive and false negative diagnoses[6].

ETT is gaining popularity in both medicine and dentistry, with a surge in its use for dental research[10–11]. This study investigated the performance of postgraduate dental students (PGs) and dental interns in detecting abnormalities in pediatric panoramic radiographs using ETT. We explored the association between different eye-tracking (ET) measurements and students' odds of correct detection. Finally, we assessed participants' experiences with ETT as a learning aid through semi-structured interviews. ETT allows for objective recording of participants' visual attention, enabling researchers to identify factors influencing decision-making and sources of misdiagnosis[7–10].

Beyond enhancing learning and clinical skills for dentists, ETT holds exciting possibilities for improving the dentist's work environment. By tracking eye movements during procedures, researchers can identify areas of visual focus and potential ergonomic challenges[12]. This information can guide the design of dental equipment and operatory layouts that optimize efficiency, reduce fatigue, and minimize musculoskeletal disorders among practitioners. Similarly, ETT can benefit patient care. A study analyzing where pediatric patients look upon entering a dental clinic highlights the potential of ETT to inform strategies for reducing patient anxiety and fostering trust, ultimately leading to better patient engagement during dental treatment[13–14].

ETT offers innovative opportunities for dental education. By tracking how students look at various instructional materials and educators, ETT can provide insights into their attention patterns, comprehension levels, and areas of difficulty[2–6]. This technology can also be used to assess learning outcomes by comparing the viewing patterns of students at different stages of their dental education. Vogel *et al.* observed a positive correlation between viewing time and completeness in reading radiographs, with participants being more likely to detect important findings with increased viewing duration. Additionally, those with more education and experience exhibited a more thorough scan pattern[2]. By understanding how students engage with different clinical materials over time using ETT, educators can develop more effective teaching methods, ultimately leading to improved patient care[15]. Thus, the aim of this study was to explore the association between different eye-tracking measurements and students' detection accuracy and to assess participants' experiences with ETT as a learning aid.

METHODS

This mixed-method observational study received ethical approval from the Ethics Committee at King Abdulaziz University Dental Hospital (KAUDH) (#112-10-22) and adhered to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines[16]. Due to the limited number of actively enrolled PG pediatric dental students, our target population encompassed all of them. Consequently, we employed a census approach (enrolling all members) instead of a sample. All PGs were invited via email to participate voluntarily; 30 agreed, while 2 declined. To ensure a comparable control group, we concurrently recruited and enrolled 32 dental interns. Written informed consent was obtained from the participants before they began the computer-based test. To maximize participation, we offered a gift card as an incentive.

STIMULI

To create a test set of panoramic dental radiographs, we extracted images from the KAUDH electronic health system, specifically the database of the oral pathology department. This database included patients diagnosed with jaw lesions between 2015 and 2018. To minimize student recognition of any patient, radiographs treated within the past three years were excluded. All cases required the presence of at least one abnormality. A

panel of three pediatric dentists and an oral radiologist (all with at least five years of clinical experience) from KAUDH reviewed the selected cases to ensure good radiographic quality.

The nine (n = 9) panoramic radiographs used in this study presented various abnormalities designated as areas of interest (AOIs). These included dentigerous cysts, odontogenic keratocysts, fibrous dysplasia, periapical radiolucencies, rudimentary teeth, retained primary teeth, and dilacerated roots. No image manipulation was performed. The type of lesion for each patient was documented and confirmed using their dental records. All identifying information was removed, and each case was assigned a de-identified serial number.

RELIABILITY

To assess the test's reliability, we adopted exam question items from two ET studies in the field of dental education[8-9]. The test was divided into three scoring sections: lesion detection, lesion location, and total score. For lesion detection, a score of 0 was assigned for reporting "No abnormality" and 1 for "Yes, there is an abnormality." Lesion location scores ranged from 0 to 6, with 0 indicating "no abnormality reported," 1 indicating an abnormality in one sextant, and so on. Test-retest reliability was measured with a two-week interval between assessments. Cronbach's alpha was used to analyze reliability for lesion detection, lesion location, and total score.

Nineteen (19) out of the 22 students recruited for testing internal consistency and reliability completed the test-retest process. All participants were dental interns rotating at KAUDH and were excluded from the main experiment. Cronbach's alpha for internal consistency was 0.77 for lesion detection, 0.89 for lesion location, and 0.84 for the total score. These results indicate good internal consistency and reliability of the test for this population.

PROCEDURE

The experiment took place in a quiet, dimly lit room with no distractions in the participant's field of view. Participants viewed the radiographs independently on a 15.6-inch laptop screen (Latitude E6530, Dell Corporation, Round Rock, TX, USA) positioned at their eye level using a magnetic strip. The RED-m® SMI software (Sensomotoric Instruments, Teltow, Germany) tracked their gaze movements as they looked at the x-rays on the screen. AOIs were pre-identified on each radiograph using BeGaze software (Sensomotoric Instruments, Teltow, Germany) (Figures 1D and F). This software recorded various ET parameters including entry time, fixation time, fixation counts, revisit counts, and dwell time.

Before starting the experiment, all participants completed a five-point eye tracker calibration to ensure accurate data collection. Individuals with gaze deviation ≥ 1° were excluded. Inclusivity was maintained by allowing students wearing contact lenses or glasses to participate.

Participants received a verbal explanation of the experiment procedures and instructions before being seated comfortably. The laptop screen was positioned perpendicular to the floor, with a viewing distance of 50–75 cm between the participant's eyes and the screen. The experiment began with a section collecting demographic information: age category, sex, years of clinical experience, and academic level (dental intern or PG). Finally, the radiographic images were presented in the same random order to each participant.

Participants viewed the radiographs at their own pace. Upon finishing the slides, they were presented with an optional survey asking if they were interested in receiving their test reports and participating in an interview about their experience using ETT as a learning aid. A blank screen then appeared, signifying the completion of the test. The entire procedure took approximately 20 minutes. Data collected for each participant was automatically transferred from the experimental software (Experiment Center 3.3, Sensomotoric Instruments, Teltow, Germany) to the analysis software (BeGaze).

MEASURES

Participants viewed and analyzed one panoramic radiograph at a time, followed by a series of three questions assessing their ability to identify and localize abnormalities. The first question was a binary choice: "Are there any abnormalities in the radiograph?" The second question asked participants to either locate any abnormalities by selecting the affected sextants on the image or indicate if the radiograph appeared normal. Finally, if an abnormality was identified, participants

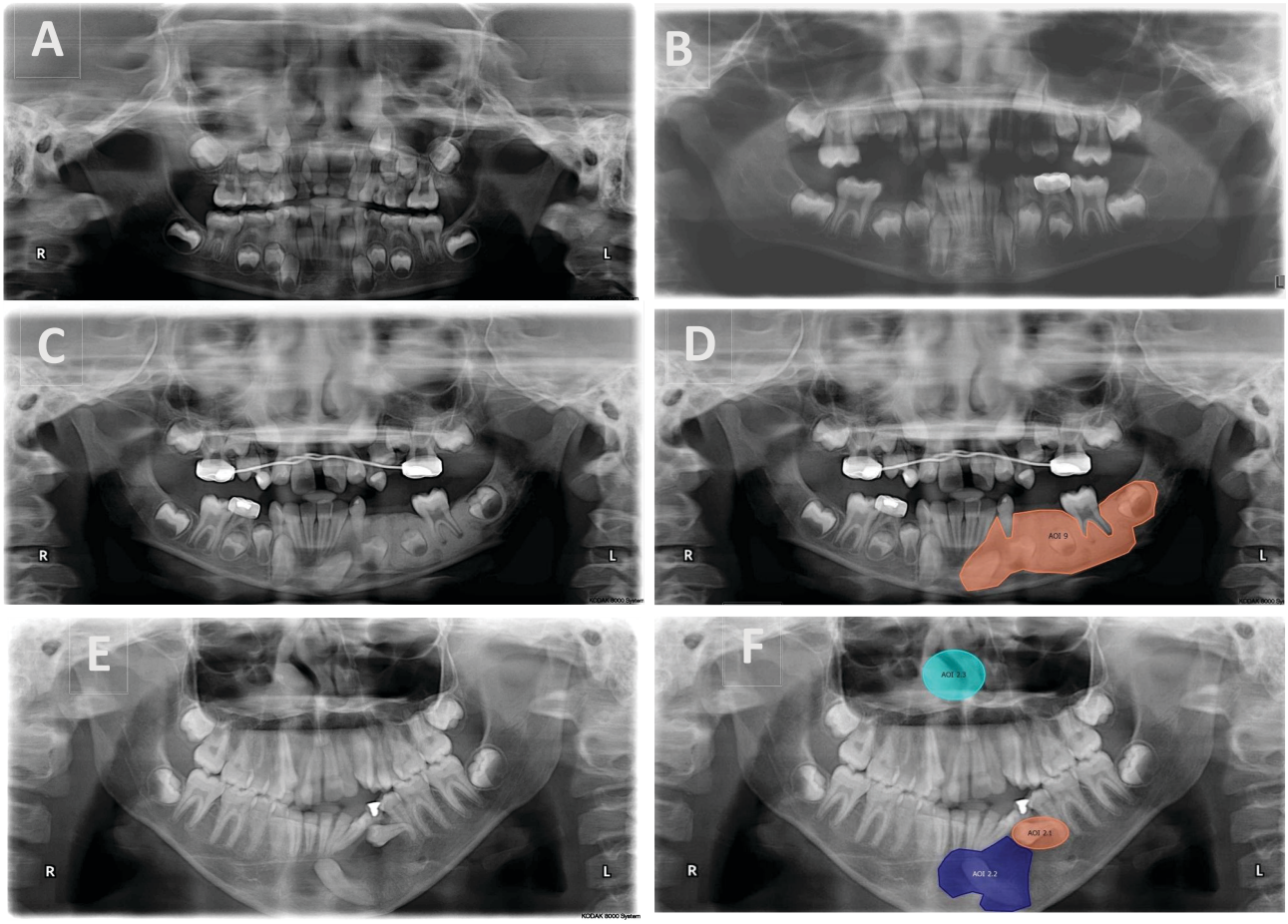

Figure 1. **(A, B) Normal panoramic radiograph; (C) Abnormal panoramic radiographs; (D) Illustrate the areas of interest (AOIs) in (C) in which a radiopacity is extending from the left ramus to the midline (Fibrous dysplasia) that causes impaction to tooth #33, 34, 35; (E) Abnormal panoramic radiographs; and 4(F) Illustrate the areas of interest (AOIs) in (E) in which AOI 2.1: Dilacerated and impacted tooth # 34, AOI 2.2: Impacted canine surrounded by a radiolucency (Dentigerous cyst), AOI 2.3: Deviated nasal septum and enlarged nasal conchae.**

were asked to describe it or provide a differential diagnosis to confirm their understanding. The last question was to ensure the participant described the targeted abnormality.

Several eye-tracking metrics were obtained using BeGaze software to assess participant interaction with radiographs. These metrics included: **entry time**—the time in milliseconds it took for a participant's gaze to first land on a specific AOI on a radiograph[17]; **dwell time**—the total time in milliseconds a participant spent looking at a specific AOI (encompassing fixation time and saccades within that area); **fixation time**—the total time in milliseconds a participant's gaze remained fixated on a specific AOI; **revisit counts**—the number of times a participant's gaze returned to a specific AOI after initially looking away; and **fixation counts**—the total number of times a participant's gaze fixated on a specific AOI[18].

INTERVIEWS

One week after the test, follow-up qualitative interviews were conducted with participants who expressed interest in receiving their test results and discussing their experiences using ETT. After obtaining informed consent for recording the interview, the interviewer confirmed the participants' understanding of the correct lesion locations used in the assessment. A semi-structured, one-on-one interview then explored the participants' experiences with ETT, including their perspectives on the potential benefits and challenges of incorporating ETT into dental education. Each interview lasted approximately 10–15 minutes.

ANALYSIS

Univariate analysis was conducted to characterize participant demographics and performance on the ET assessment. Descriptive statistics included frequencies, medians, and the associated 25th and 75th percentiles (interquartile range).

Bivariate analyses employed the Mann-Whitney U test to compare ET measures between pediatric PG dental students and dental interns. To account for multiple comparisons, the significance level (alpha) was adjusted using Bonferroni correction from .05 to .005. Additionally, a two-sample test of proportions compared the correct identification rate of radiographs between the two groups.

Multivariable analysis employed adjusted multilevel binary logistic regression to assess factors associated with correctly identified radiographs and AOIs. The dependent variable was the correct identification of normal radiographs or abnormal AOI (identified vs. unidentified). Independent variables included participant academic rank, sex, and other ET measures (revisits, fixation count, and dwell time). Based on the bivariate analysis and to avoid multicollinearity with fixation count or dwell time, fixation time was excluded from the regression model. A multilevel analysis addressed the lack of independence between observations, with students at level 2 and AOIs at level 1. The adjusted odds ratio (AOR) and its corresponding 95% confidence interval (95% CI) were used as the measure of association. Both the two-sample test of proportions and the multivariable analysis used a significant level (p - value) of less than .05. Data analysis was performed using STATA/IC version 15.1.

The qualitative analysis addressed all potential participant concerns before the interviews, including the interview format, recording process, and data confidentiality. Recorded interviews were transcribed, and thematic analysis with coding was used to generate qualitative data. Interview transcripts were anonymized, and then themes were independently highlighted by two investigators (S.ALS. and S.ALM.). Highlighted themes were grouped, with any discrepancies resolved through negotiation. NVivo 11 (QSR International Pty Ltd., Doncaster, Vic, Australia) was used for word cloud generation and subtheme development.

RESULTS

PARTICIPANTS' CHARACTERISTICS AND ET PERFORMANCE

The study recruited 30 pediatric PG students and a matching number of dental interns (n = 32). A total of nine radiographs were used, with two being normal. The abnormal radiographs contained several AOIs ranging from one to eight. For normal radiographs, the entire image was designated as a single AOI by the ET software. The ET system recorded key performance indices (KPIs) for each AOI separately, resulting in a total of 1,240 observations from the 62 participants. Table 1 summarizes the characteristics of the stimuli used.

Table 1. Panoramic radiograph characteristics.

Radiographs Characteristics	Number of Radiographs	Type of Radiographs	Number of AOI[a]	Number of Participants	Total Recorded Observation[b]
By Case					
Case 1	1	Normal	1	62	62
Case 2	1	Abnormal	3	62	186
Case 3	1	Abnormal	8	62	496
Case 4	1	Normal	1	62	62
Case 5	1	Abnormal	2	62	124
Case 6	1	Abnormal	2	62	124
Case 7	1	Abnormal	1	62	62
Case 8	1	Abnormal	1	62	62
Case 9	1	Abnormal	1	62	62
Overall					**1,240**

Note. [a]AOI: Area of Interest. [b]Total recorded observation: Number of participants multiplied by the AOI.

Table 2 presents the participant demographics, including sex, age, academic level, and clinical experience. Among the 62 participants, 42 (67.7%) were female, and most were under 30 years old. PG pediatric dental students constituted 48.4% of the participants, with 73.3% being female. Most of the PGs (66.7%) were aged 26-30 and had 2-4 years of clinical experience (56.7%).

Figure 2 illustrates the percentage of correctly identified radiographs by academic level. Overall, participants correctly identified 90.1% of the radiographs. Breaking this down further, PG students achieved a higher identification rate (91.9%) compared to dental interns (88.5%). Interestingly, for normal radiographs, the detection rate was significantly higher for interns (67.2%) compared to PG students (65%). However, a statistically significant difference ($p = 0.003$) was found in the identification of abnormal radiographs, with PG students achieving a higher rate (99.5%) compared to interns (94.6%).

Table 2. Participants Characteristics.

Participants Characteristics	*n* (%)		Total
	Pediatric Postgraduate Dental Students	Dental Interns	
Overall	30 (48.4)	32 (51.6)	62 (100.0)
Sex			
Female	22 (73.3)	20 (62.5)	42 (67.7)
Male	8 (26.7)	12 (37.5)	20 (32.3)
Age Category (Years)			
20–25	0 (0.0)	27 (84.4)	27 (43.6)
26–30	20 (66.7)	5 (15.6)	25 (40.3)
31–35	7 (23.3)	0 (0.0)	7 (11.3)
36–40	3 (10.0)	0 (0.0)	3 (4.8)
Clinical Experience (Including Internship Year)			
1 or less years	0 (0.0)	29 (90.6)	29 (46.8)
2–4 years	17 (56.7)	1 (3.1)	18 (29.0)
5 or more years	13 (43.3)	2 (6.3)	15 (24.2)

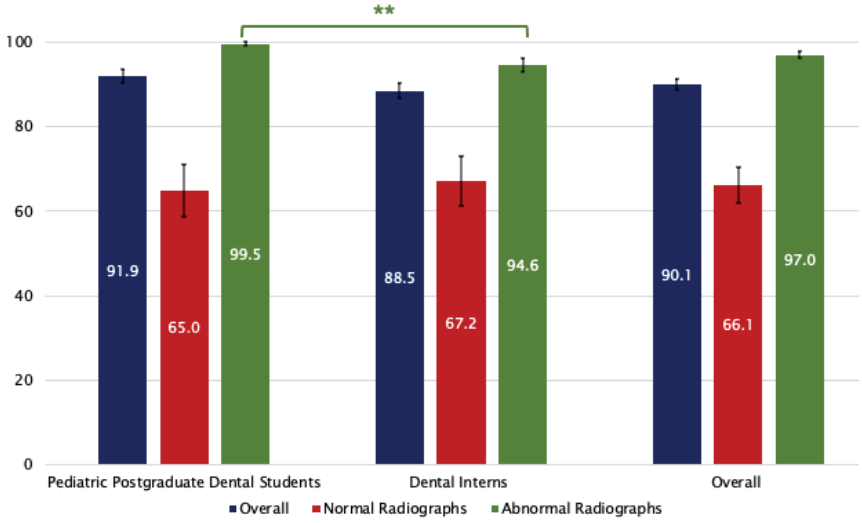

Figure 2. Percentage of correctly identified radiographs stratified by participant's' academic level. *Note.* (**): The Two-sample test of proportions indicates a statistically significant difference between the proportion of correctly identified abnormal radiographs by pediatric postgraduate dental students compared to dental interns (*p* - value = 0.003).

Table 3. Eye tracking key measures for each recorded AOIs, stratified by participant's academic level

Eye-tracking Measures	OPG	Median Score (25th - 75th IQR)			Mann-Whitney U Test *p*-value*
		Overall	Pediatric Postgraduate Dental Students	Dental Interns	
Revisits (Count)	Normal	0.0 (0.0–1.0)	0.0 (0.0–0.5)	0.0 (0.0–1.0)	0.607
	Abnormal	3.0 (1.0–7.0)	4.0 (1.0–8.0)	3.0 (1.0–6.0)	0.003
Fixation Count (Count)	Normal	98 (65.5–136.5)	114 (75–148.5)	89.5 (50.5–121)	0.011
	Abnormal	3 (0–10)	4 (0–11)	3 (0–9)	0.029
Entry Time (Milliseconds)	Normal	4.2 (1.9–6.8)	4.55 (1.75–6.9)	4.1 (1.9–6.8)	0.729
	Abnormal	3,120.8 (1,026.6–11,449.9)	3,551.9 (1,073.3–11,721.4)	2,914.4 (1,018.5–10,671.8)	0.269
Fixation Time (Milliseconds)	Normal	30,824.25 (18,947.65–45,188.8)	34,806.05 (23,554–46,715.35)	27,528.95 (15,147.85–45,034.55)	0.069
	Abnormal	1,213.95 (0–3,479.05)	1389.15 (0–3591.9)	997.1 (0–3308.15)	0.083
Dwell Time (Milliseconds)	Normal	33,478.5 (20,487.7–47,971.05)	37,191.9 (26,261.5–50,525.55)	29,665.7 (16,469.95–46,185.65)	0.031
	Abnormal	1,230.75 (0–3,521.05)	1414.15 (0–3696.35)	997.1 (0–33,66.55)	0.076

Note: *OPG: Panoramic Radiograph. IQR: Interquartile Range. AOI: Area of Interest. Bold font indicates statistical significance. (*): Alpha level is 0.005 after Bonferroni Correction.*

Table 4. Adjusted multilevel binary logistic regression analysis of the correctly identified radiographs and areas of interest

Predictors	AOR of correct detection [95% CI]	*p*-value
Participants Characteristics		
Academic Level		
Dental interns	Reference	
Pediatric postgraduate dental students	1.58 [0.64–3.90]	0.324
Sex		
Female	Reference	
Male	0.44 [0.18–1.10]	0.079
Eye Tracking Measures		
Revisits (counts)	1.17 [1.04–1.31]	0.009
Fixation count	1.00 [0.98–1.03]	0.776
Dwell Time (Milliseconds)	1.00 [0.99–1.00]	0.077

Note: *AOR: Adjusted Odds Ratio. CI: Confidence Interval. Bold font indicates statistical significance.*

Table 3 summarizes the key ET measures for the recorded AOIs by participant level. While PG students exhibited a higher number of fixations on normal radiographs compared to interns, this difference was not statistically significant (p = 0.011). Interestingly, PG students also tended to be slower in identifying normal radiographs, reflected by slightly longer entry times (p = 0.729). However, for abnormal radiographs with predefined AOIs, PG students revisited each AOI significantly more often than interns (p = 0.003).

Table 4 presents the results of the adjusted multilevel binary logistic regression analysis examining factors associated with correctly identified radiographs and AOIs. While PG students had a higher likelihood of correctly identifying radiographs compared to dental interns (AOR 1.58, 95% CI [0.64-3.90]), this difference was not statistically significant (p = 0.324). However, the analysis revealed a statistically significant association (p = 0.009) between revisit count and correct identification. For each additional revisit, the odds of correctly identifying a radiograph increased by 1.17. Fixation count and dwell time were not statistically significant predictors of correct radiograph identification.

QUALITATIVE RESULTS

Twenty students (10 PGs and 10 dental interns) participated in the follow-up interviews. A word cloud (Figure 3) visually presents the most frequently used terms and language from the interviews.

Seventy percent of the interviewees reported a positive experience with ETT, and for 45%, this study was their first encounter with the technology. Half of the participants (n = 10) indicated no significant

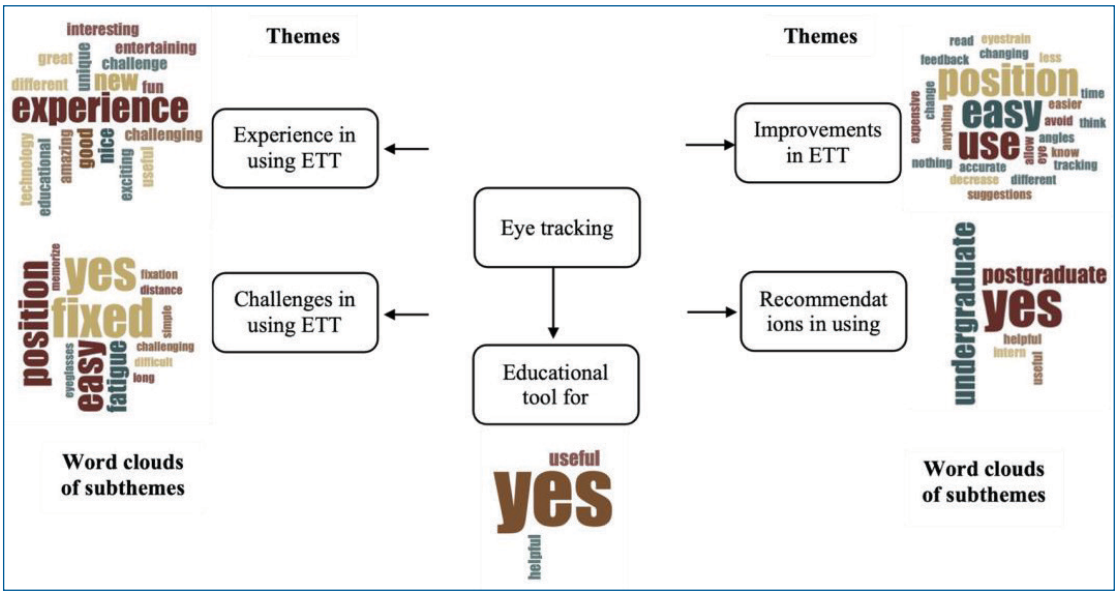

Figure 3. **Word cloud of the sub-themes of the participants' responses regarding the use of eye-tracking technology.**

difficulties using ETT. The most common challenge reported by 40% of respondents was maintaining a fixed position throughout the text.

While most respondents (n = 14) believed ETT could be a valuable educational tool, one participant expressed concern about its cost as a limiting factor. Out of the 20 interviewees, 11 suggested no improvements, while 7 proposed advancements such as more user-friendly software. Two participants recommended including fewer cases per test.

All respondents endorse the use of ETT in dental programs to improve patient care. Six specifically recommended its integration into undergraduate education to promote the early development of image interpretation skills. The remaining respondents suggested its use for assessing PGs when reviewing cone-beam computed tomography scans.

DISCUSSION

This mixed-method study investigated the visual parameters of dental students at two educational levels (PG students and interns) using ETT while viewing pediatric panoramic radiographs. This study aimed to compare the ET performance of PG students and dental interns in detecting abnormalities in panoramic radiographs, explore the relationships between different ET measures, determine the odds of abnormality detection in radiographs, and assess participant experience using ETT as a learning aid.

Building upon a previous study by our team that evaluated PG students' ability to identify abnormalities in panoramic radiographs[19], this research incorporates a control group (dental interns) for a comparative analysis of ET performance between the two distinct groups. We aimed to explore the impact of various ET metrics on detection accuracy and delve into the participants' experiences using ETT as a learning tool. Our findings revealed that PG students had a higher probability of correctly identifying abnormalities and revisiting AOIs significantly more often than dental interns. Additionally, the results suggest a link between revisit count and the likelihood of detecting a radiographic lesion.

Our findings suggest that PGs were more cautious than dental interns, potentially leading to a slight overreporting of abnormalities in normal radiographs. This is reflected by a longer trend of fixation and dwell times on normal radiographs for PGs. It is worth noting that this difference may be due to the AOI encompassing the entire radiograph for normal cases, whereas the ET system recorded separate KPIs for each AOI in abnormal radiographs.

While not statistically significant, PG students, who generally have more experience than interns, exhibited

longer entry times for both normal and abnormal radiographs. This aligns with the findings of Bahaziq *et al.*[9] who reported that expert orthodontists spend more time examining panoramic radiographs for incidental findings compared to novice orthodontists[9]. However, this contradicts another study where experienced observers demonstrated faster scanning times with potentially less thoroughness than inexperienced observers[7].

Our observations showed that PGs were slower to confirm their identification of normal radiographs compared to dental interns, resulting in slightly longer entry times. Additionally, PGs demonstrated greater consistency in correctly detecting abnormalities. This aligns with a previous study where senior dental students, despite slower scanning times, achieved higher diagnostic accuracy compared to juniors[2]. Additionally, juniors in that study more frequently missed abnormalities in peripheral areas and bones[2].

Furthermore, PG students revisited each AOI significantly more often than interns when analyzing abnormal radiographs. Our multivariable analysis revealed a positive association between revisit and the odds of accurate detection, with a 1.17 increase in odds for each additional visit. Interestingly, PG students exhibited lower specificity (more false positives in normal radiographs) but higher sensitivity (more true positives in abnormal radiographs) compared to dental interns. This data can inform training programs to emphasize the differentiation between normal and abnormal radiographs, particularly for specialists, to minimize overdiagnosis. It is important to consider that desirability bias might have contributed to the overdiagnosis observed in this study.

Innovative teaching modalities like ETT hold great promise, especially when combined with other modalities. For instance, a previous study examined how massed practice, an instructional modality that involves focused learning of a specific material type, influences the panoramic radiograph interpretation and student diagnostic performance[20]. The study evaluated dental students' performance before and after massed practice using ETT. Their findings suggest that massed practice is a valuable tool for enhancing students' ability to detect anomalies and focus on commonly overlooked areas[20]. Additionally, ETT can be beneficial for undergraduate students needing remediation in radiology courses or for specialized groups like PG dental radiology programs[6–21]. Therefore, ETT's effectiveness as a teaching tool can be gradually introduced and continuously evaluated to ensure its added value for dental students.

The second phase of this study employed semi-structured interviews with volunteer participants to gather qualitative data on their experiences using ETT. While the quantitative portion effectively measured student performance, information on ETT's impact on the learning process may not be well-suited for quantitative research. Although most dental research on ETT has utilized quantitative methods, the participants in this study provided valuable insights into their experiences, challenges, and recommendations for integrating this technology into dental education. These topics, including user perception, challenges, and future directions, are not only underexplored in relation to ETT but are also difficult to capture quantitatively due to their anecdotal nature. This exploratory section followed established protocols for in-depth interviews, the most prevalent and reliable qualitative data collection method in healthcare settings[22].

This study breaks new ground by evaluating the performance of PGs and dental interns in detecting abnormalities in pediatric panoramic radiographs. ETT provided a contemporary approach to understanding participants' visual scanning patterns and interpretation skills. Additionally, the mixed methods design offered a comprehensive assessment of participants' performance and experiences from multiple perspectives. This multifaceted approach yielded valuable insights into both the strengths and limitations of using ETT for dental education. Another key strength lies in the study's real-world setting, utilizing actual patient records without time constraints for viewing radiographs. Furthermore, the research was conducted at one of the country's largest dental schools, accredited by both the National Dental Association and the American Dental Association.

Several limitations are important to consider. First, while participation from PGs was high, the overall study population was relatively small. Second, the ET machine's range limited participant movement, potentially impacting natural viewing patterns. Additionally, some PGs may have approached the assessment with a sense of being tested, possibly contributing to the observed overdiagnosis of normal radiographs. Furthermore, participants reported the text length and fatigue associated with viewing

multiple radiographs. Presenting the radiographs in the same order for all participants partially addressed this concern. Finally, although the study was conducted at a prominent public dental school, it remains a single institution. Future studies should consider incorporating random samples from various dental schools across the country.

CONCLUSION

Pediatric PG dental students demonstrated greater accuracy in detecting abnormalities within pediatric panoramic radiographs compared to dental interns. However, a slight trend of overdiagnosis was observed in normal radiographs among PGs. Based on these findings, this study recommends the incorporation of ETT as a supplementary teaching tool in dental education. Future research should investigate PGs' performance in interpreting other types of dental radiographs.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the contents of the manuscript, and there is no financial interest to report. We certify that the submission is an original work and is not under review at any other publication.

DISCLOSURE

The authors did not receive any form of commercial support, either in the form of compensation or financial assistance, for this case report. The authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

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DATA AVAILABILITY STATEMENT

Data will be made available on a reasonable request.

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ORIGINAL ARTICLE

Unravelling the Versatile Nature of *Pseudomonas aeruginosa*: Challenges and Innovations in Infection Management

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Abstract

This review delves into the intricate and multifaceted nature of *Pseudomonas aeruginosa (P. aeruginosa)*, a notorious pathogen known for its adaptability, virulence, and resistance mechanisms. *P. aeruginosa* presents formidable challenges in both healthcare and community settings due to its ability to thrive in diverse environments, form biofilms, and acquire antibiotic resistance. The range of infections it causes, varying from mild to severe, highlights the urgent need for effective management strategies. Key aspects of *P. aeruginosa* pathogenesis, transmission, and associated risk factors are discussed, underscoring the significance of infection control measures, particularly in healthcare settings. The emergence of multidrug-resistant strains further emphasizes the necessity for innovative treatment approaches. Alternative therapies, including phage therapy; antimicrobial peptides; and natural products offer promising avenues for combatting *P. aeruginosa* infections, especially those caused by multidrug-resistant strains. Additionally, antibiotic combination therapy, incorporating antivirulence compounds, demonstrates potential in both treating infections and curbing the spread of antibiotic resistance. Despite these promising alternatives, challenges persist in the development and implementation of these approaches, encompassing efficacy, safety, and regulatory considerations. Further research, experimentation, and clinical trials are imperative to refine these strategies and address the ongoing threat posed by *P. aeruginosa* and other multidrug-resistant organisms. In summary, this review provides valuable insights into the current challenges and advancements in managing *P. aeruginosa* infections, with a specific focus on exploring novel therapeutic options and enhancing patient outcomes. Continued efforts in research and development are paramount for effectively combating this formidable pathogen and mitigating its impact on public health.

Keywords

Pseudomonas aeruginosa, Multidrug resistance (MDR), Antimicrobial resistance, Alternative therapy, Phage therapy, Antimicrobial peptide, Combination therapy

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacilli shape, aerobic bacterium, oxidase-positive, and lactose non-fermenters known for its versatile metabolism and resistance to antibiotics[1,2]. It commonly inhabits soil, water, and plant surfaces but is also a notorious opportunistic pathogen causing a wide range of infections[3], particularly in immunocompromised individuals and those with cystic fibrosis[3]. Its ability to form biofilms and acquire resistance mechanisms poses significant challenges in clinical settings, making it a critical focus of research in antimicrobial therapy and infection control[3,4].

Pseudomonas aeruginosa poses a significant threat in both hospital and community settings, contributing to infections such as healthcare-associated and community-acquired bloodstream infections. Delays in administering appropriate antibiotics and elevated mortality rates are often associated with these infections[5].

The aim of this review is to highlight the virulence and resilience of *Pseudomonas aeruginosa* as a pathogen and to explore current and emerging strategies for combating infections caused by this formidable organism. We will assess the challenges posed by *P. aeruginosa* in healthcare settings and the community, examining its ability to develop resistance mechanisms and form biofilms. Furthermore, we will discuss the existing arsenal of treatment options and identify areas where gaps in therapy persist. Ultimately, this review aims to provide insights into the ongoing efforts to develop novel therapies and improve infection control measures to better manage *P. aeruginosa* infections.

PSEUDOMONAS AERUGINOSA PATHOGENESIS

Pseudomonas aeruginosa is a highly adaptable and opportunistic pathogen capable of causing a diverse array of infections, particularly in individuals with compromised immune systems or underlying health conditions[4,6]. Its pathogenesis is multifaceted and involves several key factors. Firstly, *Pseudomonas aeruginosa* utilizes various adhesins and surface structures to adhere to host tissues, facilitating colonization of mucosal surfaces such as the respiratory tract, urinary tract, and skin[7]. Additionally, it exhibits a remarkable ability to form biofilms, structured communities of bacteria encased within a self-produced matrix, enhancing bacterial persistence and resistance to host immune defenses and antimicrobial agents, thereby contributing to chronic and recurrent infections[8,9]. The secretion of virulence factors further exacerbates its pathogenicity, including exotoxins like exotoxin A, which disrupts host protein synthesis and immune evasion; and pyocyanin, a pigment with pro-inflammatory and cytotoxic effects. Enzymes such as elastase; proteases; phospholipases; and lipases degrade host proteins and disrupt cell membranes, promoting tissue invasion and destruction. *Pseudomonas aeruginosa* also produces siderophores like pyoverdine and pyochelin to scavenge iron, facilitating bacterial growth and survival in iron-limited environments like host tissues[10,11]. Moreover, its intrinsic and acquired antibiotic resistance mechanisms, including impermeable outer membranes and efflux pumps, pose significant challenges to treatment, complicating infections and contributing to their severity[12,13]. Overall, the pathogenesis of *Pseudomonas aeruginosa* infections involves a complex interplay of bacterial adherence; biofilm formation; virulence factor secretion; and antibiotic resistance, allowing it to establish and persist in diverse host environments and causing a broad spectrum of clinical manifestations, ranging from superficial to life-threatening infections[10,14,15].

Pseudomonas aeruginosa spreads primarily through environmental sources and direct contact with contaminated surfaces or infected individuals[16]. Environmental exposure *to Pseudomonas aeruginosa* is common, as the bacterium thrives in natural environments like soil, water, and vegetation[3]. Healthcare-associated infections (HAIs) also pose a significant risk, especially for immunocompromised individuals or those with indwelling medical devices like catheters or ventilators[17]. These infections can be transmitted via contaminated medical equipment, invasive procedures, or contact with healthcare personnel. While less common, person-to-person transmission of *Pseudomonas aeruginosa* can occur, particularly in settings where individuals have compromised skin integrity or are in close contact with infected individuals, such as households or long-term care facilities[11,18]. Various risk factors predispose individuals to *Pseudomonas aeruginosa* infections, including immunocompromised states; underlying health conditions like cystic fibrosis or COPD; hospitalization; and residence in long-term care facilities[2]. Infections caused by *Pseudomonas aeruginosa* can vary in severity, ranging from mild urinary tract or skin infections to life-threatening

conditions such as pneumonia or bloodstream infections, particularly in high-risk individuals[19]. Early recognition of risk factors and implementation of appropriate infection control measures are crucial for preventing and *managing Pseudomonas aeruginosa* infections, especially in vulnerable patient populations[20].

PSEUDOMONAS AERUGINOSA EPIDEMIOLOGY

According to surveillance data on antimicrobial resistance, a significant prevalence of carbapenem resistance in *Pseudomonas aeruginosa* isolates has been observed throughout Europe. This prevalence varies widely, with rates ranging from less than 5% in countries like the Netherlands and the United Kingdom to as high as 66% in Romania. On average, the rate of carbapenem resistance across Europe is nearly 18%[21]. A significant study conducted in the United States, utilizing data from a nationally representative sample collected by microbiological laboratories, revealed that approximately 22% of *Pseudomonas aeruginosa* strains causing pneumonia exhibited multidrug resistance[22]. A study in China focused on pneumonia patients revealed that *Pseudomonas aeruginosa* strains causing hospitalized pneumonia exhibited resistance rates of 35.7% to imipenem and 22.9% to meropenem[23].

Understanding the epidemiology of MDR *P. aeruginosa* is crucial for effectively managing infections. It has been shown that there are significant regional and interregional differences in the reported prevalence of MDR *P. aeruginosa* in general clinical samples. Egypt has reported the highest prevalence at 75.6%, while Morocco has reported the lowest prevalence at 0%. In Saudi Arabia and Qatar, the prevalence stands at 7.3% and 8.1%, respectively[24]. It's noteworthy that in the Kingdom of Bahrain, carbapenem-resistant MDR *P. aeruginosa* isolates predominantly carry blaVIM, similar to neighboring countries. However, they uniquely harbor blaNDM, a trait rare or absent in the region except for Saudi Arabia, Iraq, and Egyp[25,26]. Moreover, a study has shown that MDR *P. aeruginosa* prevalence in ICU samples across MENA region countries exhibited significant variation, showing an opposite trend compared to general clinical samples. Saudi Arabia (61%) and Syria (54%) had the highest rates, contrasting with Egypt (22.5%), Libya (36.4%), Lebanon (33.3%), and Morocco (28.5%). Moderate resistance to piperacillin-tazobactam was observed in Iraq (42.3%), Jordan (37.8%), Libya (37%), and Lebanon (22%), while lower rates were recorded in Oman (7%) and Saudi Arabia (17.2%)[24].

INFECTIONS CAUSED BY *PSEUDOMONAS AERUGINOSA*

Infections caused by *Pseudomonas aeruginosa* can range from relatively mild, superficial infections such as urinary tract infections and skin infections to severe, life-threatening conditions including pneumonia, bloodstream infections (septicemia), and soft tissue infections, particularly in high-risk individuals[19]. Early recognition of risk factors and implementation of appropriate infection control measures are essential for preventing and managing *Pseudomonas aeruginosa* infections, especially in vulnerable patient populations.

Pseudomonas aeruginosa is a formidable pathogen known for its ability to cause a spectrum of infections, particularly in individuals with compromised immune systems or underlying health conditions[20]. In hospital settings, it poses a significant threat as a cause of hospital-acquired pneumonia, especially among patients undergoing mechanical ventilation or those with pre-existing lung diseases[27]. Additionally, *Pseudomonas aeruginosa* can lead to urinary tract infections, often affecting individuals with indwelling catheters or structural abnormalities of the urinary tract. Skin and soft tissue infections[20], including hot tub folliculitis and cellulitis, are also common, particularly among burn patients[28]. Furthermore, invasive infections caused by *Pseudomonas aeruginosa* can result in bloodstream infections and sepsis, posing life-threatening risks, especially to immunocompromised individuals[29]. The bacterium can also manifest as otitis externa[30], eye infections[2], and chronic respiratory infections, notably in individuals with cystic fibrosis or chronic obstructive pulmonary disease[31,32]. Moreover, burn patients are at high risk of *Pseudomonas aeruginosa* infections, often leading to severe complications and delayed wound healing[2] (Table 1).

RESISTANCE MECHANISMS

Pseudomonas aeruginosa is renowned for its intrinsic and acquired antibiotic resistance mechanisms, bolstering its survival in diverse environments and facilitating persistent infections[33]. Among its arsenal of resistance strategies, *Pseudomonas aeruginosa* employs efflux pumps, actively expelling antibiotics from the bacterial cell to reduce their intracellular concentration and render them ineffective against a

Table 1. Infections caused by *Pseudomonas aeruginosa*

Disease Caused by *Pseudomonas aeruginosa*	Reference
Urinary tract infections	19
Skin infections	20
Pneumonia (including hospital-acquired pneumonia)	27
Bloodstream infections (septicemia)	29
Soft tissue infections (e.g., cellulitis)	20
Otitis externa	30
Eye infections	2
Chronic respiratory infections	31, 32
Burn-related infections	2
Sepsis	29
Risks to individuals with indwelling catheters or structural abnormalities of the urinary tract	20
Risks to individuals with pre-existing lung diseases	27
Risks to individuals undergoing mechanical ventilation	27
Risks to individuals with cystic fibrosis or chronic obstructive pulmonary disease	31, 32

broad spectrum of antibiotics, including β-lactams, fluoroquinolones, and aminoglycosides[34,35]. Moreover, its outer membrane exhibits reduced permeability compared to other Gram-negative bacteria, limiting antibiotic entry and diminishing their efficacy[36]. The bacterium can also modify antibiotic targets, such as penicillin-binding proteins and DNA gyrase, impairing the affinity of antibiotics for their targets and reducing their bactericidal effects[37]. Additionally, *Pseudomonas aeruginosa* produces antibiotic-degrading enzymes, such as β-lactamases, to hydrolyze and inactivate antibiotics, conferring resistance to penicillins, cephalosporins, and carbapenems[38,39]. Furthermore, its remarkable ability to form biofilms provides a protective niche, shielding bacterial cells from antibiotics and host immune defenses, thereby fostering chronic infections[40]. Through horizontal gene transfer mechanisms, *Pseudomonas aeruginosa* can acquire antibiotic-resistance genes rapidly, facilitating the emergence of multidrug-resistant strains and complicating treatment efforts[21]. The rise of extensively drug-resistant and pandrug-resistant strains underscores the urgent need for innovative antimicrobial strategies and robust infection control measures to combat *Pseudomonas aeruginosa* infections effectively[41].

Pseudomonas aeruginosa employs a multifaceted approach to antibiotic resistance, utilizing various genes and mechanisms to evade the effects of antimicrobial agents. Among these, AmpC β-lactamase stands out as an inducible enzyme capable of hydrolyzing a broad spectrum of β-lactam antibiotics, rendering them ineffective against the bacterium[21,42]. Additionally, the production of Extended-Spectrum β-lactamases (ESBLs) further contributes to resistance, conferring the ability to hydrolyze extended-spectrum cephalosporins and monobactams. Carbapenemases, including metallo-β-lactamases (MBLs) and serine carbapenemases, pose a significant challenge by hydrolyzing carbapenem antibiotics, often considered the last resort for treating multidrug-resistant infections[4]. Furthermore, efflux pumps (MexA) actively expel antibiotics from the bacterial cell, while mutations in quinolone resistance-determining regions and the production of aminoglycoside-modifying enzymes diminish the efficacy of fluoroquinolones and aminoglycosides, respectively[34]. Changes in outer membrane porins (OprM) and the formation of biofilms further bolster resistance by impeding antibiotic entry and providing a protective environment for bacterial cells[34]. These mechanisms highlight the adaptability of

Table 2. Different resistance mechanisms against different antibiotics by *Pseudomonas aeruginosa*

Antibiotics	Resistance Mechanism	Reference
Beta lactam such as 1st, 2nd, 3rd cephalosporins, aztreonam	AmpC β-lactamase, Extended-Spectrum β-lactamases (ESBLs)	18, 32, 33
penicillins, cephalosporins, carbapenems	Carbapenemases (including metallo-β-lactamases and serine carbapenemases)	34, 35
Carumonam, ceftriaxone, and cefotaxime	The reduced permeability of the outer membrane	36
Fluoroquinolones	Efflux pumps (MexA), mutations in quinolone resistance-determining regions	37
Aminoglycosides	Efflux pumps (MexA), production of aminoglycoside-modifying enzymes	37
Ciprofloxacin	Biofilm formation	23

Pseudomonas aeruginosa in combating antimicrobial agents, necessitating vigilant surveillance and the development of novel therapeutic strategies to address infections caused by multidrug-resistant strains. (Table 2).

ANTIBIOTIC THERAPY OF *PSEUDOMONAS AERUGINOSA*

Antibiotic therapy for *Pseudomonas aeruginosa* infections necessitates a thorough understanding of the bacterium's resistance mechanisms and the specific context of the infection. For mild to moderate cases, such as urinary tract or skin and soft tissue infections, oral antibiotics may suffice[43]. Conversely, severe or systemic infections, particularly those involving multidrug-resistant strains, often require intravenous antibiotics[44]. Commonly employed first-line therapies for susceptible strains include antipseudomonal β-lactam antibiotics like piperacillin-tazobactam, ceftazidime, and cefepime[20,39,45]. In severe infections or those involving multidrug-resistant strains, combination therapy may be necessary to enhance efficacy and mitigate further resistance development, often involving adjunctive agents such as aminoglycosides or fluoroquinolones[20,31]. In cases of carbapenem-resistant *Pseudomonas aeruginosa*, alternative agents like polymyxins, tigecycline, or newer cephalosporins may be considered, albeit cautiously due to their associated toxicities and limited efficacy[46,47]. Moreover, treatment decisions should be informed by local susceptibility patterns, patient-specific factors, and guided by culture and susceptibility results whenever feasible. Duration of therapy varies based on infection site and severity, typically ranging from 7 to 14 days, with vigilant monitoring for clinical response and resistance emergence throughout treatment[44].

ALTERNATIVE APPROACHES IN TREATING *PSEUDOMONAS AERUGINOSA*

Phage therapy, a form of targeted antimicrobial therapy utilizing bacteriophages (viruses that infect bacteria), has emerged as a potential alternative or adjunctive treatment for *Pseudomonas aeruginosa* infections, particularly in cases of multidrug-resistant strains where conventional antibiotics are ineffective[30,48,49]. Phages are highly specific to their bacterial hosts, targeting and lysing *Pseudomonas aeruginosa* cells while leaving beneficial bacteria unharmed[50].

In phage therapy for *Pseudomonas aeruginosa* infections, specific phages that target the infecting strain are isolated, purified, and formulated into therapeutic preparations[49]. These phage preparations can be administered topically, intravenously, or via inhalation, depending on the site and severity of the infection[51,52].

Studies have demonstrated the efficacy of phage therapy in treating *Pseudomonas aeruginosa* infections, including wound infections, burn infections, cystic fibrosis-associated lung infections, and urinary tract infections[32,49]. Phage therapy has shown promise in reducing bacterial load, improving clinical outcomes, and even eradicating multidrug-resistant strains[53].

However, challenges remain in the widespread implementation of phage therapy, including the need for rigorous characterization and quality control of phage preparations, potential development of phage resistance by the bacteria, limited understanding of phage pharmacokinetics and pharmacodynamics, and regulatory hurdles in some regions[30,54,55].

Despite these challenges, ongoing research and clinical trials are exploring the potential of phage therapy as a valuable tool in the armamentarium against *Pseudomonas aeruginosa* infections, offering hope for patients with limited treatment options due to multidrug-resistant strains[56]. Further studies are needed to elucidate the optimal strategies for phage selection, dosing, administration routes, and combination therapies to maximize efficacy and safety in clinical settings.

Antimicrobial peptides (AMPs) represent a promising class of molecules for treating *Pseudomonas aeruginosa* infections due to their broad-spectrum antimicrobial activity, rapid killing kinetics, and low propensity for inducing bacterial resistance[31,57,58]. These peptides are naturally occurring components of the innate immune system found in various organisms[59] including humans, plants, and animals[60]. AMPs exert their antimicrobial effects through diverse mechanisms, including disruption of bacterial cell membranes, inhibition of cell wall synthesis, and modulation of intracellular processes[61]. Due to their multifaceted mode of action, AMPs are less prone to bacterial resistance compared to conventional antibiotics[62].

Several AMPs have demonstrated efficacy against *Pseudomonas aeruginosa* both in vitro and in animal models of infection[40]. For example, peptides such as LL-37, magainin, and cecropins have shown potent activity against *Pseudomonas aeruginosa* by disrupting bacterial membranes and inducing cell lysis[63,64]. Additionally, synthetic AMPs, designed to mimic the structure and function of naturally occurring peptides, have been developed and tested for their efficacy against *Pseudomonas aeruginosa*[65].

One advantage of AMPs is their ability to synergize with conventional antibiotics, enhancing their antimicrobial activity and overcoming resistance mechanisms[66]. Furthermore, AMPs have been shown to possess immunomodulatory properties, including the ability to stimulate host immune responses and promote wound healing[67], which can aid in the clearance of *Pseudomonas aeruginosa* infections[68]. While the therapeutic potential of AMPs for treating *Pseudomonas aeruginosa* infections is promising, challenges remain, including issues related to peptide stability, formulation, and delivery[69]. Nevertheless, ongoing research efforts are focused on optimizing the design and development of AMP-based therapeutics, with the goal of providing effective and alternative treatment options for *Pseudomonas aeruginosa* infections, particularly those caused by multidrug-resistant strains.

The use of natural products in treating *Pseudomonas aeruginosa* infections has gained attention due to their potential therapeutic benefits and relatively low risk of inducing bacterial resistance. Natural products encompass a diverse array of compounds derived from plants, animals, fungi, and microorganisms, many of which possess antimicrobial properties[70,71].

Several natural products have demonstrated activity against *Pseudomonas aeruginosa*, either through direct antimicrobial effects or by modulating host immune responses to combat infection[72]. For example, plant-derived compounds such as flavonoids, alkaloids, tannins[73], and essential oils have exhibited antimicrobial activity against *Pseudomonas aeruginosa* by disrupting bacterial cell membranes, inhibiting bacterial enzymes, or interfering with essential cellular processes[74,75].

Propolis, a resinous substance collected by bees from plant buds and sap, has been investigated for its antimicrobial properties against Pseudomonas aeruginosa. Studies have shown that propolis extracts exhibit inhibitory effects against Pseudomonas aeruginosa growth and biofilm formation, making it a potential therapeutic agent for preventing and treating infections[15,76].

Another natural product with antimicrobial activity against *Pseudomonas aeruginosa* is honey. Honey contains various compounds including hydrogen peroxide, bee-derived peptides, and phytochemicals, which possess antimicrobial properties[77]. Additionally, honey exhibits anti-inflammatory and wound-healing effects, making it beneficial for managing *Pseudomonas aeruginosa* infections, particularly in wounds and burns[78].

Marine-derived natural products have also shown promise as potential therapeutics against *Pseudomonas aeruginosa*[79]. Compounds isolated from marine organisms, such as sponges, algae, and corals, have demonstrated antimicrobial activity against *Pseudomonas aeruginosa* through various mechanisms, including disruption of bacterial membranes and inhibition of bacterial enzymes[14,80,81].

While natural products offer potential benefits in treating *Pseudomonas aeruginosa* infections, challenges remain, including issues related to standardization, quality control, and pharmacokinetics[82[. Additionally, further research is needed to elucidate the mechanisms of action of natural products against *Pseudomonas aeruginosa* and optimize their therapeutic use. Nevertheless, the exploration of natural products as alternative or adjunctive therapies for *Pseudomonas aeruginosa* infections represents a promising avenue for developing novel antimicrobial agents with potential clinical utility[77].

Studies have demonstrated that a combination of antivirulence compounds, such as gallium (a siderophore quencher) and furanone C-30 (a quorum sensing inhibitor), along with four clinically relevant antibiotics (colistin, colistin, ciprofloxacin, meropenem, tobramycin), holds promise in not only treating infections but also curbing the proliferation of antibiotic resistance[83]. Antibiotics have been demonstrated as a good alternative approach to tackling multi-drug-resistant bacteria. They can attack different targets simultaneously, reduce the probability of developing resistance by bacteria and attenuate the virulence

of bacteria[84,85]. In clinical practice, the utilization of novel β-lactam combination antibiotic regimens, such as ceftazidime/avibactam, ceftolozane/tazobactam, imipenem/cilastatin/relebactam, among others, has emerged as a strategy for addressing infections caused by multidrug-resistant (MDR) or extensively drug-resistant (XDR) *Pseudomonas aeruginosa* strains. These combinations represent innovative therapeutic approaches that offer alternative treatment options in situations where traditional antibiotics may not be effective due to the development of resistance mechanisms. While these novel regimens are not typically used as first-line treatments, they serve as valuable adjunctive therapies in managing infections caused by particularly challenging strains of *P. aeruginosa* that exhibit resistance to conventional antibiotics[19]. There is emerging evidence suggesting that combinations of β-lactams, such as meropenem with aztreonam or meropenem with ceftazidime, exhibit augmented efficacy in combating infections induced by multidrug-resistant (MDR) *Pseudomonas aeruginosa* in an invertebrate model of systemic infection. These findings represent novel insights into therapeutic strategies that have not been documented previously[86].

DISCUSSION

The multifaceted nature of *Pseudomonas aeruginosa,* as a formidable pathogen, is known for its adaptability, virulence, and resistance mechanisms. The bacterium's ability to thrive in diverse environments, form biofilms, and acquire antibiotic resistance poses significant challenges in both healthcare and community settings[3,4,10,11]. *Pseudomonas aeruginosa* infections range from mild to severe, impacting various organ systems and often resulting in high morbidity and mortality rates, particularly among immunocompromised individuals[5]. The review delves into the pathogenesis of *Pseudomonas aeruginosa* infections, highlighting its adherence mechanisms, biofilm formation, and secretion of virulence factors, all of which contribute to its pathogenicity and persistence. Moreover, the review emphasizes the modes of transmission and risk factors associated with *Pseudomonas aeruginosa* infections, underscoring the importance of infection control measures, particularly in healthcare settings. The emergence of multidrug-resistant strains further complicates treatment strategies, necessitating innovative approaches such as phage therapy, antimicrobial peptides, and natural products[3,70]. Additionally, the review explores the potential of antibiotic combination therapy, including antivirulence compounds, in addressing *Pseudomonas aeruginosa* infections while mitigating the spread of antibiotic resistance[83]. Overall, the review provides insights into the ongoing challenges and advancements in managing *Pseudomonas aeruginosa* infections, aiming to improve patient outcomes and public health. Moreover, highlights the different alternative approaches and the challenges in tackling *Pseudomonas aeruginosa*. Further research, experiments, and trials are needed to develop novel and different agents for providing a potential therapy against MDR organisms.

CONCLUSION

In conclusion, the review underscores the complex and multifaceted nature of *Pseudomonas aeruginosa* as a formidable pathogen, renowned for its adaptability, virulence, and resistance mechanisms. From its ability to thrive in diverse environments to its capacity for biofilm formation and acquisition of antibiotic resistance, *P. aeruginosa* poses significant challenges in healthcare and community settings alike. The spectrum of infections it causes, ranging from mild to severe, underscores the critical need for effective management strategies. This review emphasizes the importance of infection control measures, particularly in healthcare settings. The emergence of multidrug-resistant strains further underscores the urgency for innovative treatment approaches. Alternative therapies such as phage therapy, antimicrobial peptides, and natural products offer promising avenues for combating *P. aeruginosa* infections, particularly those caused by multidrug-resistant strains. Additionally, antibiotic combination therapy, including antivirulence compounds, shows potential in both treating infections and curbing the proliferation of antibiotic resistance. However, challenges remain in the development and implementation of these alternative approaches, including issues related to efficacy, safety, and regulatory hurdles. Further research, experimentation, and clinical trials are essential to optimize these strategies and address the ongoing threat posed by *P. aeruginosa* and other multidrug-resistant organisms. Overall, the review provides valuable insights into the current challenges and advancements in managing *P. aeruginosa* infections, with a focus on exploring novel therapeutic options and improving patient outcomes. Further efforts in research and development are crucial for the continued progress in combating this formidable pathogen.

CONFLICT OF INTEREST

The author declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The author did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the author has no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

Not applicable.

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ORIGINAL ARTICLE

An Evaluation of Clinical Dietitian's Perceptions of Telenutrition Quality and Associated Factors in Saudi Arabia

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Abstract

Background: Telenutrition has been gaining popularity worldwide due to its feasibility and positive impact on improving lifestyle choices and patient outcomes. However, the telenutrition approach has not been sufficiently evaluated in Saudi Arabia. Specifically, clinical dietitian's perceptions and attitudes toward telemedicine in Saudi Arabia remain unknown.

Objectives: To assess dietitians' perception of telenutrition quality and examine associations with sociodemographic characteristics and work experience.

Methods: This was a descriptive cross-sectional study using a translated online survey comprising 36 questions distributed to 300 dietitians working in Saudi Arabia. The questions assessed the dieticians' demographic background, as well as their perception and challenges of telenutrition. All statistical analyses were done with SPSS version 25 using the Kolmogorov–Smirnov test.

Results: 108 clinical dietitians participated in the study. Of the respondents, 90 (83%) were female, and the mean age range was 25–34 years. The majority of respondents (n = 40; 63%) had experience providing phone and online consultations, with most (n = 35; 32.4%) covering referrals for weight loss/weight maintenance. The most common difficulties reported were a lack of anthropometric measures (n = 52; 48.1%) followed by technical difficulties (n = 19; 17.6%) and interpersonal or communication difficulties (n = 16; 14.8%) In addition, older client age was the only significant predictor of a higher phone counseling quality score.

Conclusion: Our findings demonstrated promising attitudes towards telenutrition despite its limitations, such as technical errors and the absence of nutrition assessment. However, the effectiveness and outcomes of telenutrition remain unknown and require further investigation.

Keywords

Telenutrition, Dietitians, Perception, Challenges, Quality

INTRODUCTION

Telenutrition has proliferated over the past few years, leading to a decrease in face-to-face counseling following the COVID-19 pandemic[1]. As COVID-19 spread in Saudi Arabia, the resulting lockdowns impacted healthcare services, as the latter shifted to be mostly remote rather than face-to-face. This virtual approach supported the population and may have relieved strains on those with limited healthcare resources by enhancing access to treatment at a reasonable cost[2]. Virtual nutrition counseling was one of the most commonly offered services during the lockdown[3], with its use increasing over time[4]. A cross-sectional study carried out in several Arab countries confirmed that health dietitians switched to the use of social/mass media platforms, transitioning from face-to-face consultation to telenutrition[5]. The Academy of Nutrition and Dietetics defines virtual nutrition counseling as telenutrition, which is "the interactive use of electronic information and telecommunications technologies by a registered dietitian (RD) to implement the nutrition care process with patients or clients in a remote location, within the provisions of the RD's state license as applicable"[6]. Telenutrition is a virtual service that involves education and training, where clients are required to apply self-reporting for a complete Nutrition Care Process (NCP) that includes nutrition assessment, nutrition diagnosis, nutrition intervention, and nutrition evaluation and monitoring[7]. Several trials have proven the effectiveness of telenutrition, particularly in weight management, when compared to face-to-face counseling, driven by the convenience and client motivation related to the former[8-13]. Other studies have also shown promising results in diabetic patients with monitoring blood glucose levels and supporting weight loss[14]. However, communication *via* video calls, e-mails, smartphones, and other types of virtual applications[15] may present several limitations and challenges[16]. One of the main factors that must be considered is the availability of technology and technical errors that occur during sessions. Another factor is the process of self-reporting[17], which is expensive and may introduce false measurements[16]. A previous descriptive cross-sectional survey distributed to 300 dietitians showed that most clients preferred using the phone instead of online platforms, where most difficulties were associated with technical issues and communication, highlighting the need for additional training and tools in telecommunication. Data have also shown that most telenutrition services are directed towards weight loss, with several limitations occurring during consultations, including the absence of anthropometric measurements and interpersonal communication[3].

OBJECTIVE

In the current study, we aimed to assess dietitians' telenutrition experiences in Saud Arabia, focusing on the characteristics of telenutrition delivered, dietitians' perception of telenutrition quality, and associated factors using a validated survey translated into Arabic[3].

MATERIALS AND METHODS

STUDY DESIGN

A cross-sectional study was conducted using a validated survey *via* the Google Survey platform distributed to RDs working in different regions in Saudi Arabia. The survey was distributed to 300 RDs working in the Riyadh, Makkah, or Eastern regions of Saudi Arabia in the past year (2022–2023) through King Abdul Aziz University social networks. 108 (36%) participants responded to the survey (108 out of 300). All participants provided informed consent prior to responding to the survey. RDs who continued to work full-time were recruited for the study. The inclusion criteria included RDs working in Saudi Arabia who had practiced nutrition counseling in the last three years. The exclusion criteria included RDs who had not practiced virtual nutrition consultation in the past three years.

SURVEY DESCRIPTION AND TRANSLATION

A validated survey from a published article was obtained for use in this study[3], which was translated from English to Arabic by a native Arabic speaker who is also fluent in English. Once translated, a bilingual speaker of both Arabic and English back-translated the translated survey into Arabic. Participants' responses were given in Arabic and then translated into English.

The survey comprised 36 questions designed to assess participants' perceptions and challenges of telenutrition *via* phone and/or online platforms. The questions addressed the education and professional characteristics of the RDs; telenutrition characteristics, including setting, clients, and types of referrals; and RDs' perceptions of telenutrition quality. The analysis also explored factors associated with a higher overall

quality score when using the phone or an online platform for counseling, based on multivariable logistic regression. A higher quality score for phone counseling was defined as a total quality score exceeding the median value of seven points, while a higher quality score for online platform counseling was defined as a total quality score exceeding the median value of eight points, for performing telemedicine using the phone. Some items were scored on a ten-point Likert scale ranging from 1 to 10, where 1 = "very low", and 10 = "very high"; whereas others were scored on a four-point Likert scale, where 1 = "very little" and 4 = "high".

DATA ANALYSIS

All statistical analyses were conducted using SPSS version 25 (IBM, New York). The Kolmogorov–Smirnov test was used to determine the normality of continuous variable distributions, which are represented as means, and standard deviations are described in terms of the interquartile range (median) due to continuous data distributions significantly deviating from normal. Categorical variables (e.g., the proportion of participants that responded in a specific way) were characterized using frequency counts and denoted as n (%). The association between the RDs' socio-demographic and occupational factors, and the overall quality of phone and online counseling, was investigated using logistic regression models with stepwise variable selection. Independent variables associated with the overall quality variable were considered for inclusion in the multivariate models at a significance level of $p \leq 0.10$. Two separate models were created, with the median value of the quality score of each type of consultation assigned as the cut-off value. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

EDUCATION AND PROFESSIONAL CHARACTERISTICS OF DIETITIANS

Out of the 300 participants, only 108 (36%) individuals responded to the survey. Table 1 describes the characteristics of the survey participants. The majority of the participants were female, with a mean age range of 25–34 years old. Furthermore, 78 (83.3%) participants held a Bachelor of Science degree, with nutrition being the field of study for all participants. Public hospitals and other work environments were the most common

Table 1. Michel's Classification System.

Characteristics	Result (n = 108)	
	n	%
Age, years		
Mean	2.59	
SD	1.01	
Sex (female)	90	83.3
Highest level of education		
BSc	78	72.2
MS	25	23.1
PhD	5	4.6
Field of highest degree obtained		
Nutrition	108	100
Public Health/Epidemiology	0	0
Business Management/Health Systems Management	0	0
Biology/Biochemistry/Chemistry	0	0
Medical Sciences	0	0
Other	0	0
Employment setting		
Public hospital	45	41.7
Private hospital	16	14.8
Self-owned clinic	11	10.2
Private or chain clinic	18	16.7
Gym/fitness center	4	3.7
Other	22	20.4
Not working	6	5.6
Years of professional experience		
Mean	1.82	
SD	1.21	
Field of expertise in nutrition counselling		
Overweight/obesity/weight loss	21	19.4
CVD	5	4.6
Diabetes	20	18.5
Oncology	1	0.9
Gastrointestinal	19	17.6
Pediatrics (aged <6 years)	18	16.7
Bariatric surgeries	2	1.9
Eating disorders	5	4.6
Sports nutrition	5	4.6
No area of clinical specialization	12	11.1

Table 1. Michel's Classification System–Continued

Number of hours/weeks performing nutrition		
Mean	25.4	
SD	14.3	
Work capacity		
Full time	47	43.5
Part time	40	37.0
Unemployed	21	18.5

BSc, Bachelor of Science; MSc, Master of Science; PhD, Doctor of Philosophy; SD, standard deviation.

employment settings. The average number of years of professional experience was 1.82 ± 1.21. The most popular field of expertise was overweight/obesity/weight loss (*n* = 21; 19.4%), followed by diabetes (*n* = 20; 18.5%), gastrointestinal (*n* = 19; 17.6%), and pediatrics (*n* = 18; 16.7%). The mean number of hours per week spent on nutrition counseling was 25.4 ± 14.3 prior to the COVID-19 pandemic, and 24.5 ± 14.8 following the COVID-19 pandemic, with no statistically significant difference. Finally, following the COVID-19 pandemic, 47 (43.5%) participants had a full-time job capacity, whereas 40 (37.0%) worked part-time.

TELENUTRITION CHARACTERISTICS

Table 2 describes the characteristics of telenutrition provided by the respondents, which include identification of consultation type (face-to-face or phone consultation, or online consultations), client age, and referral type. Mixed counseling was the most common setting, with 40 (37.0%) participants performing face-to-face counseling, phone consultation, and online consultation. In comparison, 23 (21.3%) participants used online counseling only, and 20 (18.5%) met clients in person exclusively. Furthermore, 20 (18.5%) consulted more women, and 19 (17.6%) consulted more men. Moreover, the RDs identified different types of referrals, the majority of which (n = 35; %32.4) were identified as an increase in referrals for weight loss/weight maintenance, whereas 28 (25.9%) reported more referrals for diabetes control, and 23 (21.3%) observed more referrals for eating disorders.

DIETITIANS' PERCEPTION OF TELENUTRITION QUALITY

Table 3 presents data on the RDs' perception of telenutrition quality. Prior to the COVID-19 pandemic,

Table 2. Characteristics of telenutrition provided by dietitians

Characteristics	Result (n = 108)	
	n	%
Setting		
Usual nutrition counselling (face-to-face, in-person)	20	18.5
Usual nutrition counselling and phone nutrition counselling	1	0.9
Only phone nutrition counselling	3	2.8
Usual nutrition counselling and online nutrition counselling	16	14.8
Only online nutrition counselling	23	21.3
Only phone and online nutrition counselling	5	4.6
Usual nutrition counselling as well as phone and online nutrition counselling	40	37.0
Clients		
Younger clients	14	13.0
Older clients	14	13.0
More women	20	18.5
More men	19	17.6
Other	8	7.4
Type of **referrals**		
More referrals for weight loss/ weight maintenance	35	32.4
More referrals for diabetes control	28	25.9
More referrals for pediatric nutrition	12	11.1
More referrals for eating disorders	23	21.3
More referrals for gastrointestinal problems	4	3.7
Other*	6	5.6

Frequency
**Replies for the category "other" included the following: hardly any new clients, just returning referrals, and an overall decrease in referrals.*

the majority (*n* = 68; 63%) of respondents had prior experience providing virtual consultation, whereas 13 (12.0%) had only minimal experience, and five (4.6%) had no prior experience. More than half of the RDs (n = 59; 54.6%) claimed phone and online consultations were like face-to-face consultations, whereas 27 (25.0%) believed the former to be inferior. In contrast,

Table 3. Dietitians' perception of telenutrition quality

	Result (*n* = 108)			
Question/Response	**n**	**%**	**Median**	**Interquartile Range**
Previous experience with telenutrition				
Yes	68	63.0		
Very little	13	12.0		
No	5	4.6		
I learned to use the online platforms during the coronavirus pandemic	22	20.4		
How would you compare telenutrition to face-to-face counseling?				
Superior to face-to-face counselling	8	7.4		
Similar to face-to-face counselling	59	54.6		
Inferior to face-to-face counselling	27	25.0		
Not certain	14	13.0		
Quality of counselling**				
Overall quality*			7.5	6
Technical quality*			8	3
Clinical quality*			7	3
Organizational Difficulties*			7	4
Convenience†			4	1
Future use†			4	1
Types of difficulties reported during counselling				
Technical difficulties	19	17.6		
Interpersonal communication difficulties	16	14.8		
Difficulties due to lack of anthropometric measurements	52	48.1		
Difficulties/inconveniences from conducting the session in the home environment	6	5.6		
Other	8	7.4		
All of theabove	7	6.5		

Frequency
**Items scored on a ten-point Likert scale ranging from 1 to 10, where 1 = "very low" and 10 = "very high".*
†*Items scored on a four-point response scale, where 1 = "very little" and 4 = "high".*

only eight (7.4%) participants claimed phone counseling was superior to face-to-face counseling. Frequent difficulties were reported in using phone and online counseling due to a lack of anthropometric measurements (*n* = 52; 48.1%), followed by technical difficulties (*n* = 19; 17.6%), and interpersonal or communication difficulties (*n* = 16; 14.8%). The fewest obstacles were encountered when conducting the session in the home environment (*n* = 6; 5.6%).

ASSOCIATIONS BETWEEN SOCIO-DEMOGRAPHIC AND OCCUPATIONAL CHARACTERISTICS OF DIETITIANS AND THE OVERALL QUALITY OF TELENUTRITION (BY PHONE AND ONLINE PLATFORMS)

In multivariate logistic regression, with the dependent variable being a total quality score of phone counseling greater than 7, older age was associated with a higher

Table 4. Multivariable logistic regression analysis of factors associated with a higher overall quality score when using the phone for counseling.

(*n* = 30)	OR	95 % CI	*p*-value*
Age (years)	2.717	1.028, 7.187	0.044
Academic degree (MSc and above v. BSc and below)	0.274	0.034, 2.180	0.221
Employment setting (public v. private)	0.957	0.166, 5.512,	0.961
Workload during the pandemic (partial v. full)	1.382	0.264, 7.252	0.702
Previous experience using the phone in dietetic counselling (no experience v. experience)	1.464	0.310, 6.909	0.630
Constant	0.116		0.497

BSc, Bachelor of Science; MSc, Master of Science; CI, confidence interval; OR, odds ratio
**A higher quality score was assigned a total quality score higher than the median value of seven points for performing telemedicine using the phone.*
**p < 0.05 is considered significant.*

Table 5. Multivariable logistic regression analysis of factors associated with a higher overall quality score using an online platform for counseling.

(*n* = 16)	OR	95 % CI	*p*-value*
Age (years)	1.664	0.678, 4.079	0.266
Academic degree (MSc and above vs. BSc and below)	349498895.7	0.000	0.999
Employment setting (public vs. private)	0.863	0.103, 7223	0.892
Workload during the pandemic (part-time vs. full-time)	3.195	0.431, 23.677	0.256
Previous experience using the phone in dietetic counselling (no experience vs.experience)	0.000	0.000	0.998
Constant	80639753.9		0.999

BSc, Bachelor of Science; MSc, Master of Science; CI, confidence interval; OR, odds ratio
**A higher quality score was assigned a total quality score higher than the median value of eight points for performing telemedicine using the phone*
**p < 0.05 is considered significant.*

quality score (OR = 2.717; 95% CI = 1.028, 7.187; p = 0044) (Table 4). There was no significant association between socio-demographic and occupational characteristics of dietitians, and the overall quality of telenutrition using online platforms (Table 5).

DISCUSSION

The COVID-19 pandemic prompted a rapid and sudden change, from traditional face-to-face consultations to the use of virtual consultations, in both primary and secondary care[18]. However, the extent to which RDs participated, as well as the overall quality of these consultations, remains unknown. In the current study, we presented the unique characteristics of telenutrition in Saudi Arabia. Our results were similar to those published by Kaufman-Shriqui et al. in 2021, where most of the participants had experience providing virtual nutrition counseling, with the majority specializing in overweight/obesity and diabetes[3]. Although obesity and overweight are global health issues, tackling them virtually has become more popular because of the COVID-19 pandemic. A study carried out in 2020 examined the impact of videoconferencing on weight loss; the approach enabled clients living in rural areas to access the program, leading to increased engagement and peer support[19].

Telenutrition can be delivered *via* several channels, such as phone calls, online messaging platforms, and video calls. Table 2 presents the characteristics of the telenutrition delivered by the Saudi RDs, where most RDs frequently performed mixed counseling, which included both face-to-face nutrition counseling, phone consultation, and online consultation. The participants reported an increase in weight loss/weight maintenance referrals, which is unsurprising, given that obesity has emerged as a risk factor for COVID-19-related morbidity and mortality[20] and is still considered a major diet-related issue in Saudi Arabia[21]. Although our results agreed with those of Kaufman-Shriqui et al. in 2021, our RDs reported higher numbers of older clients who

participated in online counseling. In addition, the older age group was the only significant predictor of a higher phone counseling quality score. One of the main reasons for this effect may be that the elderly rely on the convenience, cost-effectiveness, and accessibility of receiving health care services from their home [22]. However, the use of technology can be challenging, and several technical errors may occur. Table 3 presents an evaluation of the overall quality of phone and online consultations, which showed a relatively high score. Nevertheless, 59% of the participants claimed that virtual consultations were similar to face-to-face counseling in terms of quality, and meeting the outcomes and goals of the consultations. However, in a study by Kaufman-Shriqui, 65.4% of participants indicated that phone consultations were inferior to face-to-face counseling. This result may be due to differences in culture and how online consultations are perceived, including difficulties faced by clients during consultations. In previous studies, one of the major obstacles that patients faced was a lack of interest in accessing the internet for consultations, whereas RDs were challenged by the inability to complete the NCP, as evaluation and monitoring were difficult to carry out[23]. Indeed, monitoring is an essential aspect of telenutrition, as it supports clients' health by enhancing compliance with diet plans and motivates them to continue participating in remote weight-loss programs[24].

The most frequently reported difficulties for both phone and online platforms identified by RDs in the present study were a lack of anthropometric measures, technological difficulties, and interpersonal communication difficulties. These results support our hypothesis that knowledge acquisition, along with training, technical skills, and multitasking, are the most significant considerations for the effective implementation of telenutrition. As stated in the introduction, there has been very little research on telenutrition in Saudi Arabia, with our study being the first to examine telenutrition practices in the country. In agreement with our results, the use of telenutrition has increased after the COVID-19 pandemic among Arab countries using social media, with time constraints being the main barrier, yet clients have shown great interest in several areas, such as healthy eating habits, healthy recipes, nutrition and immunity, and medical nutrition therapy[5].

When comparing our results to those of Kaufman-Shriqui et al. in 2021, there were similarities in both the difficulties dietitians encountered and the relatively high overall quality rating of telenutrition. However, there were some differences. For example, dietitians had no previous experience prior to the pandemic, and due to the pandemic, there was a reduced workload, as most participants only began using virtual consultations after the COVID-19 outbreak. In contrast, in other studies, virtual consultation was initiated before the pandemic.

The use of virtual consultation has the potential to be beneficial for both patients and practitioners. Studies have shown that the ability of service users and their caregivers to consult with general practitioners digitally/online enhances access to health care for certain populations, such as the elderly, women, and employed participants[25]. Individuals are also able to express themselves more openly about health concerns *via* telenutrition[26], in addition to other benefits, such as the ability to share images, as RDs may request to see the patient's kitchen, food items, serving dishes, and medications and/or supplementations. Although a growing number of studies have demonstrated the advantages of online appointments, there are concerns that they may involve some risk clinically, and/or are less acceptable to patients or staff. Moreover, there are considerable technological, logistical, and regulatory problems, in addition to the lack of physical examinations, that prevent online appointments from fully replacing face-to-face counseling[22,27]. To improve the success of virtual nutrition care consultations, the use of telemonitoring has been suggested[28], which involves patient self-reporting and weekly follow-ups using electronic scales, blood pressure analyzers, continuous glucose monitoring, and other health-related assessment tools[14]. Although guidelines on self-reporting have been published recently regarding e-scaling and nutrition assessment (e.g., 24-hour recall), errors may still occur, as ensuring the accuracy and precision of measurements *via* self-reporting can be challenging[29].

The findings of this study can be used as a platform for future research on both dietitians' and clients' perceptions of the usage of telenutrition in Saudi Arabia and highlight how further tools are needed to improve the experience. One of the main targets is

patients who are unable to reach clinics physically due to geographical barriers, such as distance, especially in remote rural areas. The National Health Service in the UK has also evaluated digital consultation for weight management in terms of acceptability and effectiveness, with the added benefit of convenience that is not found with face-to-face consultations, and the main challenge being the influence of practitioner attributes, which plays a major role in patient's or client's commitment to treatment[30].

CONCLUSION

The current work is considered a preliminary study that examines dietitians' perception of the quality of telenutrition delivered in Saudi Arabia. The findings showed high scores related to telenutrition quality among adults and the elderly, specifically in weight loss and weight management referrals. Our study also showed that convenience was considered a crucial benefit of telenutrition, despite the technical errors encountered by the participants. In addition to the inability to provide a complete NCP due to incomplete nutrition assessment, based on our results, we suggest improving telenutrition *via* client self-reporting and telemonitoring to obtain essential measurements and cover the entirety of the NCP. Moreover, such practices may also empower the community, motivating them to lose weight *via* measurement tools and providing training to ensure the delivery of effective dietary programs. As the effectiveness of telenutrition remains largely unknown, future studies must be designed to explore this issue further.

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AUTHOR CONTRIBUTION

Noura M.S. Eid contributed to the conceptualization, methodology, writing of the original draft, review and editing, and supervision of the project. Amal Shibli, Dalia Shibli, and Raghad Alsulami were involved in validation, formal analysis, investigation, and writing the original draft. Sumia Enani handled formal analysis, data curation, and contributed to the writing of the original draft and supervision. Rana H. Mosli participated in validation, supervision, and the review and editing process.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the manuscript's contents, and there is no financial interest to report. We certify that the submission is an original work and is not under review at any other publication.

DISCLOSURE

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ETHICAL APPROVAL

Ethical approval to conduct this study was obtained from the Applied Medical Sciences research committee (Reference Letter No. FAMS-EC2022-04). A brief online description of the study and consent forms were sent to the prospective participants through their social networks.

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ORIGINAL ARTICLE

Antimicrobial Activity of *Lepidium sativum* Ethanolic Extract Against *Madurella.mycetomatis*

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Abstract

Mycetoma is a chronic subcutaneous infection caused by fungi (Eumycetes) or bacteria from the phylum Actinomycetes. This condition, characterized by granulomatous inflammation and the formation of grains containing aggregates of the causative organisms, can extend to the underlying bone, causing severe complications. The grains, discharged through multiple sinuses, are diagnostic of the condition. Treatment of eumycetoma is particularly challenging, requiring a combination of antifungal therapy and surgical debridement. Azoles, including imidazoles (e.g., Ketoconazole) and triazoles (e.g., Itraconazole), are the primary antifungal agents used, although their efficacy varies. This study aims to isolate and identify *Madurella mycetomatis* from specimens using both mycological and molecular techniques, followed by an evaluation of the antimicrobial activity of extracts from the plant *Lepidium sativum* against the isolated pathogen. Black grains were obtained from suspected specimens of eumycetoma, and mycological examination confirmed the presence of broad fungal hyphae and chlamydospores embedded in a brown cement-like substance. The isolate was confirmed as *Madurella mycetomatis* using PCR. Plant extracts were prepared from *Lepidium sativum* seeds using petroleum ether, chloroform, and ethanol. The separation of constituents of the ethanolic extract of the *Lepidium sativum* by using the BAW system of thin layer chromatography gave thirteen components. The Rf was measured for each constituent. The ethanolic extract showed significant antifungal activity against *Madurella mycetomatis*, with a minimum inhibitory concentration (MIC) of 6.25 mg/ml, while extracts from petroleum ether and chloroform showed no activity. Thin-layer chromatography was used to separate the components of the ethanolic extract, revealing thirteen distinct components. The study demonstrates the potential antifungal efficacy of *Lepidium sativum* against *Madurella mycetomatis*, although the higher MIC compared to standard antifungal drugs like Ketoconazole suggests a need for further isolation and purification of the active compounds. This paper aims to investigate for the first time the antifungal properties of *Lepidium sativum* against *Madurella mycetomatis*, providing a foundation for future studies.

Keywords

Madurella mycetomatis, Lepidium sativum, thin layer chromatography, BAW system, Agar dilution method, Ethanolic extract

INTRODUCTION

Mycetoma is a long-lasting subcutaneous infection that can be caused by either actinomycetes bacteria or fungi[1]. This infection triggers a granulomatous inflammatory reaction in the deep layers of the dermis and subcutaneous tissue, which may spread to the underlying bone. Mycetoma is characterized by the formation of grains containing aggregates of the causative organisms that may be discharged onto the skin surface through multiple sinuses[2]. Mycetoma can arise from bacteria classified within the phylum Actinomycetes or from fungi termed Eumycetoma[3,4]. Over 20 different species of fungi and bacteria are known to be causative agents of mycetoma. Table 1 below categorizes these bacterial and fungal species based on the characteristic colors of discharge observed from infected wounds[5].

The treatment of eumycetoma is challenging[6]. Anti-fungal therapy has to be combined with debridement (surgical removal of infected tissue) when possible. Various antifungal drugs have been improved for the treatment of eumycetoma, mainly the azole group[7]. Azoles are synthetic compounds of anti-fungal agents which include: imidazole and triazole[8]. These differ with respect to their chemical structures, the imidazole consists of Ketoconazole, Miconazole, and Clotrimazole, while the second group includes Itraconazole, Fluconazole, and new Voriconazole[9–11].

Table 1. Bacterial and fungal species causing mycetoma[5]

Mycetoma species	Grains Discharged Colour's
Actinomadura pelletieri	Red discharge
Acremonium strictum Actinomadura madurae Aspergillus nidulans Noetestudina rosatii Phaeoacremonium krajdenii Pseudallescheria boydii	White or Yellow discharge
Aspergillus terreus Curvularia lunata Cladophialophora bantiana Exophiala jeanselmei Leptosphaeria senegalensis Madurella grisea Madurella mycetomatis Pyrenochaeta romeroi	Black discharge
Nocardia asteroides Nocardia brasiliensis	Yellow discharge
Streptomcyes spp (Streptomcyes Somaliensis	Yellow or red discharge

Cress (*Lepidium sativum*), often called garden cress to differentiate it from other plants with similar names, is a fast-growing, edible herb[12]. The name "cress" comes from the old Germanic word *cresso*, meaning sharp or spicy. This herb is closely related to watercress and mustard, sharing their distinctive peppery and tangy taste and aroma. In various regions, it is also referred to as mustard and cress, garden pepper cress, pepperwort, peppergrass, or poor man's pepper[13,14].

Lepidium sativum has long been used in traditional Indian medicine to treat various ailments[15]. A cold infusion of its seeds is commonly used to alleviate hiccups, while the seeds themselves are beneficial in cases of chronic liver and spleen enlargement and serve as a carminative adjunct to purgatives. When bruised and mixed with lime juice, the seeds are applied topically to relieve inflammatory and rheumatic pains. The seeds possess a variety of properties, including bitter, thermogenic, depurative, rubefacient, galactagogue, emmenagogue, tonic, aphrodisiac, and diuretic[16]. They are also useful as poultices for sprains and are applied in the treatment of leprosy, skin diseases, dysentery, diarrhea, splenomegaly, and asthma[17].

A seed powder mixed with fine sugar is an effective remedy for indigestion, diarrhea, and dysentery[18]. Another household preparation made from seeds, butter, and sugar is commonly used as a restorative for general debility[18,19]. To relieve flatulence and boost milk secretion in postpartum women, seeds are boiled in milk to form a soft, thin mass, to which sugar or jaggery is added to create a confection. This mixture is also beneficial for treating sexual debility, leucorrhea, and lower back pain caused by rheumatism[20].

The possible mechanism of action of quinoxaline derivatives likely involves multiple pathways that target bacterial cell structures and metabolic processes. Quinoxalines are known to disrupt bacterial DNA and generate reactive oxygen sepsis (ROS)[21,22].

This paper, to the best of our knowledge, is the first to aim at the isolation and identification of *Madurella mycetomatis* from specimens utilizing both mycological techniques (direct examination and culture methods) and molecular techniques (PCR). Following the successful isolation, plant materials will

be extracted using petroleum ether, chloroform, and ethanol as solvents. The study will then evaluate the activity of antimicrobial of these plant extracts against the isolated *Madurella mycetomatis*.

MATERIALS AND METHODS

MADURELLA MYCETOMATIS ISOLATION

Specimens Collection

Cultures of *Madurella mycetomatis* were prepared from black grains from five specimens with eumycetoma attending to the Mycology Lab, "National Health Laboratory". The black grains were collected in sterile normal saline using sterile swabs, and the infection was confirmed by mycological examination and molecular technique (PCR).

Mycological Examination

Collected grains from the specimens were washed with normal saline mixed with 20% Chloromphenicol several times to remove the bacterial contamination, and then The grains were examined microscopically in wet preparation using 20% KOH.

Then the grains were cultured on ordinary blood agar and were incubated at 37°C. The resulting growth was cultured onto slopes of Sabouraud's dextrose agar containing 0.1% Chloromphenicol and incubated at 37°C for 2-3 weeks. Lastly, for more mycologically confirmation, the needle mount technique was used for each culture isolate.

Molecular PCR Examination

The isolates were identified specifically as *Madurella mycetomatis* using a specific primer with PCR technique[21].

DNA Extractions and Purification (CTAB DNA Protocol)

DNA was extracted from all isolates using CTAB (hexa decyltrimethyl ammonium bromide, Sigma, U.S.A). The isolates were subcultured on Sabouraud's agar and incubated for 10 days at 37°C. About 1cm of the mycelia was scraped and ground to a fine powder with liquid nitrogen in a sterile mortar and pestle. The powdered mycelia were transferred to a propylene tube, and 4ml of CTAB lysis buffer (2% CTAB, 100 mM Tris-HCL, 10mM EDTA, 0.7 M NaCl) was added, which has been pre-heated at 65°C. The mycelia were dispersed gently using a pipette, and immediately, 40 µl of 2-mercaptoethanol was added. The tubes were incubated at 65°C for 30 minutes and inverted every 10 minutes to ensure adequate mixing. After incubation, 4 ml of chloroform/isoamyl alcohol (24 ml chloroform: 1 ml isoamyl alcohol V/V) was added to each tube. The tubes were shaken for 20 minutes, and different phases were separated by cold centrifugation at 9500 rpm for 20 minutes. The aqueous phase was removed to new tubes, and an equal volume of chloroform/isoamyl alcohol was added again. The centrifugation step was repeated, and the aqueous phase was separated, and 0.5 volume isopropanol was added to it. The tubes were left on ice for 5 minutes and the DNA was precipitated by cold centrifugation at 9500 rpm for 20 minutes. The pellets were washed with 70% ethanol and centrifuged. The pellets were left to dry at room temperature by inverting the tubes on tissue paper for 30 minutes. Finally, the pellets were re-suspended into 100µl Tris EDTA (TE) buffer (10mM Tris-HCL, 1mM EDTA). The extracted DNA was stored at -20°C pending usage for PCR.

DNA Quantification (Gene Quant pro, England)

In a separate micro centrifuge tube (1.5 ml), 10 µl DNA was mixed with 90 µl H_2O. The combining mixture was vortexed and left to stand for 10 min at room temperature to ensure complete diffusion of DNA throughout the solution. This represents 1/10 dilution. Total genomic DNA concentration was determined using a Gene Quant spectrophotometer (Amersham) according to the manufacturer's recommendation. The absorbance was measured at 260 nm, and the purity of the samples was further assessed by calculating the 260/230 and 260/280 ratios.

PCR Test

PCR was carried out using two *Madurella mycetomatis* species-specific primers, 26.1 A and 28.3 A (Alpha DNA, Montreal, Canada, USA).

The sequences of the two primers were:

- [5'-AATGAGTTGGGCTTTAACGG-3']
- [3'TCCCTGTGATGTGATGGCCCT-5'] respectively.

The reaction volumes of the PCR test were 50µl per sample in 0.5 ml thin-walled PCR tubes. The PCR mixture consisted of 5µl of 10X reaction buffer (500mM KCl, 100 mM Tris-HCl, 0.1% Triton™X-100) (Vivantis, Malaysia), 5µl of 10mM dNTP mix (Vivantis, Malaysia),

3µl of 25mM MgCl2, 3µl from each two primers (100 picomol each), 0.3 µl of thermo-stable DNA polymerase (Vivantis, Malaysia), and 5 µl of template DNA(using telsar mimi-v/PCR). Then the PCR mixtures were completed to 50 µl with double distilled water. The PCR program consisted of 40 cycles on a PCR thermocycler (Bio-Rad). Initial denaturation was done by holding the tubes at 94°C for 4 minutes. Each cycle consisted of denaturation at 94°C for 1 minute, annealing of primers at 58°C for 1 minute, and enzymatic extension at 72°C for 1 minute. The PCR products were examined by electrophoresis in 1% a garose gel stained with ethidium bromide.

Preparation of Crude Extracts of the Plant

Plant material

The *Lepidium sativum* seeds were obtained from the Omdurman market and identified by a taxonomist in the botany department, Faculty of Sciences, Khartoum University. The seeds were cleaned, shade-dried, and powdered by a mechanical grinder.

Extraction Methods

About 1000 gm of *Lepidium sativum* seed was extracted according to Harborne with Petroleum ether, Chloroform, and Ethanol in Soxhlet apparatus for 36 hr, respectively. The extract obtained was filtrated by using filter paper. The extracts were then concentrated under reduced pressure at a controlled temperature of 40-50°C using a rotary evaporator. The crude extracts were stored in a refrigerator at 4°C until further analysis.

In vitro sensitivity test of the isolates of Madurella mycetomatis to the herbal extracts

The sensitivity of the isolates of *Madurella mycetomatis* to the herbal extracts was performed using the agar dilution method. Five hundred milligram herb extracts (petroleum ether, chloroform, and ethanol) were dissolved in 2 ml of DMSO then dissolved in 18 ml of melted Sabouraud's dextrose agar medium at 42˚C. Serial dilutions of this preparation were done by adding 10 ml of this preparation to 10 ml of melted Sabouraud's dextrose agar medium at 42°C and repeating this procedure to other tubes. By doing so, the final concentration obtained was 0.0976mg/ml. Two control media were prepared, one with relevant solvent (DMSO) and the other without solvent. The media were left in a slanting position and left to solidify. The fungus was inoculated by taking a small portion of the *Madurella mycetomatis*, about 2x2 mm, from the periphery of the colonies and then inoculated on the agar surface. After inoculation, cultures were incubated at 37˚C for 15 days. The test was read when good growth was seen in the control bottles, and the minimum inhibitory concentrations (MIC) were estimated by the comparison with the controls[22].

Thin layer chromatography technique

The technique was used to separate the plant constituent by using different solvent systems. The procedure was carried out on aluminum foil coated with a thin layer of silica gel as the stationary phase. A small drop of the sample solution was applied to the plate, approximately 1.5 centimeters from the bottom, using a capillary. The solvent was allowed to fully evaporate to avoid interference with the sample's interaction with the mobile phase in the subsequent step. This process was repeated to ensure sufficient analyte was present at the starting spot for a visible result. Then, the plate was inserted into a screw-top jar containing a small amount of an appropriate solvent (mobile phase) to a depth of less than 1 centimeter. When the solvent had risen to near the top of the plate via capillary action, the plate was removed and dried, and the spots were visualized by projecting ultraviolet light onto the sheet. Chemical processes can also be used to visualize spots; a mixture of sulfuric acid and vanillin was used to clear the reaction, then the thin layer chromatography plate was incubated in a hot air oven at 110˚C for a few to clear the reaction[23]. To obtain clear separation of the components, different solvent systems were used: ethyl acetate + chloroform (8:2), chloroform + petroleum ether (7:3), chloroform + ethanol (7:3), ethanol + chloroform (1:1), chloroform + ethanol (1:1), and the BAW system (n-butanol + acetic acid + water, 4:1:5, with two layers used).

RESULTS

MYCOLOGICAL EXAMINATION

All five specimens showed positive results in the direct microscopic examination of the grains. Broad fungal hyphae and chlamydospores embedded in a brown cement-like substance were observed. All strains were cultured from black grains on blood agar, and Sabouraud's dextrose agar gave positive cultures. On blood agar, the isolates produced white to grey mycelia. On the Sabouraud's dextrose agar, the fungus produced brown pigments, and the colonies themselves were brown, buff to yellow, or grey in colour. Colonies were

flat; some developed raised centers. They were velvety or short cottony. Needle mount of all isolates grown on Sabouraud's dextrose agar revealed broad segmented, hyphae with terminally and itercallary chlamydospores.

MOLECULAR PCR EXAMINATION

Fungal DNA samples extracted with the DNA extraction CTAB method gave positive PCR results with one sample (No 4) compatible with *Madurella mycetomatis*. The isolate showed an amplicon, which estimated roughly to be 420 bp according to the DNA ladder based on the PCR product size. The isolates were identified as *Madurella mycetomatis* (Figure 1)

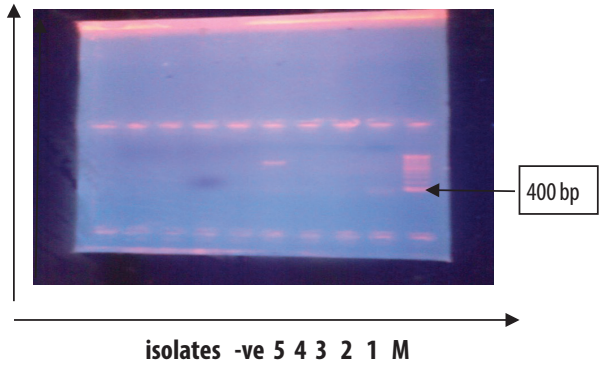

Figure 1. PCR amplification of *Madurella mycetomatis* isolates using primer 26.1A and 28.3A·

Lanes 1 to 5 show *Madurella mycetomatis* isolates; lane labeled M contains 50 b.p.DNA lader; the arrow indicates 400 b.p, and lane label (-ve) contains negative control.

Lane labeled 4 contains a DNA sample that gave a positive result compatible with *Madurella mycetomatis*. The lanes labeled 1, 2, 3, 5 contain DNA samples not compatible with *Madurella mycetomatis.*

IN VITRO SENSITIVITY TEST OF MADURELLA MYCETOMATIS TO THE HERBAL EXTRACTS (AGAR DILUTION METHOD)

The *Madurella mycetomatis* isolate was tested against the *Lepidium sativum* extracts (petroleum ether, chloroform, and ethanol). The ethanolic extract of *Lepidium sativum* showed high activity with MIC 6.25mg/ml (Figure 2), while the Petroleum ether and chloroform extracts did not show activity against *Madurella mycetomatis*.

Figure 2. *In vitro* antifungal susceptibility of *Madurella. Mycetomatis on Sabouraud's dextrose agar containing different concentrations* of ehanolic extract showed high activity with MIC 6.25 mg/ml (from left to right 50,25,12.5,6.25,3.12,1.6,0.78, 0.39,0.195,0.0976 mg/ml respectively). The first two tubes from the left control tubes, one for the viability of fungi and the other for the activity of DMSO).

THIN LAYER CHROMATOGRAPHY

Different systems were used to separate the constituent of ethanolic extract of the *Lepidium sativum*. The best system that gave the best separation was the BAW system (Figures 3 and 4). Thirteen components were separated. Some of them were fluorescent with the long UV light; eight of them gave a blue fluorescent color, and one of them gave a faint red colour. The Rf is measured for each constituent (Table 2).

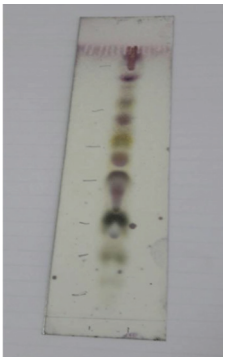

Figure 3. Thin layer chromatography techniques using the BAW system for separation of ehanolic extract components (mixture of sulfuric acid and vanillin was used to clear the components spots).

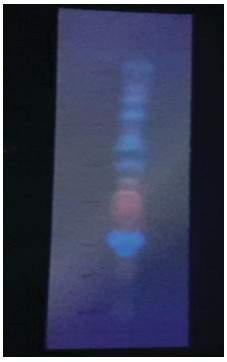

Figure 4. Thin layer chromatography techniques using the BAW system to separate the ehanolic extract components (Long U.V light was used to clear the component's spots)

Table 2. The Rf value of ethanolic extract components by using the BAW system

Component	Rf Value	Observation
1	1.8/16.5 = 0.10	No fluorescence (Long U.V light)
2	3/16.5 =0.18	No fluorescence (Long U.V light)
3	4.2/16.5= 0.25	Fluoresced deep blue colour (Long U.V light)
4	4.7/16.5= 0.28	Fluoresced deep blue colour (Long U.V light)
5	5.3/16.5= 0.32	Faint red (Long U.V light)
6	6.7/16.5= 0.41	Faint red (Long U.V light)
7	7.8/16.5= 0.47	Faint red (Long U.V light)
8	9/16.5= 0.55	Blue colour (Long U.V light)
9	10/16.5= 0.60	Blue colour (Long U.V light)
10	12.7/16.5= 0.69	Blue colour (Long U.V light)
11	12.7/16.5=0.76	Blue colour (Long U.V light)
12	14.5/16.5= 0.87	Blue colour (Long U.V light)
13	15.5/16.5= 0.93	Blue colour (Long U.V light)

DISCUSSION

In this study, for the first time, we showed that the ethanolic extract of *Lepidium sativum* plant showed high activity against *Madurella mycetomatis* with MIC 6.25 mg/ml. The research affirms the antifungal efficacy of the ethanol-based extract originating from this specific plant, a conclusion supported by the discoveries made by Solomon et al., who studied the anti-fungal activity of Lepidium sativum against three types of fungi (Aspergillus niger, Fusarium oxysporum, and *Fusarium solani*)[24]. Furthermore, this outcome aligns with the findings reported by another study, wherein they investigated the antifungal effectiveness of the ethanol-based extract against nine distinct fungal strains (*Aspergillus parasiticus, Aspergillus niger, Yersinia aldovae, Candida albicans, Aspergillus effusus, Fusarium solani, Macrophomina phaseolina, Saccharomyces cerevisiae* and *Trichophyton rubrum*)[25]. Other findings are also in agreement with the finding of[26], who tested the antifungal activity of ethanolic extract of Lepidium sativum seeds against Fusarium equiseta, Aspergillus flavus, and *Alternaria* alternate. A similar finding was obtained in another study that studied *in vitro* antifungal and antibacterial activity of ethanolic extracts of *Ferula assafoetida* resin, *Grewia asiatica* leaves, *Ipomoea hederacea* seeds, *Lepidium sativum* seeds, *Nigella sativa* seeds, and *Terminalia chebula* fruits against nine fungal strains (*Aspergillus parasiticus, Aspergillus niger, Yersinia aldovae, Candida albicans, Aspergillus effusus, Fusarium solani, Macrophomina phaseolina, Saccharomyces cerevisiae,* and *Trichophyton rubrum*)[25].

The ethanolic extract of *Lepidium sativum* exhibited antifungal activity against Madurella mycetomatis with MIC 6.25mg/ml, while the MIC for anti-fungal drugs (Ketoconazole and Itraconazole) was 0.16 µg/ml and 1.25µg/ml, respectively. The high MIC of *Lepidium sativum* and low MIC of Ketoconazole are most probably due to the purity of these drugs since the active ingredient of this plant needs further isolation and purification from the ethanolic extract mixture.

The higher MIC observed for *Lepidium sativum* extract, relative to the lower MIC of ketoconazole, can likely be attributed to the purity of the compounds involved. While ketoconazole is a purified and highly concentrated pharmaceutical compound, the ethanolic extract of *Lepidium sativum* is a mixture that contains a variety of bioactive compounds, with the active ingredient likely present in lower concentrations. Further isolation and purification of this active component from the extract could enhance its antifungal potency, potentially reducing the MIC and making it more comparable to the pharmaceutical standards. This highlights the need for additional research to refine and concentrate the active constituents within *Lepidium sativum* to fully realize its antifungal potential.

In alternative medicine, this plant is used topically for the treatment of mycetoma, which may give the possibility to synthesize topical treatment of mycetoma to reduce the risks of the side effects of drugs that are given orally or systemically.

Studies have demonstrated that *Lepidium sativum* exhibits significant anti-inflammatory and immunomodulatory properties. Specifically, research has shown a marked reduction in the levels of key inflammatory markers, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1

(IL-1). Additionally, the production of nitric oxide (NO) was notably decreased, alongside a reduction in the expression of inducible nitric oxide synthase (iNOS), and heme oxygenase-1 (HO-1). These findings suggest that *Lepidium sativum* not only attenuates the inflammatory response by downregulating these pro-inflammatory mediators, but also modulates the immune response, further supporting its potential therapeutic applications in managing inflammation-related conditions. The ability of *Lepidium sativum* to influence such a broad range of inflammatory markers underscores its potential as a natural anti-inflammatory agent with promising implications for future research and clinical use[27]. Recent studies highlight the potential of plant-based synthesis of silver nanoparticles (AgNP). They explored the antimicrobial effects of *Lepidium sativum* (curly garden cress) combined with AgNPs. This combination enhanced antibacterial activity, particularly against ESKAPE pathogens. The LS-AgNP bio-composites showed pH, time, and concentration-dependent antimicrobial actions, confirming their potential as broad-spectrum disinfectants and wound care agents[28]. Two extracts from *Lepidium sativum* L. seeds were analyzed for their phenolic content, as well as their antioxidant and antibacterial properties. The seeds were extracted using 80% ethanol with ultrasonic assistance and distilled water with microwave assistance. The total phenolic and flavonoid contents were measured using Folin-Ciocalteu reagent and AlCl3, respectively. The extracts showed significant antioxidant activity, achieving 54.66% in the β-carotene/linoleic bleaching assay, and demonstrated antibacterial effects, with a 20 mm inhibition zone against *Salmonella Enteritidis*. These findings suggest that the extracts could serve as natural preservatives in the food and pharmaceutical industries, providing a potential alternative to synthetic antioxidants like TBHQ. The results from this study may contribute to the development of natural antioxidants and bioactive agents for enhancing human health[29]. In another study, researchers found that *Lepidium sativum* extracts exhibit both antioxidant and antibacterial activities. Methanol extracts, in particular, showed significant antioxidant properties, while both ethyl acetate and methanol extracts demonstrated antibacterial activity against *Rhodococcus equi*. The antioxidant and antimicrobial effects are likely due to the presence of flavonoids and tannins, as confirmed by chemical tests. These findings suggest that *Lepidium sativum* could be a valuable source of natural antioxidant and antibacterial agents, making it suitable for medical and nutraceutical applications. This research provides scientific validation for the traditional use of these plant extracts in homemade remedies and highlights their potential in treating microbial-induced conditions[30]. Further studies could pave the way for their development as safe alternatives to synthetic antimicrobial drugs. This includes in vivo studies such as rat models to assess the *in vivo* efficacy. Moreover, pharmacokinetics and pharmacodynamics are important for drug development; thus, these experiments are vital to implement.

One of the primary limitations of this study is the use of only a few isolates of *mycetoma*-causing organisms. Due to the rarity of mycetoma, obtaining a larger number of isolates is inherently challenging. This limited sample size may affect the generalizability of the findings, as it may not fully represent the diverse genetic and phenotypic variations present in different strains of *mycetoma* pathogens. Consequently, the outcomes observed in this study, including susceptibility profiles and potential synergistic effects of drug combinations, might differ when tested against a broader range of isolates.

CONCLUSION

In this study, we have demonstrated for the first time that the ethanolic extract of *Lepidium sativum* exhibits significant antifungal activity against *Madurella mycetomatis*, with a minimum inhibitory concentration (MIC) of 6.25 mg/mL. While this MIC is higher compared to the much lower MICs of standard antifungal drugs like ketoconazole (0.16 µg/mL) and itraconazole (1.25 µg/mL), this difference is likely due to the mixed nature of the plant extract, which contains various bioactive compounds. The BAW system gave the best result among the systems that were used for the separation of exanolic extract components of *lepidium sativum* plant (Thirteen components). These findings suggest that further isolation and purification of the active components from *Lepidium sativum* could enhance its antifungal potency, bringing it closer to the efficacy of pharmaceutical drugs. This research not only validates the potential of *Lepidium sativum* as a natural antifungal agent, but also opens the door for developing topical treatments for mycetoma, reducing the side effects associated with systemic antifungal drugs.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The authors did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

Not applicable.

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ORIGINAL ARTICLE

Exploring the Combined Efficacy of 3-Hydrazinoquinoxaline-2-thiol and Flucloxacillin Against Methicillin-Resistant *Staphylococcus aureus*

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Abstract

The escalating resistance observed in different antimicrobial agents, especially those considered as last-line options, highlights the urgent required for innovative ways and novel agents to combat MRSA infections. Utilizing antibiotic combinations can enhance efficacy, broaden the spectrum against bacteria, and mitigate the risk of resistance development, offering a promising strategy to address the rising challenge of antimicrobial resistance. Resistance has emerged against nearly all antibiotics, making drug discovery difficult and costly. Developing a significant number of effective antibiotics to combat resistance in a short period is nearly impossible. This paper aims to explore the probability of synergistic effects between 3-hydrazinoquinoxaline-2-thiol (3HX) and flucloxacillin (FLX) versus a different range of MRSA clinical isolates, which may provide valuable insights into the combination of the antimicrobial activity. Broth microdilution was conducted on 22 clinical MRSA isolates to assess the Minimum Inhibitory Concentrations (MICs) of both 3HX and FLX. Following by a checkerboard assay was subjected to assess the interaction activity between the two agents, focusing on the Fractional Inhibitory Concentration Index (FICI). The MICs of FLX and 3HX were evaluated for 22 clinical MRSA strains, with FLX displaying MICs ranging from 128 to 512 µg/ml, while 3HX MICs varying from 16 to 64 µg/ml. Surprisingly, the combination of 3HX and FLX demonstrated a synergistic effect, leading to a considerable reduction of MIC up to 64-fold. The potential of combining 3HX with FLX as an effective way versus MRSA appears promising. However, further rigors, testing, and experimentation are imperative to establish its practical utility.

Keywords

Antimicrobial resistance, *Staphylococcus aureus*, MRSA, FLX, Antibiotic combination therapy, 3HX, MIC, FICI

INTRODUCTION

The global rise in antimicrobial resistance presents substantial health and economic obstacles, with its rapid emergence underscoring pressing challenges. This predicament stems from both the improper utilization of antibiotics and the dearth of novel antimicrobial agents in development[1]. AMR infections have resulted in approximately 700,000 fatalities worldwide, and projections indicate that these resistant bacteria could be responsible for up to 10 million deaths by 2050. This trend also signifies a substantial economic toll due to lost benefits[2]. Specifically, there is an immediate worldwide concern regarding multidrug-resistant (MDR) bacteria, such as *Staphylococcus aureus*, due to their ability to resist multiple drugs and their high virulence potential[3,4]. Infections attributed to *S. aureus* are both severe and recurrent, with the situation further compounded by the emergence of antibiotic-resistant variants[5]. Antibiotic resistance has become a pervasive issue, with resistance emerging against nearly all classes of antibiotics currently available. This situation poses significant challenges in the field of drug discovery, which is both difficult and expensive. The process of identifying, developing, and bringing new antibiotics to the market involves extensive research, rigorous testing, and substantial financial investment. Additionally, the timeline for developing a new class of antibiotics that is effective against resistant strains is lengthy, often spanning several years or even decades. As a result, it is nearly impossible to develop a considerable number of effective antibiotics to combat the rising tide of antibiotic resistance within a short period. This underscores the urgency for innovative approaches and alternative strategies in addressing this critical global health threat[1,6].

β-lactam antibiotics are frequently prescribed to combat bacterial infections due to their favorable efficacy, minimal toxicity, and wide-ranging effectiveness against various bacterial strains[7]. The yearly spending on β-lactam antibiotics is estimated at around $15 billion USD, constituting approximately 65% of the entire antibiotics market[8]. In MRSA, resistance mechanisms are associated with the presence of the *mecA* gene, which codes for penicillin-binding protein2A (PBP2A). PBP2A is notable for its decreased affinity to most β-lactam antibiotics, except 5th-generation cephalosporins (ceftaroline), thereby reducing their efficacy against MRSA strains[9–11]. New approaches are essential to address the challenge of antibiotic resistance and rejuvenate the efficacy of existing antibiotics[6]. One potential strategy involves identifying novel targets essential for bacterial resistance mechanisms[6]. The discovery of a small molecule inhibitor targeting such a new target is anticipated to exhibit synergistic effects when combined with an antibiotic. Another viable approach is the repurposing of older antibiotics through the utilization of synergistic combinations with current antibiotics[6].

Considering the use of antibiotic combinations provides numerous advantages, such as improving treatment effectiveness, broadening the spectrum of targeted pathogens, lowering the risk of adverse effects by reducing dosage and toxicity, and diminishing the likelihood of resistance emergence[12,13]. Augmentin stands out as a successful example of combining β-lactam antibiotics, illustrating the revival of β-lactam effectiveness. This combination includes amoxicillin, a β-lactam antibiotic, paired with clavulanic acid, a β-lactamase inhibitor. Clavulanic acid exhibits strong binding affinity to diverse bacterial β-lactamases, shielding amoxicillin from degradation by these enzymes, thus ensuring its efficacy. Consequently, this combination enables the utilization of amoxicillin in treating bacterial infections that demonstrate resistance to β-lactam antibiotics[14]. This spurs us to seek out a substance exhibiting antimicrobial properties, with the aim of revitalizing the effectiveness of β-lactam antibiotics.

FLX, a novel isoxazole penicillin, effectively combats penicillinase-producing strains of *S. aureus* and demonstrates high absorption rates in humans when administered orally or intramuscularly. In comparison to other isoxazole penicillins like oxacillin, cloxacillin, and dicloxacillin currently employed in clinical settings, FLX exhibits superior activity against Gram-positive cocci, including strains resistant to penicillin. As, this antimicrobial agent is resistant to penicillinase, an enzyme pivotal for breaking the beta-lactam ring in penicillins, thereby rendering them inactive. FLX, formulated for both oral and injectable delivery, exerts bactericidal effects. It is the preferred pharmaceutical form for administration[15,16].

Among the benzodiazine family, quinoxaline stands out as a significant six-membered ring template. These compounds including 3HX are of great significance in pharmacology due to their diverse and intriguing biological activities, such as antibacterial, antitubercular, antimalarial, antiviral, and anti-HIV

properties[17]. In their earlier work, Elfadil et al. showed the significant efficacy of quinoxaline derivatives, particularly 3HX, in enhancing the action of penicillin versus various clinical MRSA strains[18]. Expanding on this discovery, we are intrigued by whether 3HX possesses the potential to augment the effectiveness of FLX against diverse MRSA clinical strains. Put differently, we seek to explore if 3HX synergistic effects are exclusive to penicillin or if they extend to FLX in combating MRSA.

This study aims to assess the *in vitro* antimicrobial effectiveness of combining 3HX with FLX against various clinical MRSA strains.

MATERIAL AND METHODS

BACTERIAL ISOLATES AND GROWTH CONDITIONS

In this study, we examined 22 MRSA isolates obtained from King Abdulaziz University Hospital in Jeddah, Saudi Arabia. These isolates were stored in glycerol at -80°C. Prior to testing, they were thawed and cultured on blood agar from HiMedia, India, and then incubated overnight at 37°C in an aerobic environment. Colony identification was performed using standard procedures, including catalase and tube coagulase tests. Sample collection was performed in agreement with the ethics and research committee of the Faculty of Applied Medical Sciences at King Abdulaziz University (No. 38-712-456) and complied with the Declaration of Helsinki.

ANTIMICROBIAL AGENTS

This study evaluated medications designed to combat MRSA, including a 3HX compound obtained from Fluorochem Ltd in the United Kingdom and FLX powder acquired from Sigma.

BROTH MICRODILUTION ASSAY

Both drugs were initially prepared as 10 mg/ml stock solutions. To assess antibiotic and antimicrobial sensitivity, a broth microdilution test was carried out. This entailed diluting the drugs in Mueller Hinton Broth from Sigma-Aldrich in the United States in a two-fold manner (starting with 1024 µg/ml). Then, 100 µl of each drug solution was dispensed into the wells of 96-well plates from Corning, USA.

The density of the inoculum suspension was carefully set to 0.5 McFarland using a Biosan Densitometers DEN-1B turbidity detector. Subsequently, 5 µl of the inoculum was added to each well containing varying concentrations of antibiotics. The plates were left to incubate overnight at 37°C. Antibiotic susceptibility testing was conducted in triplicate, and the resulting mean values were recorded for analysis.

The Minimum Inhibitory Concentration (MIC) signifies the lowest concentration of a drug that prevents the visible growth of a microorganism. MIC results for both antibacterial agents were determined through the broth microdilution method and interpreted in accordance with guidelines from the Clinical and Laboratory Standards Institute (CLSI)[18,19].

CHECKERBOARD ASSAY

In order to assess the interaction of 3HX and FLX checkerboard assay was used. For this, we prepared a two-fold serial dilution of each antibiotic in Muller-Hinton broth (MHB) and dispensed 50 µl of each dilution into 96-well plates from Italy Inc. The density of the inoculum suspension was precisely adjusted to 0.5 McFarland using a Biosan Densitometers DEN-1B turbidity detector. Then, 5 µl of the diluted bacteria were added to each well of the 96-well plates. To assess interactions, we calculated the fractional inhibitory concentration index (FICI) using the formula: (MIC of drug A in combination / MIC of drug A alone) + (MIC of drug B in combination / MIC of drug B alone). A FIC index of ≤ 0.5 indicated a synergistic effect[20].

RESULTS

MICS OF FLX AND 3HX AGAINST MRSA CLINICAL STRAINS

In preparation for the checkerboard assay experiment, it is necessary to determine the MICs of both FLX and 3HX. The MICs for FLX vary between 128 and 512 µg/ml (Table 1), with higher MIC values expected when dealing with MRSA strains. Conversely, the MICs for 3HX range from 16 to 64 µg/ml (Table 1). The MIC was defined as the lowest concentration that inhibits bacterial growth[6].

Table 1. Minimum Inhibitory Concentrations (MICs) in µg/ml of FLX and 3HX against MRSA strains

Number of Strain	MRSA	MIC 3HX	MIC FLX
1	70	32	512
2	72	32	512
3	91	32	512
4	80	16	512
5	90	32	512
6	75	32	512
7	73	32	512
8	93	64	512
9	95	32	512
10	96	32	512
11	97	32	512
12	106	64	128
13	104	32	256
14	102	32	512
15	100	32	512
16	105	32	256
17	107	32	256
18	98	32	128
19	92	32	128
20	101	32	256
21	1	32	128
22	2	32	128

3HX SYNERGIZES FLX AGAINST DIFFERENT MRSA CLINICAL STRAINS

To investigate the potential synergy between 3HX and different beta-lactam antibiotics, such as FLX, against MRSA strains, we employed a checkerboard assay. Interestingly, when FLX was administered alone, it failed to inhibit MRSA growth. However, when combined with 3HX, a significant decrease in the MICs of FLX was observed, with reductions of up to 64-fold in some cases. Similarly, the MICs of 3HX were notably lower, up to 16-fold, when used in combination with FLX (Figure 1).

Moreover, this combination exhibited a synergistic interaction across 22 distinct clinical MRSA strains, as evidenced by FICI values consistently below 0.5 with 100% synergy (Table 2). The results strongly imply that 3HX can effectively enhance the effectiveness of FLX in fighting against MRSA strains, indicating the

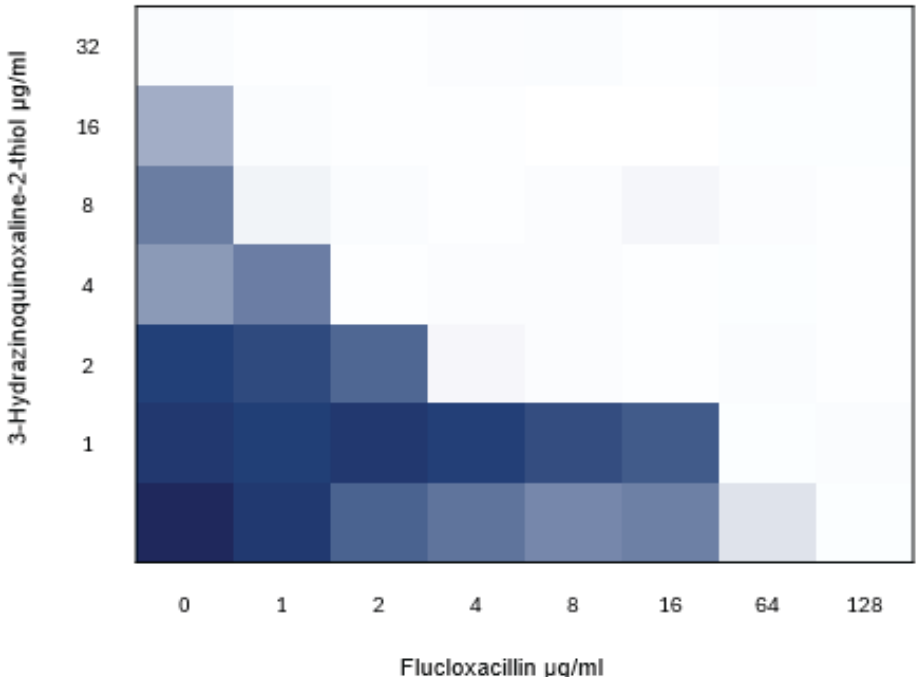

Figure 1. Checkerboard test showing the synergistic effect of flucloxacillin with 3-hydrazinoquinoxaline-2-thiol against MRSA 2. The white color represents 0 % growth, and the dark blue color represents 100% growth.

Table 2. Synergistic Interaction of 3HX and FLX Across 22 Clinical MRSA Strains. Fraction inhibitory concentration (FIC), Fraction inhibitory concentration index (FICI).

Number of Strain	MRSA	FIC 3HX	FIC FLX	FICI
1	70	0.250	0.014	0.264
2	72	0.146	0.032	0.178
3	91	0.25	0.066	0.316
4	80	0.25	0.032	0.282
5	90	0.094	0.047	0.141
6	75	0.167	0.043	0.21
7	73	0.146	0.034	0.18
8	93	0.146	0.073	0.219
9	95	0.104	0.057	0.161
10	96	0.188	0.094	0.282
11	97	0.175	0.131	0.306
12	106	0.208	0.062	0.27
13	104	0.135	0.109	0.244
14	102	0.089	0.057	0.146
15	100	0.146	0.13	0.276
16	105	0.208	0.086	0.294
17	107	0.104	0.045	0.149
18	98	0.135	0.115	0.25
19	92	0.104	0.042	0.146
20	101	0.146	0.043	0.189
21	1	0.156	0.071	0.227
22	2	0.109	0.074	0.035

possibility for enhanced treatment options. Moreover, this implies that the synergistic effects of 3HX are not exclusive to penicillin but also apply to other beta-lactam antibiotics.

DISCUSSION

Combining antibiotics in bacterial infection therapy has demonstrated its effectiveness in tackling the obstacles presented by multidrug-resistant pathogens. The successful application of combination therapy can be seen in the treatment of Mycobacterium tuberculosis[21]. The advantages of combination therapy include ensuring adequate drug delivery to infection sites, enhancing bacterial clearance, preventing resistance emergence, and inhibiting toxin synthesis, which diminishes bacterial virulence[13, 22]. Our prior research demonstrated promising outcomes with the combination of penicillin and 3HX against MRSA[18]. This prompts us to investigate whether hydrazinoquinoxaline-2-thiol can synergize ant staphylococci beta-lactam, such as FLX, against clinical MRSA strains or if this synergy was specific to penicillin.

The combination of FLX and 3HX demonstrates synergistic interaction against 22 MRSA clinical strains, leading to a remarkable reduction in MICs of FLX by up to 64-fold. Similarly, when paired with FLX, 3HX derivatives also exhibit a substantial decrease in MICs. This potent cooperative effect was consistently observed across different clinical MRSA strains in our experiments. These results strongly suggest that the combined therapy of FLX and 3HX produces a more robust response against MRSA strains compared to the efficacy of each drug alone. Also, these results suggest that 3HX synergise other beta-lactam drugs (FLX) not only limited to penicillin.

Considering the potential for toxic effects related to elevated concentrations of 3HX, FLX, and other antibiotics, employing reduced doses of each drug in a synergistic approach presents a promising strategy to mitigate potential toxicity[23]. Our research findings support this concept: while FLX alone required 512 µg/ml to impede MRSA growth, an intriguing discovery emerged when combined with 3HX. In this combined regimen, only 16 µg/ml of FLX was sufficient to inhibit the growth of the same MRSA strain. This finding suggests the feasibility of achieving a comparable therapeutic effect with lower drug doses, potentially reducing the risk of adverse effects. However, further investigations are warranted to validate this promising outcome.

Our study has demonstrated that FLX alone was ineffective in inhibiting MRSA growth. However, when combined with 3HX, it gained the ability to suppress MRSA growth. This observed effect can be attributed to the synergistic interaction between FLX and 3HX, highlighting the potential of combination therapy to address the inherent challenges associated with MRSA infections. Furthermore, it has been suggested that the introduction of a second antibiotic in the treatment regimen can compensate for the limitations of the first antibiotic[24]. This may explain why the two antimicrobial drugs in combination exhibit greater efficacy than each drug alone.

The synergy observed between 3HX and FLX seems to stem from their action on distinct pathways. While 3HX interferes with DNA synthesis[25], FLX

inhibits penicillin-binding proteins (PBPs). Inhibiting of PBPs results in abnormalities in bacterial cell wall structure, including elongation, lesions, compromised permeability, and eventual cell lysis[26]. Additionally, FLX exhibits activity against various β-lactamases, including penicillinases and cephalosporinases[27]. Consequently, FLX may inhibit the activity of penicillinases in degrading itself, thereby enhancing the effectiveness of FLX in the combination therapy.

An alternative explanation for the enhanced efficacy of combining FLX and 3HX versus different MRSA strains could involve the increased creation of reactive oxygen species (ROS). These ROS disrupt target-specific cellular processes, ultimately leading to cell death[28]. It is widely recognized that bactericidal antibiotics, particularly β-lactams, can stimulate ROS generation, which is pivotal in bacterial cell eradication[29]. Intriguingly, 3HX is also known to possess the ability to generate reactive oxygen species[30]. Therefore, we assume that the observed synergy among FLX and 3HX may be associated with the heightened production of ROS. Another possible reason for the increased effectiveness of combining FLX and 3HX against different MRSA strains could be the intercalation impact of 3HX on MRSA DNA[31, 32]. The synergism may have resulted from the cumulative impact of FLX inhibiting peptidoglycan production, so enhancing the absorption of 3HX, which disrupts DNA synthesis. Nevertheless, further experimentation is required to substantiate this conjecture.

Time-kill analysis will provide more information about the bactericidal activity of the combined drug[6]. Moreover, resistance assay can reveal whether this combination inhibits the development of new resistance[23]. *In vivo,* the model will analyze the pharmacokinetic and pharmacokinetic of the combined drug as well as the efficacy *in vivo*[33].

CONCLUSION

Our study presents groundbreaking evidence of the synergistic efficacy of combining 3HX with FLX against a wide range of clinical MRSA strains. These findings suggest a promising avenue for the clinical application of this synergy. However, significant steps are needed to translate the combined antibiotics into clinical practice. Further research and clinical trials are essential to fully evaluate their therapeutic potential, refine dosing strategies, and ensure their safety and effectiveness in real-world medical contexts.

ABBREVIATIONS

MHB, Muller-Hinton broth, MRSA, Methicillin-resistant Staphylococcus aureus, MIC, minimum inhibitory concentration, FIC, Fractional inhibitory concentration, FICI, Fractional inhibitory concentration index, ROS, Reactive oxygen species, PBP2A, penicillin-binding protein 2A, PBP, Penicillin-binding proteins

CONFLICT OF INTEREST

The author declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The author did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the author has no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

This study received approval from the Ethics and Research Committee of the Faculty of Applied Medical Sciences at King Abdulaziz University (Approval No. 38-712-456).

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CASE REPORT

Prolonged Primary Hyperparathyroidism: Are We Overtreating our Patients?

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Abstract

Primary Hyperparathyroidism is increasing in incidence likely due to enhanced screening and diagnostic methods. Current recommendations encourage surgical management for patients who are symptomatic or who have evidence of organ involvement. However, in some cases who have had long-standing disease with minimal impact, a more conservative approach may be prudent. This case report presents a 68-year-old retired physician with type 2 diabetes managed in the Endocrinology clinic, incidentally discovered to have long-standing primary hyperparathyroidism (PHPT) during routine lab investigations. Despite a history of recurrent kidney stones and elevated parathyroid hormone (PTH) levels over 40 years, the patient remains relatively asymptomatic with minimal end-organ involvement. Current diagnostic and therapeutic recommendations suggest surgical intervention; however, the patient, given his lengthy disease course, opts for a conservative approach. This case prompts a reconsideration of individualized management strategies in the era of precision medicine.

Keywords

Hypercalcemia, Recurrent kidney stones, Parathyroid hormone, Osteoporosis, Type 2 diabetes, Endocrinology

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a condition characterized by elevated serum calcium levels due to excess parathyroid hormone secretion. The most recent recommendations published after 2014 include Guidelines for the Management of Asymptomatic Primary Hyperparathyroidism: Summary Statement from the 4th International Workshop published in JCEM 2014, Primary Hyperparathyroidism: review and Recommendations on evaluation, diagnosis, and management, a Canadian and international consensus published in Osteoporosis International in 2017, hyperparathyroidism (primary): diagnosis, assessment and initial management published by the National Institute of health and care excellence in 2019 as well as evaluation and management of primary hyperparathyroidism: summary statement and guidelines from the 5th international workshop published in JBMR in August of 2022. The latter, most recent guidelines recommend that individuals with elevated serum calcium adjusted for albumin with elevated or inappropriately normal intact parathyroid hormone on two occasions at least two weeks apart is diagnostic for hypercalcemic primary hyperparathyroidism (Bilezikian, 2022). This case explores a patient with a long-standing PHPT and the challenges of reconciling historical management norms with evolving contemporary approaches, emphasizing the importance of personalized care.

CASE PRESENTATION

This is a 68-year-old retired physician presenting to the Endocrinology clinic for the management of type 2 diabetes. During the visit, a review of his recent lab investigations incidentally revealed a mildly elevated calcium level of 11 mg/dl. His past medical history was significant for type 2 diabetes, dyslipidemia, ischemic heart disease post-stenting several years ago, hypothyroidism, and a previous history of kidney stones. On further history taking, it was revealed that he had a remote history of over 40 years ago of multiple kidney stones; he reported that his first kidney stone occurred in 1981, followed by multiple kidney stones every few years after that, with the last documented kidney stone in 2014. He was also told at that time that he had elevated PTH and questionable parathyroid hyperplasia which was never further followed up or investigated as he was unwilling to undergo a Sestamibi scan at the time of the discussion. He also reports a history of low bone density diagnosed several years ago which was not followed up or treated since then. He was offered treatment with cinacalcet, which he declined. His current medications include dulaglutide, insulin degludec, metformin, empagliflozin, Gliclazide MR, rosuvastatin, valsartan, bisoprolol, clopidogrel, pantoprazole 40 mg BID, vit D 5,000 IU daily, vitamin B12, and thyroxine. He has no known allergies, is a non-smoker, and reports no family members with a similar history of kidney stones. Further review of his current lab investigations revealed mildly decreased calculated EGFR at 72 µmol/ml. As he continues to refuse nuclear imaging at this time, we proceeded with a neck ultrasound and a follow-up DEXA scan to evaluate his bone density.

Current recommendations for the diagnosis and management of hyperparathyroidism suggest that for initial investigations, serum calcium should be measured as well as vitamin D levels, PTH level, 24-hour urine calcium, or urine calcium to creatinine ratio. Additionally, it is important to assess for possible end-organ damage resulting from hypercalcemia. As this case occurred over 40 years ago, not all these investigation results are available to me when writing this case report. Available relevant laboratory investigations grouped by date were as follows:

- 2008: 25(OH) Vitamin D 17.05 nmol/L, TSH 6.7 mIU/L, Serum Calcium 10.98 mg/dl, Serum Phosphorous 1.07 mmol/L.
- 2015: PTH 3.28 pmol/L, 25(OH) Vitamin D 45.39 nmol/L, Serum Calcium 10.82 mg/dl, Serum Phosphorous 1.06 mmol/L, Serum magnesium 1.48 mg/dl, Alk phosphatase 31 U/L, Albumin 4.5 g/dl, 24 hr urine calcium 8.04 mmol/24 hrs, 24 hr urine oxalate 31.25 mg/24 hrs.
- Investigations done in a different facility (Figure 1)

Thyroid Ultrasound in 2007 and repeated in 2024 did not show any parathyroid adenomata or hyperplasia. A DEXA scan in 2015 (Figure 2).

Repeat investigations at the time of writing this report showed the following results: PTH 11.04 pmol/L (normal range 1.59-7.24), creatinine 117 µmol/L (62-115 µmol/L), calcium 2.54 mmol/L (2.08-2.65 mmol/L), serum phosphate 0.7 mmol/L (0.78-1.65 mmol/L).

Repeated BMD in 2024 was reported as shown in Figure 3.

Accumulative Results

Creatinine

03/03/21	01/10/18	29/09/18	20/09/18	30/11/15	15/09/15	20/08/15	12/08/15	10/08/15	01/02/15
110	104	104	104	102	88	86	87	105	80

Accumulative Results

Vitamin D total (25-OH-Vitamin D3)

03/03/21	20/09/18	30/11/15	10/08/15	01/02/15	21/05/14	20/09/08
132.1	70.0	80.63	51	65	45.19	63.4 (NR greater or = 75 nmol/l)

Accumulative Results

Parathyroid Hormone (PTH)

20/08/15	10/08/15	01/02/15	20/09/08
4.040	6.916	4.8	3.71

Accumulative Results

Calcium

03/03/21	20/09/18	30/11/15	15/09/15	20/08/15	12/08/15	10/08/15	01/02/15	21/05/14	20/09/08
2.55	2.61	2.69	2.72	2.65	2.79	2.54	2.75	2.69	2.53

Figure 1. **Laboratory results from a patient's biochemical profile.**

Findings:

The calculated BMD of the AP spine L1 through L4 is 1.075 gm/sq. cm.
- T-score of - 1.2
- Z-score of - 0.8.

The calculated BMD of the left femoral neck is 0.899 gm/sq. cm.
- T-score of - 1.3.
- Z-score of - 0.4.

The calculated BMD of the right femoral neck is 0.864 gm/sq. cm.
- T-score of - 1.6.
- Z-score of - 0.6.

The calculated BMD of the left forearm is 0.738gm/sq.cm.
T-score of - 0.8.
Z-score of - 0.4.
The calculated BMD of the right forearm is 0.754 gm/sq.cm.
T-score of - 0.6.
Z-score of - 0.2.

Impression:
·According to WHO guidelines, this patient is considered osteopenic with moderate risk of fracture.

Figure 2. **DEXA scan results**

Bone mineral density of the lumbar spine is 1.103 g/cm2
T-score -0.1
Z-score 0.4

Bone mineral density at the left femoral neck is 0.891 g/cm2
T-score -1.1
Z-score 0.2

Bone mineral density at the right femoral neck is 0.846 g/cm2
T-score -1.4
Z-score -0.1

</CONCLUSION/>

The patients bone mineral density is within osteopenia. The estimated risk for major osteoporotic fracture 3.4% and for hip fracture 1.1%.

Figure 3. **BMD measurement**

DISCUSSION:

Currently, there are several published recommendations for the diagnosis and management of primary hyperparathyroidism. These recommendations emphasize establishing the presence of elevated serum calcium adjusted for albumin, elevated or non-suppressed PTH level, normal vitamin D levels, the absence of hypocalciuria, and assessment for potential organ involvement such as nephrolithiasis, nephrocalcinosis, renal impairment, and osteoporosis.

Most recommendations agree that symptomatic primary hyperparathyroidism should be treated surgically with parathyroidectomy and intraoperative examination of the other parathyroid glands (Khan, 2016), with the suggestion that parathyroidectomy has beneficial effects on the preservation of bone density, reduction in kidney stones, and stabilization of renal function (Khan, 2016). In less overt cases, surgical management should be offered to patients who have one or more of the following criteria regardless of symptoms: serum calcium 1mg/dl or 0.25 mmol/L above the upper limit of normal, evidence of skeletal involvement by DEXA scan diagnostic of osteoporosis or radiographic images showing reduced bone density, kidney involvement in the form of reduced creatinine clearance or eGFR < 60ml/min or nephrolithiasis evident on any imaging modality, increased urinary calcium excretion, or age less than 50 years (Bilezikian, 2022).

Based on the above recommendations, the patient presented in this case report should have been treated with parathyroidectomy at several points during the past 40 years, had these current recommendations been implemented at that time. Since the widespread implementation of current diagnostic and therapeutic recommendations worldwide, long-standing untreated primary hyperparathyroidism is uncommonly encountered in the clinical setting. A 2008 study followed 116 patients with mild primary hyperparathyroidism for 15 years, 59 of which were treated with parathyroidectomy and 57 of which remained untreated. After 15 years of observation, the authors concluded that while biochemical and BMD parameters remained stable for the first eight to ten years of follow-up of both patient groups, the effects of prolonged untreated hyperparathyroidism in the second group eventually started catching up with them in the form of loss of cortical bone mainly in the distal radius and femoral neck (Rubin, 2008). Remarkably, our patient continued to maintain his bone mineral density

over the almost 10-year interval between his two DEXA scans, despite having had at least one recorded kidney stone during that time. His calcium level continues to fluctuate, as does his PTH level, with the last measured PTH level being the highest reading available to me. Additionally, despite the frequent kidney stones and prolonged history of what is most likely primary hyperparathyroidism, he continues to have minimal renal involvement. Given this overall lack of significant organ involvement, and considering his long history with his condition, he remains reluctant to undergo a parathyroidectomy unless deemed necessary. This brings us to the question of whether we should reconsider the need for surgical intervention in some of our patients with primary hyperparathyroidism, especially those who have already had a long course with minimal impact on their end organs. In the age of precision medicine, we are moving away from treating our patients with a one-size-fits-all approach. We perhaps should be spending more time obtaining a more global picture of the patient's condition as well as their goals of treatment and preferences. Despite being an unconventional approach, in hindsight, it appears that the management route taken did not affect this particular patient detrimentally, raising the possibility that conservative management with close observation could be an option for select patients despite meeting surgical criteria.

This case prompts a critical reflection on the evolving landscape of primary hyperparathyroidism management. While current guidelines advocate for surgical intervention based on established criteria, the presented patient challenges this paradigm, demonstrating a lengthy disease course with minimal end-organ involvement. In the era of precision medicine, adopting a more individualized approach, considering the overall impact on the patient's quality of life, and respecting patient preferences become paramount. Continued close monitoring and shared decision-making with the patient align with contemporary trends in tailoring treatments to each patient's unique circumstances.

CONCLUSION

This case encourages further exploration of nuanced management strategies for primary hyperparathyroidism, recognizing that not all patients may fit the traditional treatment paradigm.

CONFLICT OF INTEREST

The author declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The author did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the author has no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of the KAUH in Jeddah, Kingdom of Saudi Arabia with number Ref-60-24.

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