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PHILIPPINE JOURNAL OF PATHOLOGY

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IN THIS ISSUE:

THE PHILIPPINE BOARD OF PATHOLOGY

ANNOUNCES THE DATES OF THE

2026 DIPLOMATE EXAMINATIONS

ANATOMIC PATHOLOGY - FEBRUARY 22, 2026 (SUNDAY)
CLINICAL PATHOLOGY - MARCH 1, 2026 (SUNDAY)
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The Philippine Journal of Pathology (PJP) is an open-access, peer-reviewed, English language, medical science journal published by the Philippine Society of Pathologists, Inc. Committee on Publications. It shall serve as the official platform for publication of high quality original articles, case reports or series, feature articles, and editorials covering topics on clinical and anatomic pathology, laboratory medicine and medical technology, diagnostics, laboratory biosafety and biosecurity, as well as laboratory quality assurance. The journal's primary audience are Filipino laboratorians, diagnosticians, laboratory managers, pathologists, medical technologists, and all other medical and scientific disciplines interfacing with the laboratory. The PJP follows the **ICMJE Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals, EQUATOR Network Guidelines,** and **COPE Guidelines.** The PJP does not charge any article processing or submission fees from authors. It does not charge any subscription fees or download fees to access content.

On behalf of the Philippine Society of Pathologists, Inc., I extend my warmest congratulations to the *Philippine Journal of Pathology (PJP)* on the release of its second issue for 2025, and on its recent inclusion in the **Directory of Open Access Journals (DOAJ)**. This milestone is a testament to the journal's growing stature and to the dedication of its editorial team, reviewers, contributors, and readers who continue to uphold the highest standards of scientific publication.

The PJP stands as our Society's flagship platform for advancing the science and practice of pathology in the country. Its continued development, through the use of an online editorial management system and its expanding international indexation, ensures that the work of Filipino pathologists is made visible and accessible to the global scientific community.

As we celebrate this achievement, I call on all training institutions and residency programs in pathology to nurture a culture of research and scholarship by encouraging their residents to translate their scientific studies into publishable work. Through the PJP, these outputs can contribute meaningfully to evidence-based practice, laboratory strengthening, and the advancement of pathology and laboratory medicine in the Philippines.

The Philippine Society of Pathologists, Inc. remains steadfast in its support for the operations and sustainability of the PJP, recognizing its vital role in elevating the visibility, quality, and impact of Filipino research. Together, let us continue to build a vibrant and credible body of knowledge that reflects the excellence and integrity of our profession.

Maria Cecilia F. Lim, MD, FPSP
President, Philippine Society of Pathologists, Inc.

A Quiet Milestone: PJP Joins the Directory of Open Access Journals

The *Philippine Journal of Pathology (PJP)* has recently been accepted for inclusion in the **Directory of Open Access Journals (DOAJ)**. It is a quiet but meaningful milestone — like the ripple that follows a small stone dropped into still water, widening slowly and quietly, yet certain in its direction.

The DOAJ is an international index of peer-reviewed, open-access journals that meet recognized standards of editorial quality and ethical publishing. Managed by the Infrastructure Services for Open Access (IS4OA) in the United Kingdom, it lists over 20,000 journals worldwide that are committed to transparency, peer review integrity, and the free availability of research.

Acceptance into DOAJ follows a detailed evaluation of a journal's policies, editorial processes and openness, ensuring that the journals it lists uphold the principles of open science.

For PJP, being part of DOAJ affirms that we are on the right path. It validates the systems we have built over the years: clear editorial policies, a rigorous peer review process, and the quiet discipline of upholding ethical standards in publishing. More importantly, it allows the work of Filipino pathologists and laboratory scientists to reach a wider audience through greater visibility in search engines, institutional repositories, and other indexing services that draw from DOAJ.

The practical benefits are welcome, but the recognition is most meaningful as a reminder of what has sustained us. PJP has always been an endeavor that thrives on its contributors' active participation. Maintained by members, supported by the Philippine Society of Pathologists, and built upon the trust of its contributors and readers. The journal's progress rests on the generosity of those who give their time and expertise for the advancement of the discipline.

We mark this milestone quietly, as one might watch a ripple extend across calm water — without fanfare, but with a sense of continuity. The inclusion in DOAJ is not an endpoint, but a signpost — encouraging us to keep improving, to maintain transparency, and to hold fast to the values that have guided us from the beginning.

To our authors, reviewers, readers, and the PSP community: thank you for your continued support. May this small achievement remind us that even the gentlest motion, made in the spirit of service, can carry far.

Amado O. Tandoc III, MD, FPSP
Editor-in-Chief

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Revolutionizing the Future of
PATHOLOGY with the EMERGENCE of AI

75th Annual Convention
16-18 April 2026
Makati Shangri-La Hotel

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Attending Pathologist
Director of Head and Neck Pathology
Memorial Sloan Kettering Cancer Center

Hannah Wen, MD, PhD
Attending Pathologist
Director, Breast Pathology Fellowship
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Attending in Genitourinary Pathology
Cleveland Clinic

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Makati Shangri-La Hotel

Registration Rates

Category	In-person Early bird	In-person Regular	Online
PSP MEMBERS			
Regular Members (Diplomates and Fellows)	Php 8,000	Php 9,000	Php 7,000
Junior Members (Residents and Graduates)	Php 5,500	Php 6,500	Php 5,000
Regular Members (60 to 69 years old and PWD)	Php 6,400	Php 7,200	Php 5,600
Regular Members (70 years old and above)	FREE but must register before 28 January 2026		
NON-MEMBERS			
Local Delegates			
Physicians (Consultants)	Php 8,500	Php 9,500	Php 7,500
Physicians (Residents and Fellows in Training)	Php 5,500	Php 6,500	Php 5,000
Allied Health	Php 5,500	Php 6,500	Php 5,000
Foreign Delegates	USD 225	USD 250	USD 200

ADD-ON: INTERACTIVE MICROSCOPY SESSIONS

Limited to 50 participants per session

TOPICS – Anatomic Pathology	Early bird IN-PERSON RATE	Regular IN-PERSON RATE
Breast	Php 1,500	Php 2,000
Female Genital Tract	Php 1,500	Php 2,000
Head and Neck	Php 1,500	Php 2,000
Genitourinary	Php 1,500	Php 2,000

REGISTRATION PERIOD

EARLY BIRD & ONLINE: 01 November 2025 to 28 January 2026 (5 PM)
REGULAR: 03 February 2026 to 13 March 2026 (5 PM)

SCAN TO REGISTER

Revolutionizing the Future of PATHOLOGY with the EMERGENCE of AI

75th Annual Convention
16-18 April 2026
Makati Shangri-La Hotel

Registration Mechanics

1. Timeline for registration is as follows:
   a. **Early Bird and Online (Virtual) Registration** 01 November 2025 to 28 January 2026 (5 PM)
   b. **Regular Registration** - 03 February 2026 to 13 March 2026 (5 PM)
2. Registration should be done only within the abovementioned dates and only through the **PSP Events Page**: bit.ly/432ASz4.
   a. There will be **NO LATE AND ON-SITE REGISTRATION.**
   b. Persons under proxy registration should likewise register within the abovementioned timeline.
   c. Graduates who will take the diplomate exams in 2026 may ***initially*** register as Junior Members. If they pass the exam, the Regular Member (Diplomate) rate shall apply, and they must settle the remaining balance within a certain period. Further details regarding this shall be announced as the results of the diplomate exams are released. The graduate must register within the early bird period to avail of the discounted rates.
   d. For individuals who do not have an account, please visit the PSP Events Page, click **Sign-up**, and follow the on-screen instructions to create your account.
3. Online registration form must be accomplished first before payment.
   a. There are **limited slots** for **in-person** attendance that will be given on a **first come, first served basis.**
   b. For in-person registrants, please fill out the section of the form on dietary restrictions/food allergies.
4. For payment, please refer to further instructions detailed on the site.
   a. For a single transaction covering multiple individuals (e.g., person under proxy registration), please indicate the names of the persons covered in the proof of payment/receipt. Then, distribute it to the persons covered, and they may proceed with individual registration using this proof of payment.
   b. Please wait for **three (3) to five (5) business days** for the secretariat to confirm your payment.
5. **No reimbursement or refund arrangements shall be entertained by the society.**
6. Online (Virtual) registrants who wish to change their registration to in-person attendance may only do so BEFORE the secretariat confirms the payment.
   a. If payment has not yet been made, please cancel the previous registration attempt and create a new one choosing the preferred mode of attendance.
   b. In case that the registrant has already paid for the previous registration, he/she must immediately inform the secretariat and wait for them to confirm the remaining balance, which should be settled as soon as possible.
   c. A single photo/ file containing both transaction receipts/ proof (original and additional payment) should be used for the new registration.
7. Individuals who registered AND paid for in-person attendance will **NOT** be able to change their registration to online (virtual) even if the secretariat has not yet confirmed the payment.
8. Once payment is confirmed, an email notifying the successful registration shall be sent to your registered email address.
9. **Confirmed registrations cannot be changed, cancelled, and refunded.**
10. Regular members who registered for online (virtual) attendance will be able to join the business meeting. They must be individually logged-in during the business meeting to be considered present.
11. In case of difficulties during registration, kindly contact the PSP secretariat through the following channels:

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Hope for the Future of Laboratory Quality in the Philippines

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Editor's Note: *In this issue of the Philippine Journal of Pathology, we spotlight a principle at the core of diagnostic medicine: quality. Though much of pathology's work is unseen, the accuracy of laboratory results guides every clinical decision. Few have advanced this cause as strongly as* ***Dr. Sarah Jane Datay-Lim.*** *In the interview that follows, Dr. Datay-Lim reflects on her path from stepping into quality assurance with no clear roadmap to becoming a leading advocate through the Philippine Council for Quality Assurance in Clinical Laboratories (PCQACL). Her experiences highlight both the country's progress and the challenges that remain in strengthening a true culture of quality. She underscores a shift in how we view quality—no longer as a punitive exercise, but as a collaborative, systems-oriented approach that promotes learning and continuous improvement. Her insights are timely as diagnostics evolve with genomics, point-of-care testing, digital pathology, and artificial intelligence. She calls for expanded external quality assessment, harmonized standards, and sustained education to build the next generation of quality champions. Ultimately, Dr. Datay-Lim reminds us that quality is about patients and the trust they place in laboratory medicine. We hope this conversation inspires continued commitment to a stronger, safer, and more accountable laboratory system for the Philippines.*

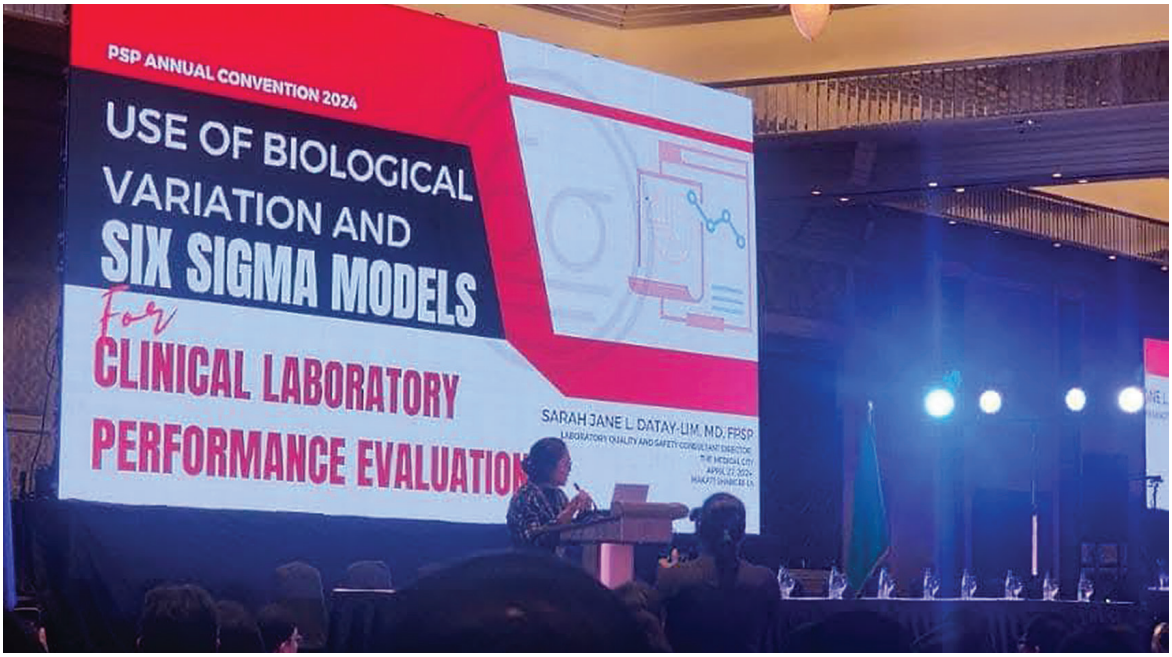
What drew you to quality assurance in medical laboratories, and how has your perspective evolved since you first started in this field?

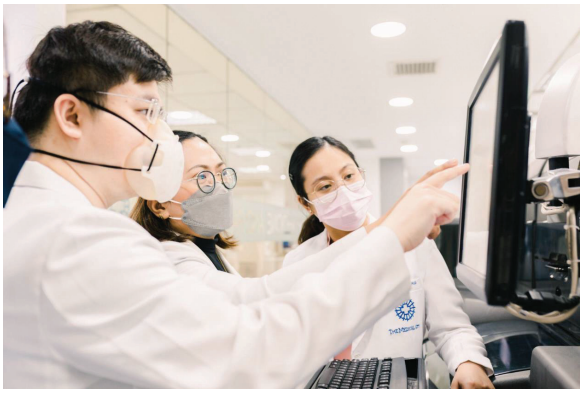
Initially, my journey into quality assurance was driven by necessity. While reviewing for the boards, our former chair, Dr. German B. Castillo Jr., offered me the position of Laboratory Quality Consultant Director at The Medical City. That opportunity became a strong motivator to pass the boards. However, once I started, I quickly realized how challenging the role was—there was no clear roadmap or guide to follow. Quality assurance is such a broad domain without clear boundaries that I often find myself in situations that make me ask, *"Is this still part of my responsibility?"*

I remember that there was even a time when my position was questioned, especially since the hospital already had its own quality department. That experience made me reflect deeply on the unique responsibilities within the laboratory and the importance of having someone dedicated to quality assurance in that specific setting.

Looking back, aside from the support by the people around me, what helped me grow in this role was volunteering with the Philippine Council for Quality Assurance in Clinical Laboratories (PCQACL). There, I was fortunate to be exposed to a network of experts both local and international… who generously shared their knowledge and best practices. Their influence was invaluable. I also took every training opportunity available such as auditor training and lean six sigma certifications… and invested in books and references to build my understanding and capabilities. I think this helped me appreciate my role in laboratory quality and gave me a clear picture on what I should aspire our laboratory to be.

In the past, quality assurance was often perceived as a restrictive and punitive process… effective only when rules were rigidly enforced and errors were penalized. But over time, as our understanding deepened and the healthcare landscape evolved, so did the approach to quality. For me, quality assurance is no longer about fault-finding or drawing hard lines between right and wrong. It's about helping laboratories identify opportunities for improvement and supporting them in building safer, more reliable systems. More often than not, errors are not the fault of individual staff members, but rather symptoms of systemic vulnerabilities. Recognizing this shifts the focus from blame to collaboration, and from punishment to progress. This is what I love about quality assurance and keeps me motivated to keep on going.





As former president of the Philippine Council for Quality Assurance in Clinical Laboratories, what moments or initiatives stand out as milestones in advancing laboratory quality in the country?

I think we made good progress with continued education and training, particularly in various disciplines of quality assurance and laboratory medicine. Seminars, webinars and workshops that focus on important topics such as internal quality control, proficiency testing, method validation, accreditation to name a few were regularly conducted, not just in the traditional platform but in other ways to cater to the changing times such as online. We also made good linkages with the international community which helped us align with the global standards. However, we need to be consistent and continue to share the good practices more so that we can influence not just laboratory professionals but also the regulatory bodies as well.

How would you describe the current state of quality assurance in clinical laboratories in the Philippines?

I believe we've made significant strides in recent years. For instance, the expansion of External Quality Assessment (EQA) schemes led by the National Reference Laboratories (NRLs) and the growing number of laboratories accredited by reputable bodies are clear indicators of progress. However, when compared to the global landscape, we still have a long way to go. Many laboratory professionals continue to face challenges with foundational aspects of quality assurance, such as troubleshooting quality control issues. Moreover, the proportion of accredited laboratories in the Philippines remains relatively low compared to other countries, highlighting the need for more widespread and sustained efforts in this area. We also see many non-compliance with good practices and international/ local standards.

In your view, what are the most persistent gaps or challenges that laboratories face in meeting international standards like ISO 15189?

The most persistent gap I think is justifying the cost of quality. One of the most persistent gaps in quality assurance is ensuring that standards are not only well-documented but also genuinely implemented in daily practice. It's one thing to have policies in place, but translating them into consistent action remains a major challenge. Another significant hurdle is resource limitation. Convincing hospital administration to invest in quality can be difficult, especially when the returns are not immediately visible. However, it's important to emphasize that quality is not

an expense… it's an investment that ultimately leads to better outcomes, improved efficiency, and greater trust in laboratory services.

Do you think the COVID-19 pandemic accelerated or complicated the journey toward better quality systems in Philippine laboratories?

I believe the COVID-19 pandemic accelerated the journey toward better quality systems in laboratories. It placed the laboratory at the forefront of healthcare, highlighting the critical role of diagnostics in patient care and public health. This heightened visibility brought with it greater expectations and accountability, pushing us to elevate our standards and performance. The impact of our work became more evident than ever, reinforcing the need for robust quality systems to ensure accurate, timely, and reliable results.

ISO 15189 accreditation is often seen as the "gold standard" for laboratories. In your experience, what is the most misunderstood aspect of ISO 15189?

I think one of the most misunderstood aspects of ISO 15189 is the belief that only large, well-funded laboratories can achieve accreditation. Many assume that the process is prohibitively expensive and complex, making it unattainable for smaller or resource-limited facilities. However, with the right mindset, commitment, and strategic planning, even modest laboratories can work toward compliance. Accreditation is not about perfection… it's about continuous improvement and building systems that ensure quality and patient safety.

What practical advice would you give to laboratories in resource-limited settings that aspire to meet ISO 15189 requirements?

I think a practical advice would be to read the standards, resource materials and attend seminars and webinars about ISO15189 as much as possible. Then perform a gap analysis to check your laboratory against the standards to be able to have an understanding of where you are and where you want to go.

How do you see accreditation impacting not only laboratory operations but also patient outcomes and trust in the health system?

Any form of laboratory accreditation can significantly enhance patient outcomes by establishing a strong framework for quality within laboratory systems. Accreditation promotes more accurate testing and reduces the likelihood of errors, which directly influences the quality of patient care and clinical decision-making. It builds trust in laboratory results and reinforces the role of diagnostics as a cornerstone of effective healthcare.

Quality assurance often requires a cultural shift within institutions. How do you foster a culture of quality among staff who may initially see QA as additional paperwork or bureaucracy?

I believe fostering a culture of quality starts with the leadership within the laboratory. Historically, quality assurance has often been viewed as punitive… focused on identifying who made a mistake rather than understanding why the mistake occurred. To shift this mindset, leaders must model a culture that encourages openness, learning, and collaboration. It's about promoting the recognition of errors not as failures, but as opportunities to improve systems and prevent recurrence. When staff see that leaders are genuinely committed to these principles (not just enforcing paperwork)… they begin to understand that quality is not an added burden, but an integral part of everyday practice.

… (ISO 15189) Accreditation is not about perfection… it's about ***continuous improvement*** *and* ***building systems that ensure quality and patient safety.***

In your opinion, what leadership qualities are most critical for driving quality improvement in the laboratory?

I believe the most important leadership quality in driving quality improvement is the ability to 'walk the talk'… to consistently model the behaviors and values we expect from our teams. Leaders must be open, approachable, and willing to listen and collaborate. Shifting the mindset of staff toward embracing quality requires more than directives: it demands authenticity, flexibility, and a shared commitment to continuous improvement. When staff see that quality is not just a checklist or paperwork, but something truly embedded in the way we work every day, they begin to take ownership of it themselves.

How can laboratory managers and pathologists mentor the next generation to be champions of quality?

The laboratory managers and pathologists can mentor the next generation to be champions of quality by involving them very early on. We must also communicate with them and make sure that they understand the vision and goals of the laboratory towards quality. This way, they do not just follow blindly but they understand why they are doing their tasks.

With new technologies such as genomics, point-of-care testing, and digital pathology becoming more prominent, what new quality assurance challenges do you foresee?

One of the emerging challenges in quality assurance is ensuring proper verification and validation of new and rapidly evolving technologies. As innovations like genomics, point-of-care testing, and digital pathology become more prevalent, laboratories must adapt their quality systems to accommodate these advancements. Preparing staff to competently manage and maintain these technologies is equally critical. Additionally, the integration of artificial intelligence into clinical laboratories presents both opportunities and challenges… we must invest time and effort to understand its capabilities, limitations, and implications for quality and patient safety.

What role should the PSP, PCQACL, and government play in harmonizing standards nationwide?

These organizations and institutions have a big role to play in harmonizing the standards as experts in the field of laboratory medicine. We must work hand in -hand to make sure that we are at par with the global standards, starting perhaps first with critical aspects of laboratory quality such as internal quality control or something emerging such as point of care testing.

If you could chart a 10-year roadmap for laboratory quality in the Philippines, what would be your top priorities?

For laboratory quality perhaps the top priorities should be expansion of EQA scheme, empowering organizations on continuing medical education and research, creation and harmonization of standards lead by experts, and promotion of accreditation.

What lessons from your own career would you want young pathologists and medical technologists to carry with them as they face the pressures of balancing quality, cost, and patient care?

When I first stepped into the role of overseeing laboratory quality, I was overwhelmed by the scope and scale of the responsibilities. I quickly realized that meaningful change doesn't happen overnight… you can't do everything all at once. Instead, you focus on what you can do, one step at a time. Small, consistent efforts can create ripples that lead to significant impact over time. When people see that you lead by example… that you truly 'walk the talk', you not only earn their respect but also their support in driving quality improvement initiatives. In balancing all the demands of the role, I've learned that patient care and safety must always come first. That guiding principle helps keep everything in perspective. Lastly, always learn to be humble and accept mistakes because we are all human after all.

What gives you hope about the future of laboratory quality in the Philippines?

What gives me hope about the future of laboratory quality is whenever I see laboratory professionals very interested to learn about quality. I am happy to know we get overwhelming support in all the endeavors of PCQACL especially the seminars, conventions, webinars and other activities. It goes to show that everyone is interested to learn and grow.

Development of Core Curriculum for Cytopathology Fellowship Training in the Philippines

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ABSTRACT

Cytopathology in the Philippines is currently practiced by board-certified anatomic pathologists who receive only a three-month rotation in the discipline during their four-year combined Anatomic and Clinical Pathology residency training. At present, the country lacks a formal fellowship program in cytopathology, and no national core curriculum has been developed for this subspecialty. Local pathologists with advanced training and extensive experience in cytopathology recognized by the Philippine Society of Pathologists, Inc. (PSP), developed the first national core curriculum for cytopathology fellowship training. This initiative marks the first locally developed guideline for cytopathology fellowship training in the Philippines, representing a significant milestone in the advancement of subspecialty pathology education in the country.

Key words: cytopathology, core curriculum, core competencies, fellowship training

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INTRODUCTION

Cytopathology is a subspecialty of pathology concerned with the study and diagnosis of disease at the cellular level, utilizing specimens obtained through exfoliative cytology or interventional techniques such as fine-needle aspiration (FNA). It plays a critical role in the diagnosis of a broad spectrum of conditions, including neoplastic (benign and malignant), infectious, inflammatory, and other non-neoplastic diseases.

In the Philippines, cytopathology is currently taught as part of a three-month rotation within the four-year combined Anatomic and Clinical Pathology residency program. While this provides introductory exposure, it remains insufficient to achieve subspecialty-level proficiency. Gaps persist in several competency areas, including limited knowledge and practical experience, underutilization of ancillary diagnostic tests, inadequate training in FNA procedures, and lack of structured patient interaction during diagnostic interventions. At present, the country lacks a formal fellowship program in cytopathology, and no national core curriculum has been developed for this subspecialty.

ESTABLISHMENT OF THE PHILIPPINE SOCIETY OF CYTOPATHOLOGY AND THE CYTOPATHOLOGY FELLOWSHIP TRAINING PROGRAM

Recognizing this gap, the Philippine Society of Pathologists, Inc. (PSP) initiated the subspecialty recognition of fellows with advanced training and extensive experience in cytopathology. Six such fellows subsequently founded the Philippine Society of Cytopathology (PSC), the first subspecialty society formally recognized by the PSP. In line

with its mandate to ensure a sustainable pipeline of future subspecialists, the PSC has developed a formal one-year fellowship program for cytopathology fellowship training.

The overarching objective of the Cytopathology Fellowship Training Program is to produce competent cytopathologists capable of independently diagnosing diseases and conditions based on cytologic specimens. The core curriculum is designed to cultivate a deep understanding of cellular morphology, diagnostic techniques, and the clinical implications of cytologic findings. Fellows are expected to acquire the knowledge, technical skills, interpretative expertise, and professional attitudes necessary to function independently as cytopathologists in clinical, academic, and research settings.[1,2] Through hands-on experience, didactic instruction, and exposure to a diverse range of cases, the program seeks to produce graduates who can advance cytopathology practice in the Philippines.

Specifically, the program shall:

1. Equip fellows with advanced knowledge and interpretative skills in exfoliative and fine-needle aspiration cytology.
2. Develop technical proficiency in specimen collection, preparation, processing, and application of ancillary diagnostic methods.
3. Enhance communication skills and professional conduct in patient interaction and multidisciplinary collaboration.
4. Instill values of ethical practice, lifelong learning, and evidence-based decision-making in cytopathology.
5. Prepare fellows for leadership roles in academic, clinical, and laboratory-based cytopathology practice.

CURRICULUM DEVELOPMENT

The development of the fellowship curriculum followed a consensus-driven process among the founding members of the Philippine Society of Cytopathology. Deliberations were conducted en banc to define the key result areas, competencies, learning objectives, teaching strategies, training activities, and methods of evaluation.

The framework was organized according to the domains of knowledge, skills, and attitudes, consistent with outcome-based education principles.[3] International standards, particularly those from the Accreditation Council for Graduate Medical Education (ACGME),[1,2] were reviewed and adapted to align with the Commission on Higher Education (CHED) regulatory framework and the local training environment.[3] The resulting curriculum reflects both global best practices and the unique needs of cytopathology practice in the Philippines.

CORE CURRICULUM

In the context of fellowship training, *competency* refers to the fellow's demonstrated ability to integrate knowledge, technical skills, and professional attitudes in the effective practice of cytopathology. The curriculum emphasizes the delivery of high-quality patient care within both academic and clinical settings.

Table 1. Core Competencies for Cytopathology Fellowship

Core competency	Description
Patient care	Provision of safe, effective, accurate, patient-centered diagnostic services, including fine-needle aspiration (FNA) and handling of specimens.
Medical knowledge	Demonstration of knowledge applicable to the practice of cytopathology like cytomorphology, disease mechanisms, and various testing.
Professionalism and ethical standards	Commitment to carry out professional responsibilities following highest ethical standards, respect, and self-awareness.
Practice-based learning and improvement	Evaluation of the fellow's own practices, appraisal and assimilation of scientific evidence, and improvement based on self-evaluation and feedback
Interpersonal and communication skills	Effective interaction and communication with clinicians, patients, and families, writing and issuance of succinct cytology reports, and good performance as part of the health care team.
Systems-based practice	Knowledge on the healthcare system including its resources most especially to laboratory operation, quality assurance, compliance in regulations, and collaboration.

Competencies are mapped to specific learning objectives and reinforced through a variety of teaching modalities, including didactic lectures, case-based discussions, supervised diagnostic sign-outs, FNA procedures, laboratory rotations, multidisciplinary conferences, and research activities. Assessment tools, ranging from direct observation and case log reviews to written and practical examinations, are employed to ensure mastery.

The program's structured design ensures that graduates are not only proficient in accurate cytologic diagnosis but also capable of applying their expertise toward comprehensive patient management, collaborative clinical practice, and the advancement of cytopathology as a discipline in the Philippines.

CORE COMPETENCIES

There are six (6) **Core Competencies for Cytopathology Fellowship** (Table 1).[1]

LEVELS OF COMPETENCY

It is important to understand the levels of competency for both trainers and fellows in cytopathology to track the progression of fellows from novices to experts. Based on the ACGME milestone framework, there are five levels of competency (Table 2).[1]

INSTRUCTIONAL DESIGN MATRIX[1,2,4]

The instructional design matrix (Annex 1) is a tool used to systematically align the learning objectives, learning content, activities and strategies as well as evaluation tools with the core competencies.[5] It is a strategic framework to guide the design, delivery, and evaluation of the entire training program ensuring that every learning activity has a clear purpose aligned with the core competencies that can be assessed properly. This alignment creates a competency-based curriculum that is goal-driven, measurable, and assessment-ready. The matrix can be used by various stakeholders, i.e. program directors, fellows (trainees), trainers (cytopathologists, faculty, and attending

Table 2. Levels of Competency for Cytopathology Fellowship Training Program

Competency Level	Description
Level 1	The novice who starts training and is expected to know the basic concepts in cytomorphology and reviews the cellular features in health and in disease. He is guided and supervised in preparing and triaging of specimens.
Level 2	The developing fellow who applies his knowledge and skills learned under close supervision of the trainers. These entail generation of cytologic diagnoses using the standard nomenclatures for reporting and the ability to explain the basic report terminologies to clinicians. The fellow likewise can participate in the performance of FNA under close supervision of the trainer.
Level 3	The competent fellow who manages the assigned tasks with limited supervision. He can diagnose both gynecologic and non gynecologic samples accurately, performs FNA and rapid on-site evaluation (ROSE), has knowledge of appropriate utilization of ancillary tests, and can effectively communicate with clinicians in most instances.
Level 4	The proficient fellow who is ready to graduate for independent practice. He can confidently render cytopathologic diagnoses of complicated cases and discuss these with clinicians. He can confidently perform FNAs and ROSE and has good judgment in test utilization. He participates in tumor board discussions and teaching activities.
Level 5	The fellow who has achieved mastery of the subspecialty and can be recognized as an expert in the field. He is capable of leading quality improvement initiatives and research projects related to cytopathology.

pathologists), department heads, quality assurance committees, and accreditation bodies.

In practice, the matrix will be used in curriculum planning to design or revise it so that each learning objective is delivered through appropriate teaching methods and assessed using valid tools. It is useful in structuring learning activities not only dependent on lectures but also in a variety of educational methods such as case-based learning, hands-on procedures, and slide review. Likewise, the matrix can be utilized in competency-based assessment ensuring fair and consistent evaluation of each fellow across the domains of knowledge, skills, and professional attitudes and behavior. The fellow can also find the matrix helpful in tracking his own progress enabling himself to identify areas which need improvement. Faculty guidance and standardization can help attending pathologists know what and how to teach, and how to assess. In program evaluation and accreditation, the instructional design can help provide a structured competency-aligned curriculum and identify the strengths and areas of program improvement.

The rotational grid in the matrix (Annex 2) is a planning tool used in the education program design to map how the specific competencies, learning activities, and evaluation tools are covered during the rotations. Its application in a one-year program helps provide that all core competencies are addressed, learning objectives are met using diverse educational experiences with balanced and progressive instruction as well as systematic assessment of the fellow. The grid in a 12-month curriculum follows a step-by-step guide showing the key rotations and duration which is usually continuous once started, using the learning activities, and incorporating the evaluation tools.

Table 3. Requirements for Cytopathology Fellowship Training Program Applicants

1. Must hold a valid medical license issued by the Philippine Regulation Commission (PRC) to practice medicine in the Philippines.
2. Must be a bona fide member of the Philippine Medical Association (PMA).
3. Must be board-certified in Anatomic Pathology by the Philippine Board of Pathology (BOP), or the applicant may be board eligible but can only apply for certification in cytopathology after being certified in Anatomic Pathology by the Philippine Board of Pathology.

Table 4. Requirements for Cytopathology Fellowship Program Trainers

1. Achieving the objectives of the training program requires strong leadership, a dedicated faculty supported by competent and collaborative staff, and an administration fully committed to the educational goals. Additionally, the availability of appropriate facilities and resources is essential to support effective learning and professional development.
2. Trainers should promote critical thinking by engaging in educational activities, self-directed learning (reading of suggested textbooks and online sources), and oral presentations at seminars and conferences. Trainers should inspire and encourage their fellows to pursue excellence and foster a commitment to lifelong learning. This commitment encompasses the completion of a faculty-supervised research project, aimed at contributing to the field through publication in reputable peer-reviewed journals.
3. The number of trainers must be sufficient to provide effective and personalized supervision for fellows participating in the training program. A trainer-to-fellow ratio of 1:2 is considered an appropriate standard to ensure quality mentorship and guidance. This ratio should reflect not only the total number of cytopathology consultants on record but also the actual, active commitment of faculty members who are directly involved in the program's execution and trainee development.
4. The training program should be overseen by a director, typically the chief of the Department of Pathology, who is responsible for overall leadership and strategic direction. The core training team should be led by a designated training officer, supported by selected faculty members who serve as mentors providing continuous support, supervision, and professional development for the fellows.

TRAINING PROGRAM REQUIREMENTS

In order to standardize the implementation of the Fellowship Training Program, the Society has updated its list of requirements for applicants (Table 3), trainers (Table 4), and training institutions (Table 5).

EVALUATION OF FELLOWS[4,5]

Evaluation of fellows during training involves the assessment of medical knowledge and professional competencies in clinical, diagnostic and interpersonal domains. The evaluation should be structured, objective, and aligned with the ACGME competencies and national standards. Methods of evaluation include sign-outs with cytopathologists and faculty, clarity and accuracy of written cytology reports, slide review logs and unknown case sets, peer-reviewed cytology cases, written exams, presentation during case conferences and seminars, direct observation and feedback from supervising pathologists during FNA and ROSE procedures, interaction with clinicians during procedures, oral communication with clinicians, colleagues, and other healthcare staff, punctuality and ethical behavior, self-assessment tools, engagement in quality improvement and research activities, and exit interview as feedback for program improvement.

Table 5. Requirements for Training Institutions for Cytopathology Fellowship Training Program

1. The training institution is the venue where the training program comes into realization and completion. The institution should provide an intellectual environment conducive to the acquisition of knowledge, skills, attitude, and values essential to cytopathology. There should be available and adequate resources in terms of physical infrastructure. Access to resources should be arranged depending on the available facilities, services, and policies. Outside rotation may be done to fulfill the minimum requirements if resources are either absent or inadequate in a particular training facility. Collaborative learning is done not only to satisfy reportorial requirements but also to encourage camaraderie among the fellows in training.

2. For administrative requirements, the following are necessary
   - 2.1. Teaching facility equipped with conference room, physical or online library, training tools such as binocular and multi-header microscopes, teaching aids such as glass slide study sets of exfoliative gynecologic smears (both conventional and liquid-based), non-gynecologic and FNA samples;
   - 2.2. Reportorial requirements to include standards and registry of procedures, schedule of conferences, records, and proceedings of conferences, attendance sheets, evaluation and grades, logbooks, and research output;
   - 2.3. Rules of conduct pertaining to the training manual, guide to evaluation, promotion, commendation, and disciplinary action;
   - 2.4. Existence of a quality management team for data collection and assessment of the training program for compliance to and improvement of the program as mandated by The Philippine Society of Cytopathology and The Philippine Society of Pathologists, Inc.

3. Clinical resources and learning avenues with specified areas and length of rotation per area should be available to enable the trainee to acquire the best possible knowledge, skills, and attitude.
   - 3.1. Gynecologic cytology (conventional and liquid-based) Papanicolaou smears with an annual case load of at least 3000;
   - 3.2. Non-gynecologic: body fluids: sputum, cerebrospinal fluid, urine, pleural fluid, ascitic fluid; peritoneal washing, bronchial washing and brushing, biliary brushing, including cytospin and cell blocks of various fluids, at least 2000 samples per year;
   - 3.3. Fine-needle aspirates of palpable lesions (thyroid, salivary gland, lymph nodes, breast cysts, etc.), preferably in both Giemsa (MGG) and Papanicolaou stains, at least 1000 samples per year and;
   - 3.4. Rapid on-site evaluation (ROSE) of aspirates of deep-seated lesions through FNA, endoscopic ultrasound biopsy (EUS), endobronchial ultrasound bronchoscopy (EBUS), with or without tissue cores, with a minimum annual case load of 200 cases.
     - 3.4.1. FNA clinic
     - 3.4.2. Laboratory: Cytopathology, General Laboratory, Histopathology, and Molecular Laboratory

4. Support departments: Internal Medicine, Obstetrics-Gynecology, Oncology, Otorhinolaryngology, Radiology, and Surgery

CRITERIA FOR COMPLETION OF FELLOWSHIP TRAINING [4,5]

The fellow should meet specific training criteria that demonstrate proficiency across clinical, procedural, academic, and professional domains and are designed to ensure that the fellow is competent to practice independently as a subspecialist in cytopathology. The minimum case log requirements should be met or exceeded in gynecologic and non-gynecologic samples, FNA cases including ROSE. The fellow should be able to correlate cytologic findings with clinical, imaging, and histologic data. He should be able to produce clear, concise cytopathology reports as well as effectively participate in cytology conferences, tumor boards, and multidisciplinary discussions. Likewise, he should be involved in teaching of pathology residents. Lastly, he should complete all educational requirements including a minimum of one research project presented in a national conference and/or published in a peer-reviewed scientific journal.

FREQUENCY OF CURRICULUM REVIEW [4,5]

Curriculum review for quality improvement should be structured, ongoing, and responsive to the goals of the institution and the performance of the fellow. Quality improvement activities should be in place throughout the academic year to ensure that the fellow is actively involved in learning while contributing to uninterrupted improvement.

CONCLUSION

Aligning cytopathology fellowship training with the domains of core competencies will elevate the program to a comprehensive and practice-ready education. At the end of the training, the fellow is prepared to become a cytopathologist who is knowledgeable, technically skilled, ethical, and an effective communicator, willing to collaborate, receptive on quality improvements, focused on safety, ready to lead and innovate in the healthcare system. Additionally, the integration of the core competencies will have a profound and practical impact on cytopathology practice with improvement of patient safety, better clinical decisions, fewer delays in care, improved laboratory operations leading to reduced turnaround times, lower costs, and more sustainable laboratory practices in keeping cytopathology practice relevant, updated, and efficient.

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ANNEXES

Annex 1. Instructional design matrix for training in cytopathology

Core Competency	Learning Objectives	Learning Content	Learning Activities	Method of Evaluation
1. Patient Care	• To collaborate with pathologists, clinicians, resident physicians, and other health care personnel in delivering patient care • To demonstrate knowledge of the application of ancillary techniques relevant to patient care • To understand and apply the best practices for performing FNA, including assessing specimen adequacy, handling samples properly, and preparing smears • To prepare smears from samples acquired from deep-seated lesions and evaluate specimen adequacy to ensure effective patient management. • To explain the FNA biopsy procedure, obtain informed consent, and discuss post-FNA care with the patient • To construct an appropriate cytopathology report that is succinct, concise, and understood by clinicians	• Knowledge on the duties and responsibilities of pathologists, clinicians, resident physicians and fellows in various departments • Familiarization and knowledge on the application of ancillary techniques to help achieve a correct diagnosis • Choice of appropriate procedure and laboratory technique in the proper clinical setting • Familiarity with instruments and materials in the performance of FNA • Knowledge on the basics of obtaining samples and smear preparation from deep-seated lesions under radiologic guidance • Familiarity with the informed consent for FNA, possible complications, and post-FNA care	• Didactics and lectures • Daily case sign-outs • Multi-disciplinary conferences, case consults, clinic-pathologic conferences • Simulation of FNA using training tools such as phantom models (gelatin of varied sizes and shapes) • Supervised procedures • Smear preparation • Rapid on-site evaluation of smears	• Consultant/faculty evaluation • Daily case sign-outs • Procedure and case logs • Direct observation • Review of pathology reports • Skills during simulated procedure and patient encounters • Interdepartmental conferences
2. Medical Knowledge	• To obtain accurate and pertinent clinical information for proper clinicopathologic correlation • To know the common and special diagnostic procedures, their medical applications, and correlation • To know the various laboratory techniques and their usefulness, such as special stains, immunofluorescence, immunohistochemistry, and FNA • To know the basics of cytology in health and in disease, appreciate normal from abnormal (reactive, dysplastic, and neoplastic) cells • To be familiar with the terminology used for reporting cytopathology in gynecologic, non-gynecologic cytology, body fluids, and fine needle aspirates of both superficial and deep-seated lesions for application in cytopathology reports • To understand the fundamentals of automated screening for gynecologic samples • To continually update medical knowledge including exposure to molecular cytopathology • To demonstrate an analytic approach to clinical conditions and their cytologic manifestations during daily sign-out activities	• Application of medical knowledge on accurate history taking and clinicopathologic correlation • Knowledge of the various diagnostic procedures and their application • Development of skills in cytopathology in terms of specimen collection, smear preparation, and relaying of results to attending clinicians • Knowledge on the basic cell structure and its function, cytogenetics, various cytologic screening programs, and the pathologic processes affecting the cells from inflammation to cancer • Knowledge of the standard nomenclatures and the composition of the cytopathology report	• Review of patient's chart, diagnostic procedures, etc. • Use of references and suggested cytopathology reading list • Didactics and lectures • Daily case sign-outs • Rotation in the cytology laboratory for familiarization in the different laboratory techniques • Cytohistologic correlations in daily cases • Presentation and when required discussion of cases during departmental and interdepartmental conferences e.g., multidisciplinary tumor board participation	• Consultant/faculty evaluation • Daily case sign-outs • Direct observation • Report review • Evaluation of skills in laboratory techniques, in simulated procedure, and in patient encounters • Performance in departmental and interdepartmental conferences

Annex 1. Instructional design matrix for training in cytopathology *(continued)*

Core Competency	Learning Objectives	Learning Content	Learning Activities	Method of Evaluation
3. Practice-based Learning and Improvement	• To apply the principles of quality control and quality assurance in cytopathology • To identify and remedy the gaps in terms of personal knowledge • To address clinicians' queries on expected cytology results, including turnaround times of various reports • To identify improvements of processes to prevent errors	• Knowledge to troubleshoot problems in the technical aspect and reporting of cases • Dissemination of information to clinicians regarding the turnaround time of reports • Application of concurrences in difficult cases and those with opposing opinions • Ability to recognize a possible omission in the report for proper issuance of an amended report • Utilization of diagnostic adjuncts like immunohistochemistry, flow cytometry, and molecular testing	• Daily review of every 10th case signed-out by pathology resident physicians • Monitoring the quality of slide preparation in coordination with the medical technology staff • Rapid on-site evaluation and Intra-operative consultations • Presentation of interesting and problematic cases, • Cytopathology unknown conferences • Pathology resident teaching (slide sessions, didactics, etc.)	• Maintain logbooks of cytohistologic correlation, intra-operative consultations (ROSE) and final histologic diagnosis • Faculty evaluation • Daily case sign-outs • Report review • Decision to use adjunct tests
4. Professionalism and Ethical Standards	• To develop an understanding of the ethical and confidentiality issues that impact patient care • To consistently demonstrate professionalism through behavior and attitude, reflecting core values such as honesty, integrity, and respect • To demonstrate leadership qualities, including a strong sense of responsibility, compassion for others, and resilience in the face of challenges	• Knowledge of basic ethical principles • Code of Ethics of professional and regulatory groups (PSP, Inc., PMA) • Good medical and pathology practice principles	• Workshops/ • Seminars • Lecture series • Code of Ethics manual • Mentoring	• Direct observation • Faculty evaluation • Peer evaluation • Incident reports
5. Systems-based Practice	• To understand the processes, guidelines, and issues in the cytopathology laboratory from specimen handling to report generation • To demonstrate the ability to access the HIS, LIS, and EMR for reports and clinical history • To collaborate effectively with clinicians and other healthcare personnel in evaluating the appropriateness and clinical utility of diagnostic tests with the goal of optimizing patient outcomes • To know the basics of effective managerial practices • To participate in faculty-supervised quality improvement and research activities • To remain informed and current on the diverse rules and regulations governing the practice of Pathology and Cytopathology	• Procedures and guidelines related to specimen handling, processing, slide preparation, staining, laboratory and hospital information systems, EMR and all other matters pertinent to the cytopathology laboratory • Flow chart in addressing issues and their resolution in the laboratory • File copies of the different administrative orders of the Department of Health, including revisions and updates on issues concerning the practice of cytopathology	• Regular system checks on processes and guidelines to ensure quality results • Filing of records on issues addressed and their resolution for improvement • Discussion with the laboratory staff, for updated issues concerning the laboratory that require immediate attention	• Direct observation • Faculty evaluation • Peer evaluation of pathology residents, laboratory personnel, and residents training in the clinical departments • Procedure and case logs
6. Interpersonal and Communication Skills	• To convey the necessary information to the patient and the other members of the management team • To implement the appropriate use of communication, whether direct, telephone, written, or email, in a timely manner • To maintain proper records pertinent to the management of patients and training • To write a cytopathology report that is complete, accurate, grammatically correct, easily understood, and in a timely manner • To demonstrate the ability to seek consultation with other members of the faculty or other subspecialists for proper cytohistologic correlation	• Basic communication skills • interpersonal communication skills • Data recording and management • Public speaking skills	• Patient encounters • Departmental and interdepartmental conferences and presentations • Discussion of interesting cases • with faculty and pathology residents • Lecture series to pathology residents	• Direct observation • Faculty evaluation • Daily case sign-outs • Peer evaluation • Procedures and case logs • Report review

Annex 2. Rotational grid for the cytopathology fellow

Activity-Learning Content per Month	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Knowledge of the cell in health and in disease, including related standard nomenclatures of widely employed reporting systems (The Bethesda System for reporting cervicovaginal cytology and The International system for fluid cytology)	*	*	*									
Cytology routine, special, and IHC stains to include troubleshooting	*	*	*									
Screening of gynecologic smears (conventional and LBC)	*	*	*	*	*	*	*	*	*	*	*	*
Screening of non-gynecologic smears and cell blocks	*	*	*	*	*	*	*	*	*	*	*	*
Knowledge of the basics of FNA and its application, simulation of procedure, and optimum smear preparation				*	*							
Review of standard nomenclatures in cytologic diagnosis of aspirates of thyroid, salivary gland, lymph nodes, and breast				*	*	*	*	*	*	*	*	*
Screening / interpretation of thyroid, salivary gland, lymph node, and breast aspirates				*	*	*	*	*	*	*	*	*
Knowledge of ROSE and subsequent interpretation of deep-seated sample aspirates obtained with radiologic imaging using the standard cytologic nomenclatures							*	*	*	*	*	*
Proposal for a research project as early as the 6th month of rotation for presentation on or before the end of the training program						*	*	*	*	*	*	*
Cytology didactics alternating with cytology slide conference are scheduled every last week of each month	*The cell in health and disease, TBS for reporting cervico-vaginal cytology and body fluid cytology	*Routine staining and other lab techniques	*Slide conference on interesting gynecologic and non-gynecologic cases	*Lecture on The Bethesda System for reporting thyroid cytopathology	*Slide conference on thyroid, aspirates	*Lecture on The Milan System for reporting salivary gland cytopathology, WHO Reporting Systems for lymph node, lung, and breast cytology	*Slide conference on salivary gland, lymph node, and breast aspirates	*Lecture on standard cytology nomenclature of aspirates from deep-seated lesions	*Slide conference on aspirates of deep-seated lesions	*Comprehensive assessment of the training program (objectives met, adequate learning/ teaching activities, rotation, fair performance evaluation)	*Written and practical feedback from fellow by pathology consultants involved in training	*Exit interview
Outside rotation in an institution with an accredited cytopathology training program, with submission of an accomplishment report and certificate of rotation									*Schedule subject to change	*Schedule subject to change		
Evaluation of performance- written and practical exams every quarter ***Evaluation of sign-outs with faculty*** ***Performance of various tasks*** ***Research output/s***			*			*			*		*	*Latest submission of research output. Performance evaluation and final deliberation by faculty

OPEN ACCESS – FEATURE ARTICLE

Molecular Diagnostic Techniques of Infectious Diseases: An Overview*

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ABSTRACT

Recent advancements in molecular techniques such as real-time PCR, isothermal amplification, next-generation sequencing, metagenomics, microarray, and CRISPR-infectious disease diagnostics have significantly evolved and improved over the past years. This overview will explore the innovations that have shaped the molecular diagnostics workflow, as well as the progress made in these innovative techniques. Additionally, it will address existing gaps, unmet needs, and the potential future directions for further enhancing diagnostic capabilities in the field.

Key words: molecular techniques, real-time PCR, isothermal amplification, next-generation sequencing, metagenomics, microarray, CRISPR-infectious-disease diagnostics

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INTRODUCTION

The World Health Organization (WHO) has reported that infectious diseases are emerging and spreading at an unprecedented rate, and that new infectious diseases are being discovered more frequently than ever before. In 2002, severe acute respiratory syndrome (SARS) emerged in China and spread to nearly 30 countries. SARS is caused by SARS-CoV-1, a strain of coronavirus that spreads through tiny infectious respiratory particles. In 2012, Middle East Respiratory Syndrome (MERS) emerged, causing a serious viral respiratory disease known as MERS-CoV. In 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which started as an outbreak in Wuhan, China, subsequently resulted in the COVID-19 pandemic.[1,2]

Infectious disease diagnostics is based on clinical presentation and laboratory findings using various techniques such as microscopy, culture, biochemical tests, to name a few. Over the past decades, we have witnessed the explosive growth of molecular diagnostics, as well as the availability of new assays to rapidly detect and characterize clinically important pathogens with a high degree of accuracy.[3] Indeed, molecular diagnostics of infectious diseases have evolved through the years and significantly advanced disease diagnostics by enabling the rapid identification of infectious disease pathogens.[4]

It is well recognized that the choice of molecular diagnostic techniques for infectious diseases depends on several factors including but are not limited to the target pathogen, turnaround time, clinical setting, and availability of resources. It is anticipated that as technology advances, newer techniques will emerge that are more suitable for the rapid identification of infectious disease pathogens, especially in resource-limited settings. Currently, the commonly used molecular diagnostic techniques for infectious disease include real-time PCR, isothermal amplification, next-generation sequencing, metagenomics, microarray, and CRISPR-infectious-disease diagnostics. A timeline of methodological discoveries is shown in Figure 1.

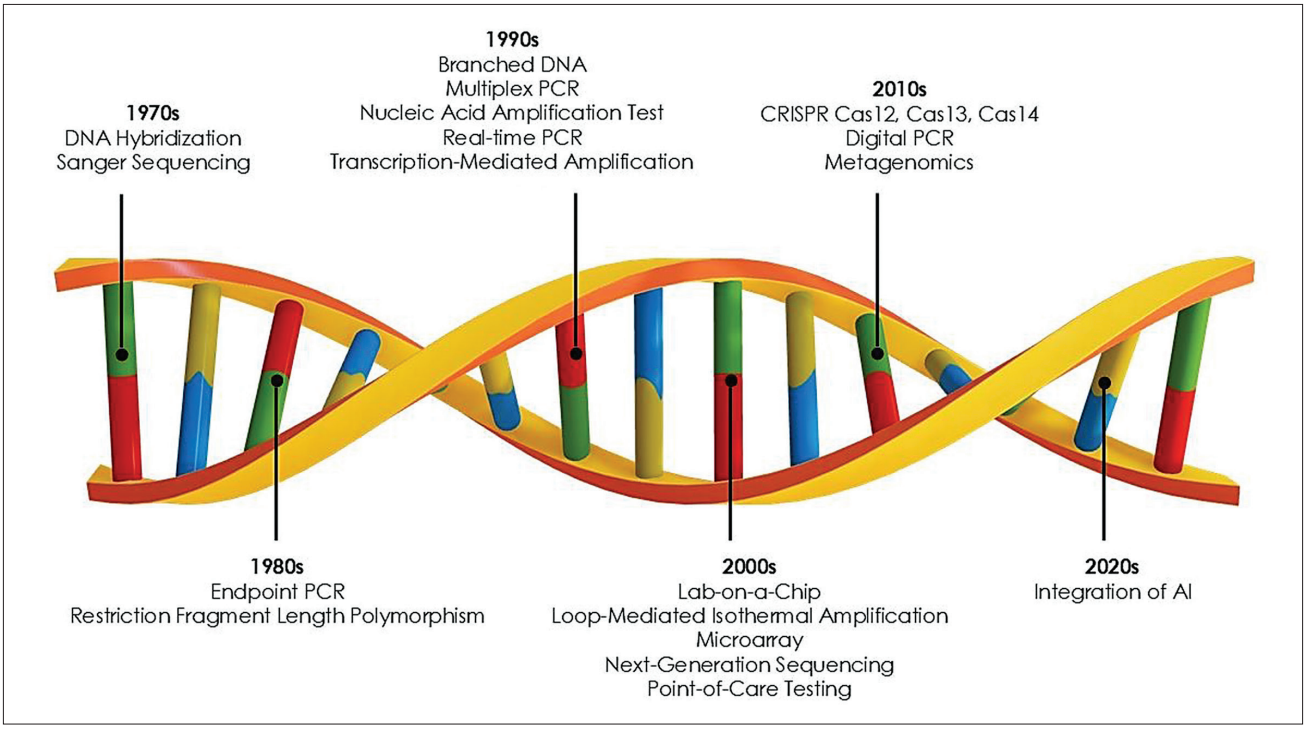

Figure 1. Timeline of major molecular diagnostic techniques for infectious diseases.

The advent of automation, integration, and consolidation in laboratory workflow has ushered in a new era for molecular diagnostics. Automation has significantly reduced handling time and turnaround times while enhancing both efficiency and productivity. Integration, on the other hand, involves the seamless combination of various analytical technologies and instruments to optimize the diagnostic process. Consolidation refers to the merging of multiple sample types and processes into a unified system using one or more equipment.[5] A notable advancement is the development of closed systems, where all phases of the molecular diagnostic workflow (e.g., detection, identification, characterization) occur within sealed containers, minimizing the risk of contamination. In particular, the sample-to-result approach, which integrates nucleic acid extraction, target amplification, and analysis into a single instrument, streamlines the diagnostic process, making infectious disease diagnostics more efficient and productive.

This article provides an overview of various molecular platforms and devices for point-of-care (POC) testing, lab-on-a-chip (LOC), and CRISPR-based diagnostics, particularly in resource-limited settings. In addition, it highlights the gaps and unmet needs in the field. The literature included in this overview was selected based on publications indexed in PubMed, Scopus, and Clarivate Analytics (formerly ISI and Thomson Reuters), ensuring the inclusion of high-quality, peer-reviewed research. This review will cover the most used techniques in molecular diagnostics of infectious diseases, as well as next-generation tools such as POC testing, LOC, and CRISPR-based assays. The future of diagnostics lies in rapid, accurate, and accessible platforms that support global surveillance, outbreak response, and personalized treatment strategies.

INNOVATIONS IN MOLECULAR DIAGNOSTICS WORKFLOW

The molecular diagnostics workflow has rapidly evolved and significantly improved through the years from sample preparation (e.g., nucleic acid extraction) to target amplification (e.g., endpoint PCR, real-time PCR, digital PCR, isothermal amplification), as well as identification of the target pathogen (e.g., colorimetric, spectrometric, fluorescence, chemiluminescence, electrochemical). Taken together, these developments are revolutionizing clinical practice by providing more accurate diagnosis and management of infectious diseases.[6]

There are several ways of extracting nucleic acids (e.g., DNA, RNA) from various clinical samples such as the use of inorganic and organic solvents (e.g., saturated NaCl, phenol-chloroform extraction), solid-phase extraction methods (e.g., spin column with a silica-based membrane) and magnetic beads. The spin column and magnetic bead-based extraction are readily scalable and amenable to automation.[7]

For target amplification, real-time PCR uses fluorescently labeled probes to quantitatively analyze the fluorescence signal of the amplicons without the need for post-PCR analysis such as gel electrophoresis. On the other hand, digital PCR uses the same primers and probes, except that the samples are partitioned into thousands of chambers, thereby increasing its analytical sensitivity. One of the advantages of digital PCR over real-time PCR is that the quantification of nucleic acids is absolute. An example of this application is the absolute quantitation of cytomegalovirus (CMV).[8]

MOLECULAR TECHNIQUES OF INFECTIOUS DISEASES

PCR-based diagnostics

Endpoint or traditional PCR amplifies specific DNA or RNA to detect the presence or absence of the target pathogen. This technique utilizes agarose-stained gel for the detection of the amplified product at the final phase of the PCR reaction. Traditionally, ethidium bromide is used to stain the gels; however, its use has been discontinued for safety reasons. Newer intercalating dyes have been developed and offer improved sensitivity for nucleic acid visualization. Real-time PCR (qPCR) is a variation of endpoint PCR wherein the amount of amplification product is measured at each reaction cycle via fluorescent dyes or probes. Real-time PCR allows for quantitative measurement of the target pathogen in each sample through real-time monitoring of DNA or RNA amplification. Sexually transmitted infections (STIs) such as *Chlamydia*, *Neisseria*, *Trichomonas*, and *Mycoplasma* can be detected using a qPCR platform. It is worthwhile to mention that a real-time PCR for the detection and identification of *Plasmodium* spp. to confirm microscopic findings has been developed.[9]

A more sensitive version of real-time PCR is digital PCR (dPCR). This technique can detect low-abundance pathogens in various clinical samples. It can be used for the detection of cell-free human papillomavirus (HPV) in plasma; identification of hetero-resistance in *Mycobacterium tuberculosis* (Mtb) and detection of viral genome integration in the host genome such as human herpes virus 6 (HHV-6).[8]

Reverse transcription PCR (RT-PCR) allows for the detection of RNA viruses such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), dengue virus, and SARS-CoV-2 by converting RNA to complementary DNA (cDNA) before amplification.

Molecular infectious disease diagnostic tests have proven to be faster and often more sensitive than their traditional counterparts.[10] In particular, a sample-to-result molecular testing based on a multiplex real-time PCR platform has been developed and validated for clinical use such as the bioMérieux BioFire® FilmArray® and the QIAGEN QIAstat-Dx panel for the detection of gastrointestinal pathogens.[11]

It is noteworthy to mention that advances in bioinformatics enabled the development of oligonucleotide primers for multiplex PCR. This enables the detection of multiple pathogens from a single sample in a single test. Bispo and colleagues (2018) developed a multiplex PCR coupled with high-resolution melting (HRM) for the rapid detection and identification of uveitis pathogens such as herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), CMV, and *Toxoplasma gondii*. This technology is important especially in cases where the infections have similar clinical signs or symptoms but require different treatment and management.[12]

Isothermal amplification

Isothermal amplification, most notably the loop-mediated isothermal amplification (LAMP), has attracted a lot of attention as a potential rapid and accurate infectious disease diagnostic platform. LAMP uses 4 to 6 primers and is a simplified version of nucleic acid amplification because it does not require the traditional thermal cycling (e.g., denaturation, annealing, extension) and thus makes it suitable for POC molecular diagnostics, especially in low-income countries and resource-constrained environments.[13-14]

The LAMP assay has various formats such as strip, paper and coated tubes. Recently, a LAMP assay for the detection of dengue, Ebola, hepatitis B virus (HBV), HIV, H1N1, HPV, influenza, MERS-CoV, and Zika has been developed.[15]

The other amplification techniques include branched DNA (bDNA), hybrid capture and transcription-mediated amplification (TMA). For bDNA, the capture probe binds to the microwell and the hybridized probe is detected by chemiluminescence reaction. Blood-borne pathogens such as HBV, HCV, and HIV can be detected using bDNA. Human CMV, HPV, *C. trachomatis*, *and N. gonorrhea* can be detected by hybrid capture, while West Nile virus (WNV), *T. vaginalis*, and *Mycoplasma* can be detected by the TMA technique.

Next-generation sequencing (NGS)

Next-generation sequencing allowed for massive parallel sequencing of DNA and RNA by enabling high-throughput sequencing of genomes of infectious disease pathogens. This technique has paved the way for various sequencing approaches including whole genome sequencing (WGS), targeted sequencing, and shotgun sequencing of clinically important pathogens. These advances have transformed the rapid identification of emerging and re-emerging pathogens. NGS is also widely applied in antimicrobial resistance profiling, disease surveillance, and outbreak investigation.[16-17] Furthermore, it has revolutionized microbiome research by elucidating the role of microorganisms in chronic diseases such as diabetes and inflammatory bowel disease (IBD).[18] Today, several NGS platforms are available, each offering distinct features and tailored applications depending on research or clinical needs (Table 1).[19-26]

Table 1. Major sequencing platforms and their various applications

Technology (Generation)	Timeline	Key Features (Company)	Selected Pathogens	Applications
Sanger Sequencing (First)	1970s to present	Chain termination method using fluorescently labeled ddNTPs (ThermoFisher ABI)	HBV, HCV, HIV, Mtb	16s rRNA, 18s rRNA, 23s rRNA, ITS sequencing, drug resistance mutations, targeted sequencing
Next-Generation Sequencing (Second)	Mid 2000s to present	Sequencing by synthesis (Illumina) Sequencing by ligation (ThermoFisher SOLiD) Semiconductor sequencing (ThermoFisher Ion Torrent)	*E. coli* O104:H4, Ebola, HCV, HIV, HPV, MRSA, Mtb, *P. falciparum*	Drug resistance, metagenomics, outbreak investigation, WGS
Single-Molecule Sequencing (Third)	2010s to present	Real-time sequencing (PacBio, Pacific Biosystems; MinION, Oxford Nanopore)	*C. difficile*, SARS-Cov2	Drug resistance, mutations, genotyping

ddNTPs: dideoxynucleotide triphosphates; ITS: internal transcribed spacer; rRNA: ribosomal RNA; WGS: whole genome sequencing

Metagenomics

Next-generation sequencing (NGS)-based metagenomic approaches enabled the detection and characterization of entire microbial communities without relying on traditional microbiological methods. This technique facilitates the identification of unknown pathogens in diverse clinical samples, even without prior knowledge of the organism. Metagenomic sequencing is particularly valuable for detecting rare and co-infecting pathogens in complex, mixed samples.[27-29] Recently, a rapid metagenomic NGS (mNGS) technique has been developed to detect pathogen cell-free DNA in various body fluids. This platform integrates automated library preparation, a hybrid Illumina and nanopore sequencing protocol, and advanced bioinformatics pipelines for comprehensive metagenomic analysis.[30]

Microarray

Microarrays or chip-based diagnostics (e.g., DNA, RNA, protein) are used as diagnostic tools for the rapid identification of pathogens. It utilizes a set of probes to detect and characterize a broad spectrum of pathogens (e.g., bacteria, viruses, parasites, fungi), as well as to determine antimicrobial resistance profiles.[31] In 2020, Ma and colleagues developed a DNA microarray assay for rapid detection of fifteen bacterial pathogens in pneumonia. This approach offers the potential to provide a faster diagnostic tool than the current standard methods.[32]

As microarrays are increasingly used in clinical practice and research, efforts are being made to validate and standardize these techniques to ensure reproducibility and accuracy. Recently, a multiplex protein microarray for antibody testing for tickborne and other infectious diseases has been developed.[33]

Point-of-care molecular diagnostics

Point-of-care testing refers to diagnostic testing performed near the site of patient care, including but are not limited to doctors' clinics and emergency rooms. The goal of POC is to provide fast results and thus enabling rapid diagnosis and treatment. The WHO ASSURED framework (e.g., affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to the end-users) has provided the roadmap for creating molecular diagnostics that meet the needs of low-income countries and are impactful in resource-limited settings.[34] This framework has evolved and been updated to REASSURED with the addition of real-time connectivity and ease of specimen collection.

Microfluidics and lab-on-a-chip

The lab-on-a-chip is a technology that integrates laboratory functions onto a small chip. The integration of microfluids and miniaturized molecular diagnostics systems has revolutionized the field as it offers a rapid, accurate, and portable platform, especially for remote areas and field settings. These innovations can extract the genetic material from a variety of clinical samples such as blood, saliva, and urine in a closed system, thus making it easier and faster to process samples with minimal hands-on time. The LOC devices can be used for various applications, including POC molecular diagnostics. Recently, a LOC for the simultaneous detection of SARS-CoV-2 RNA and SARS-CoV-2 antibodies in saliva and plasma has been developed. It utilizes an electrochemical sensor that detects the presence of nucleic acid and protein antibodies.[35]

Several LOC devices now integrate with smartphones. This allows for the remote analysis of the results, and this can be immediately shared with the requesting healthcare provider. This in turn, will significantly improve access to care, especially in low-resource settings.

CRISPR-based diagnostics

CRISPR-based diagnostics utilize enzymes to cleave DNA or RNA from target pathogens and provide a colorimetric or visual readout. This approach makes infectious disease diagnostics faster and accessible, especially in resource-constrained environments.[36] The CRISPR-based diagnostics have provided new ways of detecting and characterizing nucleic acids with a high degree of accuracy and precision.

Cas12a targets DNA with collateral cleavage of single-stranded DNA. DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) allowed for fast, accurate, and cost-effective molecular diagnostics of infectious diseases such as COVID-19.[37] Cas13a targets single-stranded RNA with collateral cleavage of ssRNA. The Specific High Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) has been used to diagnose flaviviruses such as dengue, Zika, WNV, and yellow fever.[38] When Cas12a, Cas13a, or Cas14 binds to target nucleic acid, it releases a fluorescent molecule, which in turn produces a fluorescent signal. Currently, CRISPR Cas-based detection methods include lateral flow device, fluorescence, colorimeter, and quantitative real-time PCR. Some of the advantages of the CRISPR-based diagnostics are as follows: (1) rapid onsite detection; (2) simple and portable; (3) high sensitivity and specificity; (4) timesaving; and (5) multiple detection.[39]

CRISPR-based diagnostics for infectious diseases have demonstrated acceptable technical performance in vitro; however, their real-world field performance has shown inconsistent results. This variability is often attributed to factors such as sample complexity, reagent stability, environmental conditions (e.g., temperature, humidity), and operator variability. Efforts to improve performance in field settings include the following: (1) integrated sample preparation methods such as microfluidics and paper-based extraction; (2) the use of lyophilized reagents to enhance stability and eliminate cold chain requirements; (3) smartphone-based readers for improved result interpretation; (4) standardized protocols; and (5) staff training.[37-41]

Taken together, POC, LOC, and CRISPR-based diagnostics represent interconnected technologies that are driving the next-generation of rapid, accurate, and accessible molecular diagnostics for infectious diseases.

GAPS, UNMET NEEDS AND FUTURE DIRECTIONS

Molecular diagnostics of infectious diseases have advanced significantly, but several gaps and unmet needs persist across clinical, technological, and socioeconomic dimensions. First, the detection of nucleic acids does not always mean

active infection. Thus, there is a need to distinguish between active and latent infections. Second, standardization of viral loads across nucleic acid amplification tests and sample types remains to be seen.[42] Third, there is a need to develop cost-effective molecular diagnostics in resource-limited settings.

The future directions of molecular diagnostic techniques of infectious diseases are centered around making diagnostics faster, more accurate, and smarter. First, integrate omics data (e.g., genomics, transcriptomics, proteomics) and artificial intelligence (AI) to develop innovative strategies for prevention, rapid diagnosis, and treatment. The integration of AI in molecular diagnostics of infectious diseases could significantly play a critical role in interpreting and analyzing data, which in turn will further improve the turnaround time and diagnostic accuracy.[43] Second, expand the test menu for infectious diseases (e.g., bacterial, viral, parasitic, fungal). Third, establish transdisciplinary and international collaborations to address global challenges, as well as emerging and re-emerging infections.

CONCLUSION

The molecular diagnostics workflow has been evolving, and the applications in the field of infectious disease diagnostics have seen significant advancements. Various molecular techniques have significantly improved the accuracy of infectious disease detection, identification, and characterization. Molecular techniques have transformed the landscape of infectious disease diagnostics. Addressing the gaps and unmet needs is key in advancing the ability to diagnose and treat infectious diseases in the future.

Overall, this overview contributes to the existing literature by providing an up-to-date synthesis of next-generation tools for infectious disease diagnostics, with a particular focus on POC, LOC, and CRISPR-based assays, which are especially valuable in resource-limited settings. By integrating technological advances with clinical applicability, this overview offers valuable insights for scientists, clinicians, policymakers, and decision makers seeking to improve diagnostic strategies, clinical outcomes, and healthcare delivery.

STATEMENT OF AUTHORSHIP

The author certified fulfillment of ICMJE authorship criteria.

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Datasets generated and analyzed are included in the published article.

AUTHOR DISCLOSURE

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OPEN ACCESS – ORIGINAL ARTICLE

Quantifying Total Allowable Error Violations in Serum-Sodium Quality Control: A Computer Simulation Experiment of Two- to Six-Sigma Processes

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ABSTRACT

Background. Serum-sodium reporting tolerates a total allowable error (TEa) of only ±4 mmol/L, yet many laboratories continue to operate at the marginal three-sigma level because the quantitative benefit of additional sigma capability is poorly characterized.

Objectives. The study aims to translate sigma metrics into clinically intuitive risk estimates by (1) quantifying the proportion of QC results that exceed the TEa at five sigma levels (2 – 6 σ) and (2) determining whether successive sigma gains produce statistically significant reductions in error.

Methodology. Five (5) hypothetical assays were parameterized with a common mean of 140 mmol/L and CVs corresponding to 2-, 3-, 4-, 5- and 6-sigma performance. For each assay, 1,000 Monte-Carlo iterations were run, each iteration simulating 36,500 QC results (assuming 100 runs/day for 365 days) drawn from $N(\mu = 140, \sigma = \mu \times CV)$. The error rate (the proportion of results outside ±4 mmol/L) was recorded per iteration. Distributions were summarized (mean, range, SD); differences were evaluated with one-way ANOVA followed by Tukey's HSD.

Results. Mean (±SD) error rates declined significantly with increasing sigma: Assay A (2 σ): 0.0456 ± 0.0011; Assay B (3 σ): 0.00270 ± 0.00027;Assay C (4 σ): $6.3 \times 10^{-5} \pm 4.1 \times 10^{-5}$; Assay D (5 σ): $5.8 \times 10^{-7} \pm 8.0 \times 10^{-7}$; and Assay E (6 σ): $2.0 \times 10^{-7} \pm 3.1 \times 10^{-7}$. The maximum single-iteration error rate fell from 0.0505 at 2 σ to 1.1×10^{-4} at 4 σ. The 5 σ and 6 σ processes produced zero TEa violations in ≥96 % of iterations. ANOVA confirmed a global difference ($p < 0.001$); all pairwise contrasts were significant ($p < 0.001$) except between 5 σ vs 6 σ ($p = 0.62$).

Conclusions. Each one-sigma gain yields an order-of-magnitude reduction in TEa violations until a plateau is reached at ≥5 σ, where residual analytical risk is negligible. These simulations support the recommendation that laboratories operating serum-sodium assays below 4 σ should prioritize precision improvements or enhanced QC strategies, whereas ≥5 σ assays may safely adopt less intensive QC without compromising patient safety.

Key words: Rstudio, total allowable error, TEa, quality control, six sigma, sigma metrics

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INTRODUCTION

Reliable measurement of serum sodium is critical for the diagnosis and management of a broad spectrum of disorders, from hyponatraemia-related cerebral edema to hypernatremic dehydration.[1] Analytical error leading to misclassification of sodium concentrations can precipitate inappropriate therapeutic interventions with substantial patient harm.[2,3] Contemporary laboratory quality management therefore mandates a stringent total-allowable-error (TEa) for sodium of ±4 mmol/L, one of the narrowest limits applied in clinical chemistry.[4,5]

The sigma metric has gained prominence as a unifying index that relates analytical imprecision (coefficient of variation, CV) and bias to the governing TEa.[6,7] A higher sigma value denotes fewer defect opportunities per million test results and provides operational guidance on the intensity of internal-QC (IQC) protocols. Westgard and co-workers have formalized "risk-based QC" frameworks in which the frequency and complexity of QC rules are scaled

to an assay's sigma capability.[8–10] However, despite wide acceptance, many laboratories continue to operate sodium assays below or just at the marginal 3-sigma threshold, probably partly because the quantitative benefit of additional sigma gains has not been described in clinically intuitive terms such as expected TEa violations over an annual workload.[11–13]

Computer simulation presents a practical, resource-efficient approach to closing this evidence gap. By repeatedly sampling QC data under controlled distributional assumptions, one can translate abstract sigma metrics into tangible estimates of patient-level risk—namely, the proportion of results that would fall outside TEa over time.[14] Many earlier simulation studies have primarily examined single-sigma scenarios or incorporated multirule algorithms whose complexity can make it more challenging to clearly discern the underlying relationship between sigma level and error frequency.[15–18]

The present work employs a large-scale Computer Simulation (Monte-Carlo) design to model five hypothetical serum-sodium assays spanning 2- to 6-sigma process performances, while holding systematic bias at zero to reduce complexity in analysis. Our primary objective was to estimate, for each sigma process level, the yearly proportion of QC results breaching the ±4 mmol/L TEa for the hypothetical serum sodium assays. Secondary aims were (1) to visualize the changing dispersion pattern by means of representative Levy–Jennings plots and (2) to quantify and demonstrate statistical differences in error rates across sigma levels. These data provide simulated but empirically grounded and informationally familiar targets that can assist laboratories that are seeking to balance assay-selection costs against patient-safety gains.

METHODOLOGY

Study design

A Monte-Carlo computer assisted simulation experiment using the R programming language, in RStudio integrated development environment (IDE), was performed to characterize the analytical-error behavior of five hypothetical serum-sodium assays that differ only in imprecision (coefficients of variation, CVs) and hence in sigma performance.

For each assay, 1,000 independent annual Levy-Jennings (LJ) runs were simulated, mimicking, for example, 1,000 different laboratories using the same platforms. For each of the 1,000 simulated Levy-Jennings, 36,500 quality-control (QC) results were generated, corresponding to 100 QC measurements per day (which is an average case load per day for a low to medium case-load laboratory) for 365 days. All simulations were executed in the R programming language, inside Rstudio[19,20] with the random-number generator initialized to a fixed seed to ensure full reproducibility.

Analytical specifications

The total allowable error (TEa) for serum sodium was fixed at ±4 mmol/L, in accordance with CLIA recommendations. The process mean for every assay was set to 140 mmol/L to mimic a normal level QC material. Five CV values were pre-specified to emulate 2-, 3-, 4-, 5- and 6-sigma processes (Table 1). For every simulated run the within-run standard deviation (SD) was calculated as SD = CV × mean.

Table 1. Simulated Serum Sodium Assays with CV based on assumed Sigma Metric

Assay	Sigma metric	CV
A	2 σ	0.014286
B	3 σ	0.009524
C	4 σ	0.007143
D	5 σ	0.005714
E	6 σ	0.004762

Data-generation procedure

For each assay in every iteration, 36,500 QC results were sampled from a normal distribution $N(\mu = 140, \sigma = \mu \times \text{CV})$. A result was classified as "out-of-bounds," "breach," or a "Violation" when it lay outside the symmetric TEa limits (≤136 or ≥144 mmol/L). The iteration-specific error rate was defined as

$$\text{Error rate}_{ij} = \frac{\text{Count of } |x - \mu| \geq 4}{36\,500}$$

where i denotes assay and j denotes iteration.

Visualization of a single iteration

To illustrate typical LJ behavior, one representative iteration was plotted, using R code, for all five assays (Figures 1 to 5).

Outcome measures

Across the 1,000 iterations per assay, the following statistics were computed: mean error rate, minimum, maximum, and standard deviation. Iteration-level error-rate distributions were visualized with box-and-whisker plots sharing a common y-axis, displaying distribution of the proportion of the QC results that fall beyond ±TEa.

Statistical analysis

Differences among the error rates of the different sigma-metric assays were assessed with one-way analysis of variance (ANOVA). After a significant global F-test ($\alpha = 0.05$, two-sided), pairwise differences were explored by Tukey's honestly significant difference (HSD) test, which controls the family-wise error rate. All statistical analysis procedures were performed in the R programming language inside RStudio.

Software environment

All simulations were implemented in the R programming language. A copy of the entire code is available as supplementary data to this manuscript.

Software tools employed included the R programming language[19] for simulation coding and statistical analysis, RStudio[20] as the integrated development environment, Microsoft Excel[21] for data management, Microsoft Word[22] for manuscript preparation, and ChatGPT[24] for code debugging support and manuscript language assistance and refinement.

All conceptual and creative development, experimental design, analysis implementation decisions, and manuscript drafting were solely and independently initiated by the co-primary authors. ChatGPT was utilized solely to assist in identifying coding inconsistencies, debugging, and enhancing manuscript language clarity, without substantial contributions to the study's intellectual content or experimental framework. The co-primary authors affirm full intellectual ownership and responsibility for the entirety of the content presented in this manuscript.

Ethical considerations

As the study did not involve any actual human or animal subjects, tissues, or personal information, ethical approval from the Institutional Review Board (IRB) or Institutional Animal Care and Use Committee (IACUC) was not sought.

RESULTS

Error-rate distributions across sigma levels

A total of 1,000 Monte-Carlo iterations, each comprising 36,500 quality-control (QC) results, were generated for every assay. The proportion of QC results that exceeded the total allowable error (TEa = ±4 mmol/L) is displayed as the "*error rate*." Summary statistics for the iteration-level error rates are presented in Table 2.

The error-rate distribution widened markedly as analytical precision deteriorated. Assay A (2-sigma) exhibited error rates centered on 4.6%, whereas Assays D and E (≥5-sigma) produced out-of-bounds results only sporadically. More than 95% of their iterations contained no (zero) TEa violations.

Figure 1 visually displays the distribution of the 1,000 iteration-level error rates for each assay. The width of the boxes (inter-quartile range, IQR) and the overall whisker length decreased as the sigma metric improved:

Assay A (2 σ). The median error rate was 4.56% and the IQR spanned 4.48%–4.64%. Whiskers extended to 4.17% (minimum) and 5.05% (maximum). No individual iteration fell within the allowable error limits for all 36 500 QC results, underscoring the clinical inadequacy of a 2-sigma process.

Assay B (3 σ). The distribution contracted sharply: the median error rate was 0.27% with an IQR of 0.25%–0.29%. Although outliers were still present, every iteration's error rate was <0.35%, representing an 18-fold reduction compared with Assay A.

Assay C (4 σ). The median error rate approached zero (6.1×10^{-5}), and 75% of iterations exhibited ≤1 TEa violation. Outliers—iterations with two or three violations—appeared as isolated points above the upper whisker.

Assay D (5 σ) and Assay E (6 σ). Both boxplots were heavily compressed at the zero line. For Assay D, 959 iterations (95.9%) recorded zero violations; for Assay E the figure was 992 iterations (99.2%). The few non-zero iterations for these high-sigma assays manifested as single-point outliers with error rates below 0.0005%.

The boxplots confirm the clear drop in the number of TEa violations with incremental gains in sigma performance. The graph further illustrates that the performance gap between 5- and 6-sigma assays may be considered negligible in routine operations (100 runs per day case load).

Statistical comparison

One-way analysis of variance (ANOVA) demonstrated a highly significant difference in mean error rates across the five assays (*p value* <0.001). Post-hoc Tukey testing (Table 3)

Table 2. Mean error rates of serum assays across different sigma metrics

Assay (sigma)	Mean CV	Mean error rate*	Minimum	Maximum	SD
A (2 σ)	0.014 286	0.045600	0.041700	0.050500	0.001100
B (3 σ)	0.009 524	0.002700	0.001920	0.003400	0.000270
C (4 σ)	0.007 143	0.000063	0.000000	0.000110	0.000041
D (5 σ)	0.005 714	0.000001	0.000000	0.000005	0.000001
E (6 σ)	0.004 762	0.000000	0.000000	0.000001	0.000000

*Mean of 1,000 iterations; error rate expressed as a proportion of 36,500 observations per iteration.

Table 3. Tukey HSD Post-hoc tests

Assay Comparisons	Difference in mean error rates	Lower CI	Upper CI	Adjusted *p* value
B - A	-4.28E-02	-4.2906E-02	-4.2779E-02	<0.000001
C - A	-4.55E-02	-4.5536E-02	-4.5409E-02	<0.000001
D - A	-4.55E-02	-4.5601E-02	-4.5474E-02	<0.000001
E - A	-4.55E-02	-4.5601E-02	-4.5475E-02	<0.000001
C - B	-2.63E-03	-2.6934E-03	-2.5665E-03	<0.000001
D - B	-2.69E-03	-2.7580E-03	-2.6312E-03	<0.000001
E - B	-2.70E-03	-2.7586E-03	-2.6317E-03	<0.000001
D - C	-6.47E-05	-1.2809E-04	-1.2273E-06	0.043187
E - C	-6.52E-05	-1.2864E-04	-1.7752E-06	0.040410
E - D	-5.48E-07	-6.3978E-05	6.2882E-05	1.000000

confirmed that every pairwise contrast involving Assay A or Assay B differed significantly (p <0.001). In contrast, the comparison between the two highest-sigma assays (D vs E) was non-significant (p = 0.62), indicating indistinguishably low residual error at ≥5-sigma performance.

Plots of a representative iteration of Levy Jennings charts across sigma metrics

A representative set of Levy–Jennings charts (Figures 2 to 6), from a single iteration, support our findings. All simulated LJ charts share an identical y-axis scale bounded by the

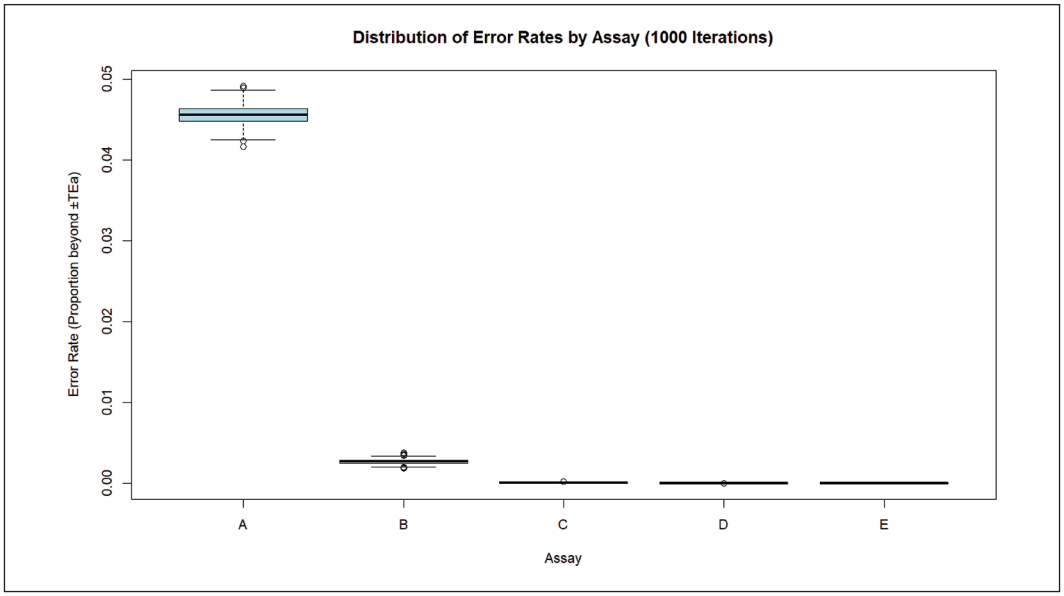

Figure 1. Box and Whiskers plot of the error rate of the 5 different simulated assays across 1000 simulations.

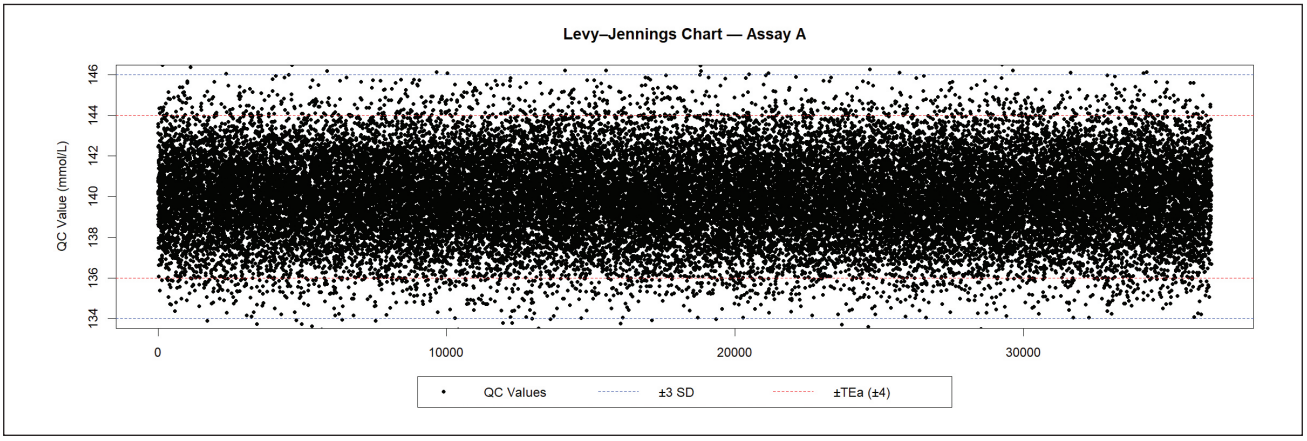

Figure 2. A representative iteration of an LJ chart for serum sodium with a 2-sigma process, showing 36,500 data points.

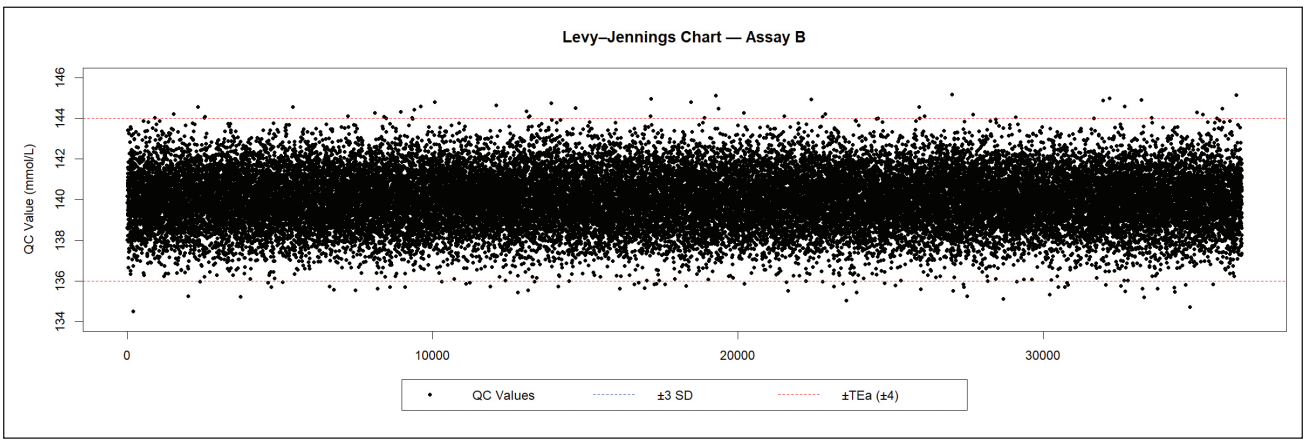

Figure 3. A representative iteration of an LJ chart for serum sodium with a 3-sigma process, showing 36,500 data points.

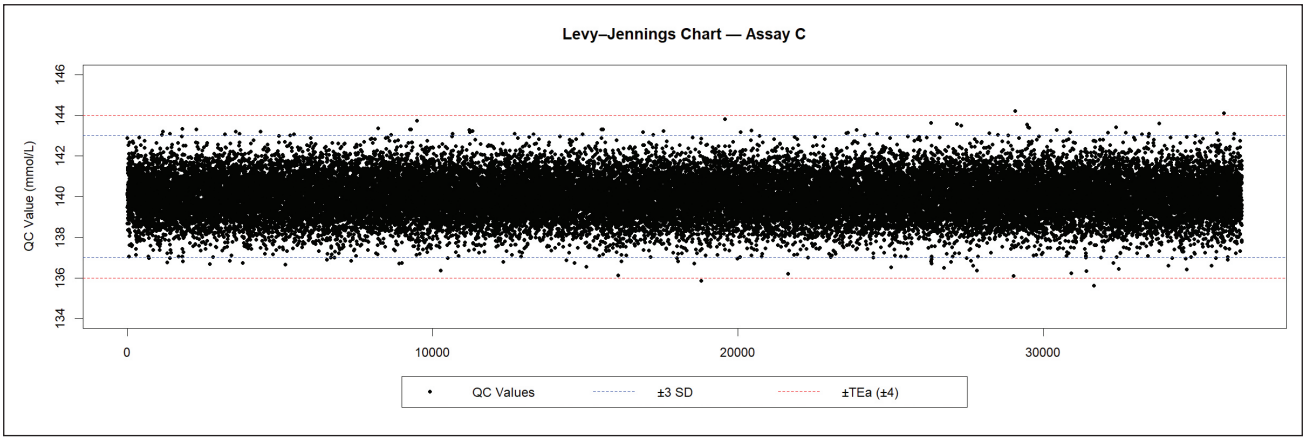

Figure 4. A representative iteration of an LJ chart for serum sodium with a 4-sigma process, showing 36,500 data points.

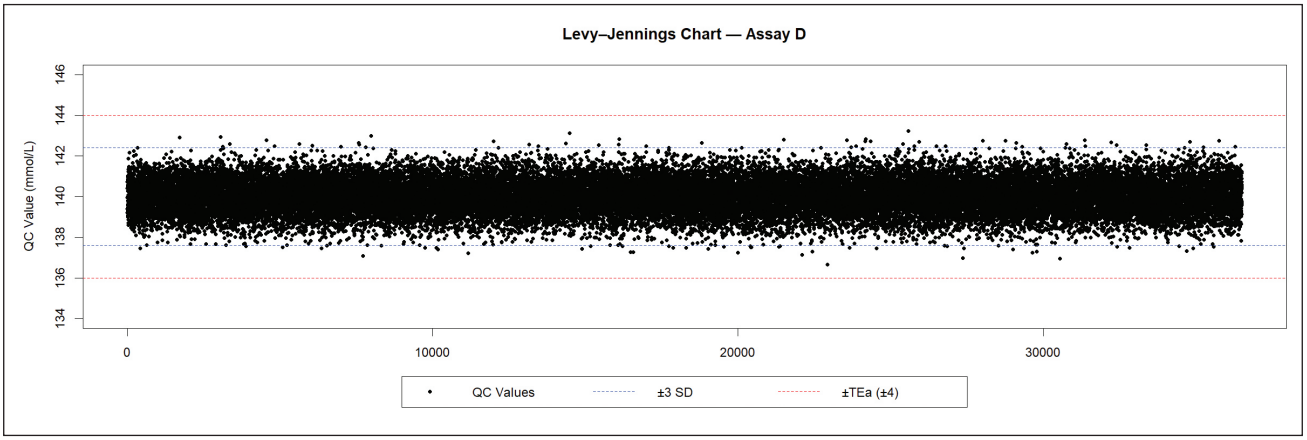

Figure 5. A representative iteration of an LJ chart for serum sodium with a 5-sigma process, showing 36,500 data points.

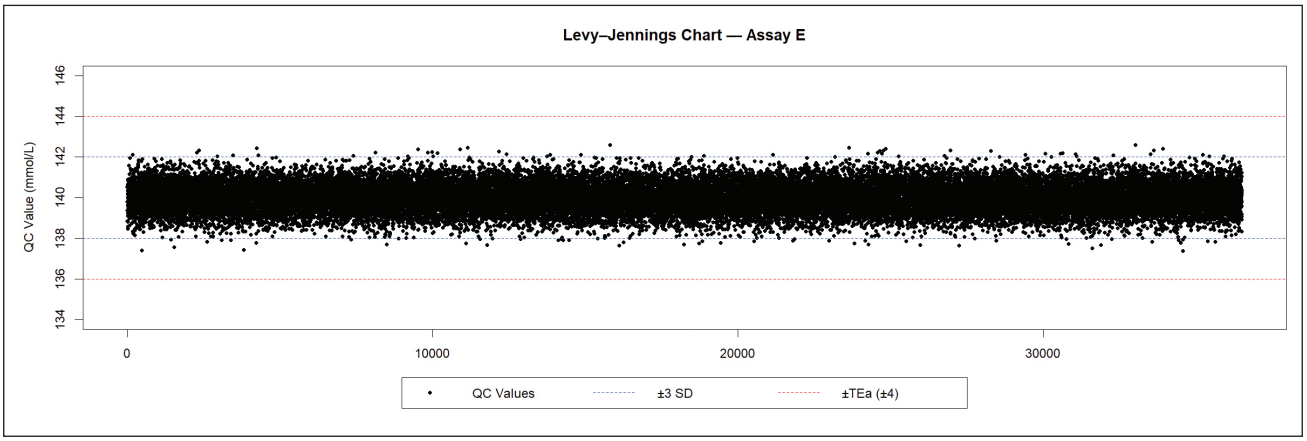

Figure 6. A representative iteration of an LJ chart for serum sodium with a 6-sigma process, showing 36,500 data points.

larger of the ±3 SD limits for Assay A or the regulatory TEa (±4 mmol/L), facilitating direct visual comparison.

In the 2-sigma chart (Assay A) QC points are widely dispersed. Frequent clusters breach both the blue dashed ±3 SD lines and the red dashed TEa boundaries, with prolonged sequences of consecutive violations evident.

In the 3-sigma chart (Assay B) most observations lie within ±3 SD. Only isolated points cross the TEa limits and visual "strings" of excursions or clusters are rare.

For the 4-sigma process (Assay C) all values are confined to a narrow band around the process mean. Occasional single-point outliers touch, but seldom exceed, the TEa lines.

The 5- and 6-sigma charts (Assays D and E) show an almost solid black band at the mean. QC points rarely approach the ±3 SD lines and no point in the depicted iteration exceeds the TEa limits.

The progressive collapse of the data cloud toward the process mean from Assay A to E provides an immediate qualitative illustration of the exponential reduction in clinically relevant error as sigma capability increases.

Collectively these results illustrate the exponential decline in TEa violations or breaches as assay precision improves from 2- to 6-sigma capability. Our results also show that once a process attains ≥5-sigma performance, further gains in CV may yield negligible additional reduction in clinically relevant errors.

DISCUSSION

This computer assisted simulation analysis experiment demonstrates a precipitous, exponential, tightly graded relation between an assay's sigma capability and its propensity to yield clinically unacceptable results for serum sodium.

Across 1,000 independent annual simulations per assay, the mean TEa violations (error rates) precipitously fell from 4.6% for the 2-sigma process to <0.0001% for the 4-sigma process and to zero for ≥5-sigma processes. One-way ANOVA confirmed that these gradients were statistically robust, and Tukey comparisons showed that each stepwise gain of one sigma level translated into a statistically, and, meaningful reduction in error, with the sole exception of the 5- versus 6-sigma contrast, where residual risk was already vanishingly small.

Representative Levy–Jennings (LJ) charts vividly illustrated this pattern. Whereas the 2-sigma assay generated frequent "runs" that fall outside of both the ±3 SD and ±Tea QC LJ lines, the 5- and 6-sigma assays plotted as a narrow ribbon around the process mean, with no violations in the illustrated iteration.

Our simulation experiment results align with Westgard's theoretical error grids, which predict ~45 000 defect opportunities per million (DPM) for a 2-sigma process and <4 DPM for a 5-sigma process when TEa is set at 1.65 SD.[9,10,14,24] While absolute rates differ because our TEa is fixed in concentration units rather than multiples of SD, the simulated decline in error as sigma rises is congruent with analytic-quality models employed by CLSI EP23-A2 and IFCC.[7,9,25] Moreover, the finding that a 3-sigma process still yields error in the order of 1×10^{-2} corroborates several external-quality-assessment reports showing that 3-sigma sodium assays fail 'total-error' proficiency challenges four- to five-times more often than 5-sigma assays.[26]

Clinical and operational implications

From a risk-management perspective, any test with ≥5-sigma capability for sodium essentially meets a "six-sigma" quality target after accounting for biological variation (≈0.5%).[2,4,5] Such performance permits less intensive QC scheduling, potentially reducing reagent waste, technologist time and instrument down-time without compromising patient safety. Conversely, a 2-sigma process is categorically inadequate. Even under idealized random-error conditions, it would deliver approximately 1,600 out-of-specification results per year at the simulated workload. A 3-sigma process, though markedly better, still produces about 1 error every four days, underscoring the need for either tighter imprecision goals or supplementary QC rules (e.g., Westgard multirules, moving averages) if this level of performance cannot be improved.

The 4-sigma scenario presents an inflection point: the average laboratory might find its residual error tolerable if additional patient-based QC[27] is in place. However, the tail of the error-rate distribution (maximum 1.1×10^{-4}) implies that sporadic TEa violations remain possible and must be weighed against the clinical consequences of sodium misclassification.

Strengths and limitations

A major strength of this work is the large simulation size—36,500 observations per iteration and 1,000 iterations per assay—providing tight confidence around the error-rate estimates.

The framework is transparent, fully reproducible and parameterized directly in concentration units of serum sodium, enhancing its practical relevance, understandability, and direct translatability to actual bench experience - features that would support use by laboratory staff and managers.

Many limitations stem from the model simplification choices we made in the simulations. First, only imprecision was varied. Other key factors such as systematic bias, drift, reagent lot effects and matrix interferences were not modelled, to keep the simulation tractable and feasible using standard computational resources. In real-world quality control, bias significantly impacts sigma metrics and is often the reason assays fall short of performance expectations despite good precision. The exclusion of bias in our model assumes idealized conditions and may thus lead to an overestimation of QC performance. Therefore, future studies should incorporate bias to more accurately reflect operational laboratory scenarios and better guide QC protocol design. These are relevant sources of variation that can and should be modelled using grounded assumptions.

Second, the assumption of normality may underestimate distribution tail behavior for real-world assays with non-Gaussian error distributions. The assumption of a normal distribution in simulating control values may underrepresent the frequency of extreme deviations. In practice, assay result distributions can exhibit skewness or heavy tails due to pre-analytical or biological variability, especially in low-sigma processes. This may lead to underestimation of error rates and overconfidence in assay reliability. Thus, caution must be exercised in applying these findings directly to routine QC planning without empirical distribution analysis. Follow-up simulations using statistical models with "fatter" tails may be informative.[28]

Third, the upper and lower TEa lines were treated as a fixed symmetric bounds, yet in practice different regulatory bodies can specify asymmetric or clinically variable goals.[5,29] This study used CLIA guidelines as the source of TEa due to their regulatory relevance and widespread use in clinical laboratory practice. However, it is important to note that using TEa derived from biological variation or percentage-based goals could alter sigma estimations. Wider TEa ranges would likely yield higher sigma values, potentially resulting in fewer TE violations. This underscores the importance of context-specific TEa selection when interpreting QC performance.

Finally, the analysis focused on a specific analyte only - serum sodium, which has a measurand with stringent TEa (±4 mmol/L). Direct extrapolation to analytes with wider or percentage-based TEa requires caution.

FUTURE DIRECTIONS

Incorporating systematic shifts and trending errors would refine understanding of QC frequency requirements, particularly for mid-tier (3- to 4-sigma) assays. Future work should also evaluate the combined effect of sigma performance and multirule QC algorithms to identify cost-efficient QC strategies under resource-constrained settings. Expanding the simulation to additional measurands with biologically driven TEa (e.g., potassium, calcium) would generalize the conclusions to a broader clinical-chemistry portfolio.

CONCLUSIONS

This simulation confirms that sigma metrics provide a powerful, quantitative gauge of analytical reliability. A 5-sigma sodium assay is effectively "zero-defect" under routine workloads, validating reduced QC burdens, whereas a 3-sigma assay, although showing a significant improvement from 2-sigma assay, still carries a measurable risk of clinically significant error and warrants enhanced QC oversight. Laboratories should therefore prioritize analytical-imprecision improvements to at least 4-sigma, and preferably at least 5-sigma, when selecting or validating serum-sodium methods.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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OPEN ACCESS – ORIGINAL ARTICLE

Prevalence and Pattern of Antifungal Drug Minimum Inhibitory Concentration (MIC) of Invasive Candidiasis and its Associated Risk Factors*

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ABSTRACT

Background. Invasive candidiasis is defined by the growth of *Candida* species in the bloodstream or other internal organs. It is a global concern due to increasing multidrug resistance and high mortality rates. This study aimed to update prevalence data on *Candida* infections in the Philippines, analyzing demographic factors (age, sex), specimen sources, and associated risk factors. We compared antifungal resistance patterns against CLSI epidemiological cutoff values (ECVs) and clinical breakpoints and examined MIC variations by underlying disease to inform potential standardized empiric therapies.

Methodology. We conducted a retrospective analytical cross-sectional study (SLMC-IERC approval, minimal risk) reviewing one year of *Candida* speciation and susceptibility results from January 2024 to December 2024 at a private tertiary hospital. All aseptically collected samples that tested positive for *Candida* species were included. Respiratory and wound specimens required a Gram stain demonstrating yeasts and hyphae prior to culture, while urine cultures were included only if they yielded ≥100,000 CFU/mL. Identification and susceptibility testing were performed using the VITEK 2 system, with results interpreted using CLSI breakpoints and ECVs.

Results. Among 266 patients with *Candida* infections, invasive candidiasis predominated in those aged ≥60 years (66.4%). *Candida albicans* (21.7%) and *Candida tropicalis* (13.5%) were more frequent in females, while *Candida parapsilosis* (13.2%) and *Candida glabrata* (5.3%) were more common in males. Blood and CSF samples strongly correlated with invasive disease and underlying risk factors. *C. albicans* was linked to infection-related conditions (13.9%), malignancy (9.0%), and cardiovascular disease (6.8%). *C. parapsilosis* (23.3%) and *C. tropicalis* (20.7%) were frequently associated with infection, malignancy, and metabolic disorders. *C. glabrata* (7.5%), noted for antifungal resistance, was isolated in patients with direct infections (3.4%) and malignancies (1.9%). Among azoles, fluconazole demonstrated greater susceptibility against *Candida* species, requiring lower concentrations for inhibition, despite a higher resistance rate (13.22%) compared to voriconazole (8.95%). Among echinocandins, micafungin showed better susceptibility than caspofungin. Amphotericin B demonstrated the highest overall susceptibility (93–100%), though MICs approached ECV limits. Most susceptible MIC values were fluconazole 0.5 µg/mL for *C. albicans* and *C. parapsilosis*, 1.0 µg/mL for *C. tropicalis*; voriconazole and caspofungin 0.12 µg/mL; micafungin 0.06 µg/mL; amphotericin B 0.5 µg/mL; and flucytosine <1 µg/mL for all species.

Conclusion. These findings support a species-specific, risk-adapted approach to antifungal therapy, incorporating demographic and clinical variables. Continuous surveillance of invasive candidiasis prevalence and antifungal MIC trends, with periodic breakpoint updates, is crucial to preserve therapeutic efficacy. Effective management of multidrug-resistant *Candida* infections also requires close collaboration between clinicians and pharmacists, as well as the development of new dosing strategies based on pharmacokinetic/pharmacodynamic (PK/PD) principles.

Key words: candidiasis, antifungal agents, drug resistance, Candida, azoles

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INTRODUCTION

Invasive candidiasis occurs among hospitalized patients when *Candida* infects the internal organs (such as the kidney or brain) or the bloodstream.[1] This infection typically happens when *Candida* species breach the damaged organ barrier, which can be caused by various factors such as cancer chemotherapy, surgery, bacterial toxins, and local vascular perfusion disorders (endogenous route). This primarily applies to species such as *C. albicans, Candida tropicalis, Candida glabrata (Nakaseomyces glabrata), Candida lusitaniae (Clavispora lusitaniae), Candida parapsilosis* and, to a lesser extent, *Candida krusei (Pichia kudriavzeveii).* Infections can also occur via an exogenous route, such as through healthcare workers' hands or central line colonization and subsequent spread into the blood. This is especially true for *Candida auris, C. parapsilosis, Candida lusitaniae (C. lusitaniae)* and *Candida haemulonii*.[2]

Besides colonization and immunosuppression, factors known to be associated with increased risk of invasive candidiasis (IC) are broad spectrum antibiotic use, total parenteral nutrition (TPN), central venous catheter (CVC), surgery, renal replacement therapy, diabetes, prolonged mechanical ventilation, severe sepsis, and high Acute Physiology and Chronic Health Evaluation (APACHE) II score,[3] which classifies disease severity and predict mortality in intensive care unit (ICU) patients. There are other early diagnostic tools to assess the risk of invasive candidiasis early in patients admitted to ICU, such as the *Candida* Colonization Index (CCI) and the *Candida* score. The use of these is debated and not validated for all populations. While the risk scoring showed sensitivity and specificity for IC of 81% and 74%, respectively, the population tested mostly included surgical ICU patients, with only 35% of admissions for medical reasons. Additionally, the tools may be less reliable for patients with nonsurgical reasons for ICU admission. Furthermore, considering these, the key management in improving patient outcome is prompt source control (i.e., the elimination of the focus of infection) through early initiation of antifungal therapy to reduce hospital mortality.[4]

The prevalence of candidiasis has been on a growing trend, which is often related to the immunological status of patients.[5] It remains one of the top 5 healthcare-associated bloodstream infections in the world and causes high mortality rates. In the Philippines, candidiasis constitutes to about 80.40% of the fungal infections in 2016.[6] Utilization of the health budget has improved over the years, but the governance and implementation challenges persist due to the fragmented nature of the system. Moreover, the country has a mixed health system with an expanding private sector. The regional and socioeconomic disparities, access to effective treatment from the subsidy of the government or availability of resources are prominent concerns especially to the marginalized communities.[7]

At present, antifungal agents are limited to three major classes: the polyenes, which bind fungal cell membrane ergosterol leading to cell lysis; azoles that inhibit ergosterol biosynthesis; and echinocandins that inhibit fungal (1,3)-β-D-glucan cell wall biosynthesis.[8] Due to indiscriminate use of antifungal prophylaxis, limited choice of appropriate antifungal drugs, delay in obtaining antifungal susceptibility testing (AFST) results and the lack of pharmacodynamic correlation between minimal inhibitory concentration (MIC) values and risk factors has caused emergence of rising numbers of multi-drug resistant microorganisms. Due to this emergence of acquired antifungal resistance, reassessing the pattern and discrepancy in clinical breakpoints (CBPs) of antifungal agents made it more complex. These problems are important to consider in updating the appropriate antifungal selection.[9]

Antifungals are associated with greater uncertainty. This is partly due to the relatively low prevalence of invasive fungal infections (mold infections) and some specific biological characteristics of fungal pathogens like the possibility of biofilm formation (especially for *Candida* species).[9]

Gaps in the overall improvements in health systems and ICU care in the last few decades, as well as the development of different antifungals and microbiological techniques, mortality rates in invasive candidiasis have not substantially improved. One of them is that early diagnosis of candidemia and deep-seated candidiasis remain a challenge due to the prolonged time to positivity of blood cultures, which can take up to 5 days to become positive, and due to the low yield of culture diagnostic tests for deep-seated candidiasis (50%). Guidelines may have existed to guide the choice of antifungal therapy, however, patients affected by invasive candidiasis need a tailored approach due to heterogeneous host factors and significant geographical variation in species distribution and antifungal drugs resistance rates. Also, the value of different treatment strategies remains to be clarified.[4]

Globally, the incidence rate of invasive candidiasis had been 3–5 per 100,000 persons in the general population, 1%–2% of all ICU admissions and 750,000 cases per year. *Candida albicans* is the most common cause of invasive candidiasis. Currently, non-*albicans Candida* species account for an increasing proportion of cases. It was known that deep-seated candidiasis, arising from direct inoculation or hematogenous dissemination of *Candida* into normally sterile body sites, is often difficult to diagnose and may affect a population as large as that of candidemia.[10]

While *C. albicans* remains the most prevalent species in many parts of the world, the past decade has seen a notable rise in non-*albicans Candida* species. For instance, *C. glabrata*, particularly among elderly patients and solid organ transplant recipients, is the second most common species in the United States, northwestern Europe, and Canada. In contrast, *C. parapsilosis* and *C. tropicalis* are more frequently reported in Southern Europe, South America, India, and Pakistan.[4] In the Philippines, a prospective study conducted in Metro Manila in 2012 reviewed 39 candidemia cases with isolated species. *Candida tropicalis* (35.9%) was the most prevalent, followed by *C. parapsilosis* (30.8%) and *C. albicans* (28.2%). The majority of the isolates were susceptible to fluconazole. The top 3 underlying conditions that lead to candidemia were cancer (28.2%), neurologic disease (20.5%) and both solid tumor and renal disease (17.9%). The most common risk factors were orotracheal intubation (33%) and intraabdominal surgery (33%).[11] In a retrospective study done in Bacolod City in

2018, 184 *Candida species* were isolated. The most frequent among these isolates were *C. albicans* (62%), *C. tropicalis* (15%) and *C. ciferrii complex* (10%). The samples submitted were 69.02% from respiratory specimens (sputum and tracheal aspirate), 20.65% from urine specimens, 7.61% from blood, and 2.72% from vaginal discharge.[6]

A study on risk prediction for invasive candidiasis by Ahmed et al. demonstrated that identifying patients at increased risk led to a meaningful reduction in fungal infections through the use of antifungal prophylaxis, as well as a significant decrease in overall mortality. This can be achieved by incorporating microbiological parameters to stratify high-risk groups, rather than relying solely on clinical indicators. One approach involves determining the MICs for a clinical isolate and interpreting susceptibility according to established CBPs, which is essential for selecting the most appropriate antifungal therapy.[12]

In this study, VITEK 2® system (bioMerieux, France), a commercial automated method that allows rapid and accurate species identification by comparison of the biochemical profile with an extensive database. Its system incorporates the YS08 antifungal susceptibility testing (AFST) cards with expanded role in yeast susceptibility test that determines *Candida* growth by attenuation of light measured through optical scanner, performing fully automated testing of susceptibility to flucytosine, amphotericin B, fluconazole, and voriconazole.[13] It has a miniaturized and automated version of the doubling dilution technique for MICs determined by microdilution method. It followed the breakpoints from Global Clinical and Laboratory Standards Institute-based (CLSI M27M44S 3rd Edition, August 2022) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (version 2020) guideline for the *Candida* species.

Therefore, this study aims to review the current prevalence of invasive candidiasis and help clinicians improve patient outcomes through knowing the MIC values of antifungal drugs against isolated *Candida* species and determine risk factors affecting it. This will further help clinicians make the choice of empiric antifungal drugs by preventing side effects as well as evolution to drug resistance. Additionally, there is no available data for some low- and middle-income countries due to the absence of hospital infrastructures for blood culture analyses and further AFST workups.[4]

METHODOLOGY

The study design was retrospective, analytical cross-sectional, performed in the Microbiology section of a private tertiary hospital in Taguig City, Metro Manila. Random data were gathered retrospectively from January 2024 to December 2024. Reference materials used were the retrieved compilation of *Candida* speciation printed records of samples (blood, body fluids, urine, sputum, and body tissues) collected aseptically in sterile appropriate containers. The archive results from VITEK 2 identified specimens with *Candida* infection and measured the Anti-susceptibility Testing (AST). This programmed machine utilized the standard procedure of the Global Clinical and Laboratory Standards Institute (CLSI) M27M44S for antifungal susceptibility testing (AFST) and derivation of minimum inhibitory concentration (MIC) values. The researchers sought assistance from the medical technology staff in the Microbiology section for the retrieval of *Candida* speciation test results upon approval of the conduct of this study. Consequently, retrospective data collection, analysis of data in Excel worksheet and statistical computations were done in a timely manner.

Inclusion criteria

Culture results dated between January 2024 and December 2024 were collected. Patients with *Candida* species as the causative agent of their infection were included in the study. Data acquisition included only the patients' accession number, age, sex, underlying risk factor of disease or diagnosis, kind or source of specimen, date of laboratory submission, identified isolates, and its susceptibility test results. The timings of reading were noted to evaluate consistency of the MIC determination. From the institutional database, all patients with *Candida* speciation requests to include Antimicrobial Sensitivity test results with MIC of each antifungal agent, were collected.

To increase the data collection, all one-year data with positive *Candida* infection was scrutinized and were considered relevant. All samples (sterile and non-sterile) were included in the study. For respiratory specimens included, gram stain results revealed presence of yeasts and hyphae. While urine cultures included were with colony growth of at least 100,000 colony-forming units (CFU) per milliliter of *Candida* species. These were processed by the medical technology staff in the Microbiology section for the specific organism identification and susceptibility testing using the VITEK 2 machine with AST-YS08 biochemical cards. The workflows were all based on the procedures of the laboratory.

Exclusion criteria

This study excluded patients with incomplete demographics and medical assessment. Also, if the causative agent was not *Candida* species it was disregarded and if the anti-susceptibility testing was terminated run in the VITEK 2 machine.

Sample size estimation

Sample sizes were obtained using the Krejcie-Morgan equation considering the essential measure of a level of accuracy as well as the required confidence level based on the existing population available for this study. The study size had a confidence level of 95% and error rate of 5%. This was due to 80% prevalence of Candidiasis in the Philippines.[6] The formula was as follows:

$$n = X^2NP1\text{-}P[e2\ N\text{-}1+X^2\ P1\text{-}P]$$

Where:

N = population size
X^2 = chi-square value at 95% confidence level with a degree of freedom of 1
P = proportion of the population
e = margin of error
n = 3.8412450.81-0.8[0.052245-1+3.8410.81-0.8]
n = 150.567 1.22
n = 123.42

Therefore, this study required at least 123 samples based on the calculation being made by the researcher and had a final 266 samples during the collection process from January 2024 to December 2024.

Data analysis

All data were analyzed using statistical tool SPSS version 27. Descriptive and exploratory data analyses were performed using absolute frequencies (n), relative frequencies (%), Measures of Central Tendency (mean and median) and had variance (standard deviation). Analysis of Variance was used to compare the antifungal drug resistance pattern of identified *Candida* species based on the Global CLSI ECVs and breakpoints. Furthermore, the differences of the Antifungal MIC of isolated *Candida* species according to risk factors of underlying disease using Analysis of Variance. The significance among identified variables was calculated based on the standard p-value of <0.05.

Ethical considerations

The study abided by the Principles of the Declaration of Helsinki (2013) and conducted in accordance with the Guidelines of the International Conference on Harmonization-Good Clinical Practice (ICH-GCP), E6 (R2) and other ICH-GCP 6 (as amended); National Ethical Guidelines for Health and Health-Related Research (NEG HHRR), 2017, and the Philippine Data Privacy Act of 2012. This protocol was approved by the SLMC Institutional Ethics Review Committee. Patient confidentiality was respected by ensuring anonymity of patient records. This research had no direct involvement with any vulnerable subjects that may breach ethical codes (e.g., children, prisoners, pregnant women, mentally challenged, educationally and economically disadvantaged), with no consent necessary due to the use only of laboratory information systems and printed records.

RESULTS

From January 2024 to December 2024, the tertiary hospital had a total of 447 requests of *Candida* speciation with susceptibility testing done in the hospital, giving an overall *Candida* infection prevalence rate of 59.51 % for a year. Of those 181 were excluded because identified were other filamentous fungi or susceptibility testing data was incomplete. A total of 266 patients with candidiasis were included for analysis. It was observed that the majority consists of admitted patients (98%) who had stayed in the hospital for at least 35 days and longer.

Candida species frequency is presented in Figure 1 by age group (0–18, 19–59, ≥60 years) to show the distribution of the species. The most common species was *C. albicans* (41.0%), and it was most prominent in the ≥60 years age group (26.7%), which may suggest that this species was more common in older people. The second most common species, *C. parapsilosis* (23.3%), also occurred most frequently in older adults (12.4%) and, to a lesser extent, in those 19–59 years of age (10.2%). This data suggests that these species may be more familiar with advancing age due to age-related changes in the host immune system or the presence of other diseases. On the other hand, uncommon species such as *C. glabrata* and *C. tropicalis* exhibited distinct age-related distribution. *Candida tropicalis* was present in 14.7% of patients who were ≥60 years old, whereas *C. glabrata* was rare and isolated from older adults (6.8%). Rare species like *C. krusei*, *C. lusitaniae*, and *C. orthopsilosis* were also seen in small numbers, in the older groups, consistent with their rarity. The table further supports the idea that age should be considered as an element in diagnosing and treating *Candida* infections because some species were more frequent in certain age groups.

Candida species distribution is presented in Figure 2 by sex, which shows the differences in species prevalence in male and female patients. The most common species remained *C. albicans* (41.0%), with slightly more in females (21.7%) than in males (19.5%). The second most common species, *C. tropicalis*, was also more prevalent in females (13.5%) than males (7.1%). *Candida glabrata*, on the other hand, presented a male prevalence, with 5.3% of the cases in males and 2.3% in females. There were minimal differences in the incidence of *C. krusei* and *C. orthopsilosis* between the two groups because these were rare in the study population. Interestingly, some species, such as *C. lipolytica* and *C. famata*, were only or mainly found in males, but they were very few. These findings highlight the need to consider the patient's sex when dealing with *Candida* infections because some species may have a different prevalence in males and females and thus require different

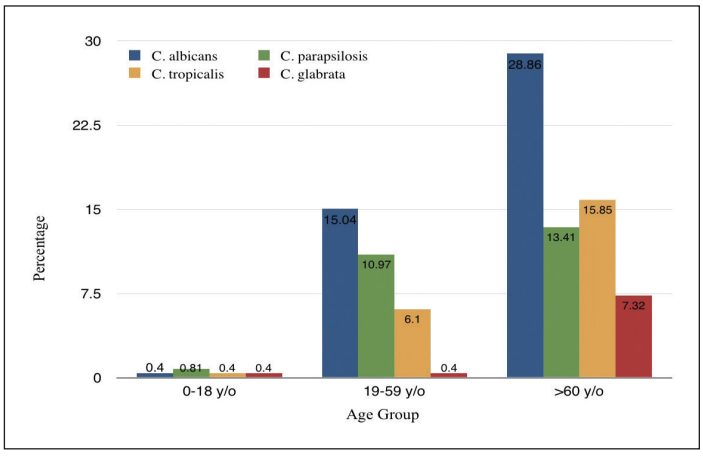

Figure 1. Age distribution of top 4 *Candida* species from January 2024 to December 2024 in a private tertiary hospital.

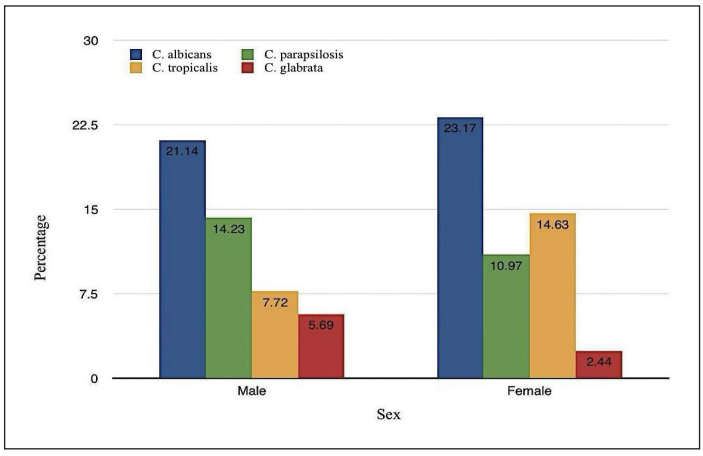

Figure 2. Sex distribution of top 4 *Candida* species from January 2024 to December 2024 in a private tertiary hospital.

approaches to diagnosis and treatment. Hence, association to anatomical, physiologic, and hormonal characteristics in relation to *Candida* growth is suggestive for further investigation.

Figure 3 shows the frequency distribution of *Candida* species according to the source of the samples and reveals considerable differences in prevalence among different sample types. The most common species was *C. albicans* (41.0%), which was most frequently found in cerebrospinal fluid (CSF, 10.2%), and in blood (9.0%). The second most common species, C. *parapsilosis* (23.3%), was strongly associated with sepsis and was isolated from blood in 18.8% of the cases. *C. tropicalis* (20.7%) also mainly recovered from CSF (8.6%).

Notably, this study identified the presence of *C. albicans* (16.8%) and *C. tropicalis* (8.6%) in respiratory specimens. These findings were included to explore the observations by Barantsevich et al., who reported strong associations between *Candida* isolated from multiple specimen types and invasive disease, supporting the Infectious Diseases Society of America (IDSA) recommendation for non-invasive diagnostics and empiric antifungal therapy guided by clinical risk, surrogate markers, and culture data.[14]

Although *Candida* species are often considered innocent colonizers of the respiratory tract, components like beta-glucan in their cell walls can act as lung pro-inflammatory agents, leading to macrophage and neutrophil dysfunction.[6] Colonization studies suggest that disruption of the bacterial flora and factors facilitating fungal translocation into the bloodstream increase the risk of invasive candidiasis. Immune status also plays a critical role, influencing both the clearance of fungal organisms and the risk of organ involvement.[1]

According to Meena and Kumar, diagnosing *Candida* pneumonia remains challenging due to nonspecific imaging findings and the lack of definitive diagnostic tools. The clinical significance of *Candida* in respiratory samples, particularly in ventilator-associated pneumonia (VAP) and community-acquired pneumonia (CAP), remains uncertain. Nonetheless, despite limited clinical evidence, the potential roles of biofilm formation, advanced genetic techniques, and fungal-bacterial crosstalk should not be overlooked.[15]

The rare species such as *C. krusei*, *C. lipolytica*, and *C. auris* were isolated from different sample types but to a minimal level in this study population. These findings also support the idea that the sample source is critical in determining the clinical relevance of *Candida* species since some pathogens are more associated with harboring areas that allow it, which helps in selecting the appropriate diagnostic and therapeutic measures.

Figure 4 highlights the distribution of *Candida* species across various risk factors, demonstrating significant associations with underlying health conditions. *Candida albicans* (41.0%) was the most prevalent species, frequently linked to infection-related conditions (13.9%), malignancy (9.0%), and cardiovascular disease (6.8%), suggesting its opportunistic nature in immunocompromised patients. Similarly, *C. parapsilosis* (23.3%) and *C. tropicalis* (20.7%) were commonly associated with infections, malignancies, and metabolic conditions, reinforcing their role in hospital-acquired and systemic fungal infections. *Candida glabrata* (7.5%), known for its antifungal resistance, was primarily isolated in patients with infections (3.4%) and malignancies (1.9%), while *C. krusei* (1.1%) was found in a smaller subset of individuals, emphasizing the clinical relevance of species identification in high-risk populations.

The data further illustrates that metabolic disorders, particularly diabetes, were strongly associated with *C. albicans* (3.4%) and *C. tropicalis* (2.3%), aligning with the known impact of hyperglycemia on fungal growth. Chronic kidney

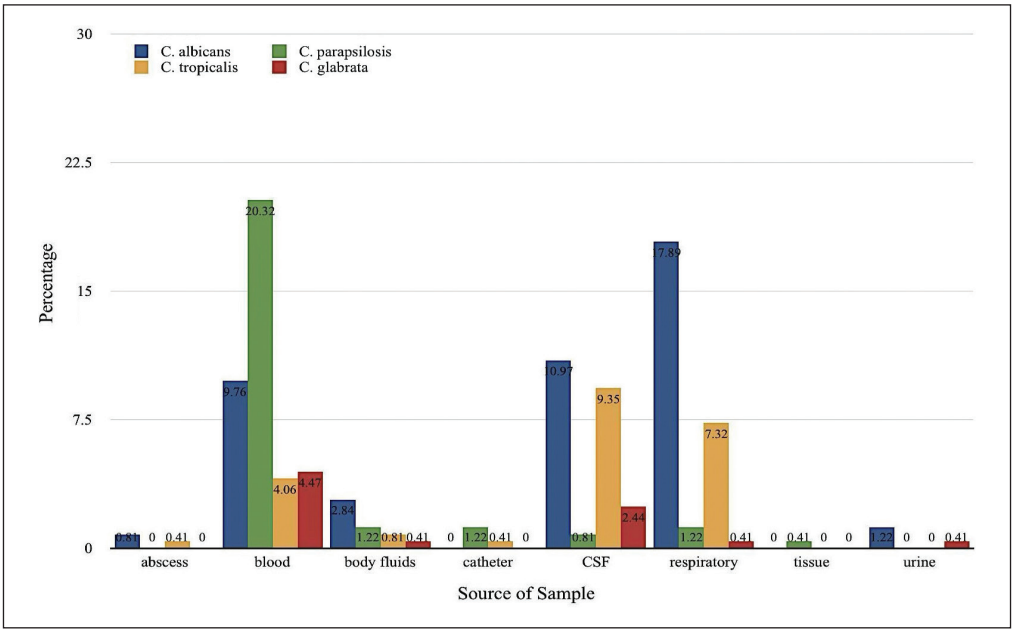

Figure 3. Distribution of sample source of top 4 *Candida* species from January 2024 to December 2024 in a private tertiary hospital.

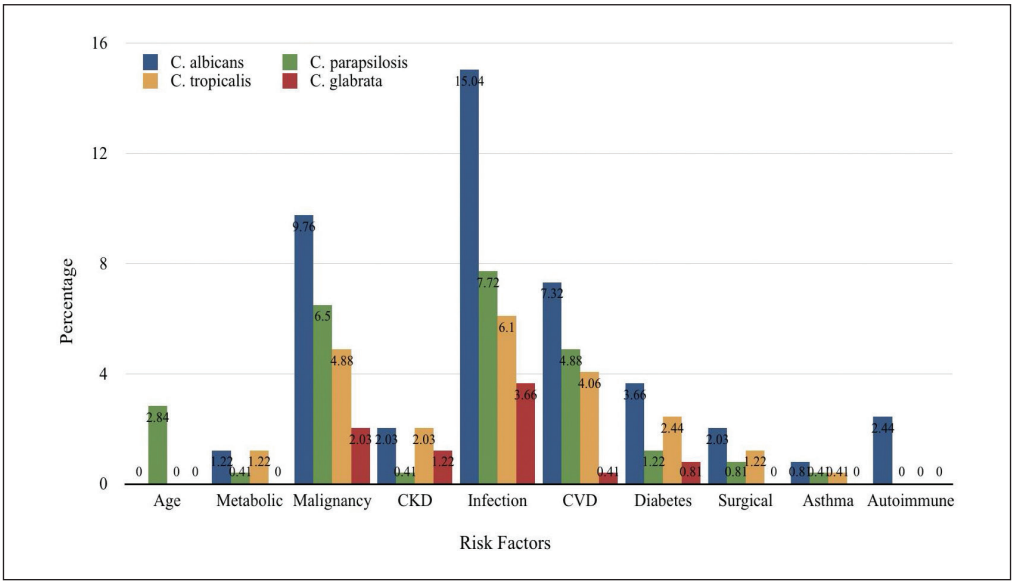

Figure 4. Distribution of risk factors for top 4 *Candida* species from January 2024 to December 2024 in a private tertiary hospital.

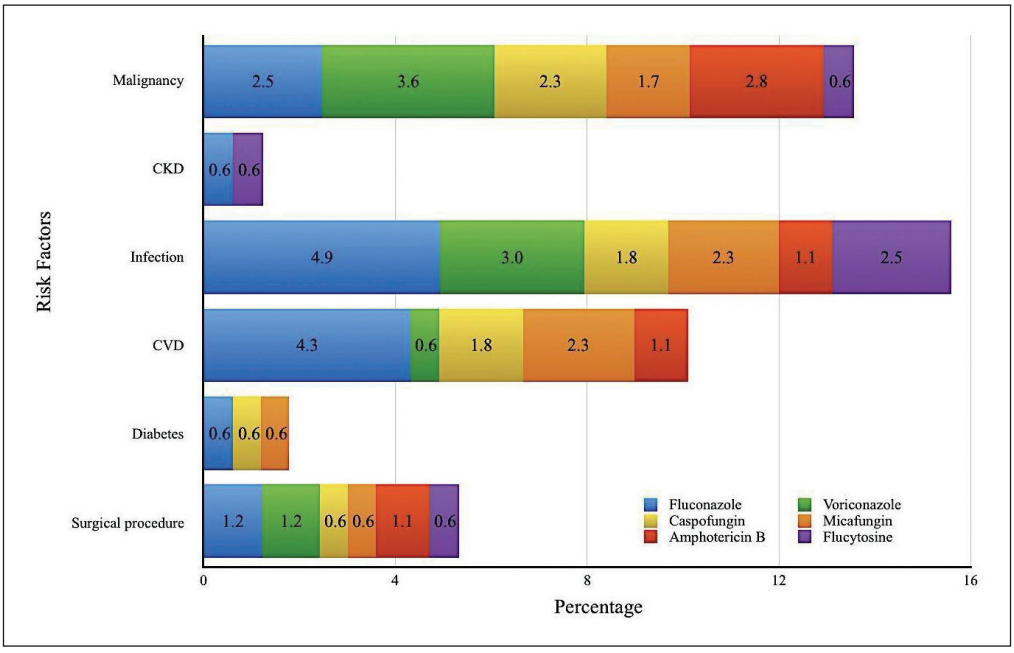

Figure 5. Antifungal drug resistance pattern according to risk factors from January 2024 to December 2024 in a private tertiary hospital.

disease (CKD) was linked to *C. albicans* and *C. tropicalis* (both at 1.9%), highlighting renal dysfunction as a potential predisposing factor. While surgical procedures and autoimmune diseases had lower associations with *Candida* infections, their presence suggests that immunosuppression or prolonged hospitalization may still contribute to fungal colonization. These findings underscore the need for targeted surveillance and early antifungal intervention in high-risk groups, particularly those with malignancies, diabetes, or CKD, to prevent complications associated with resistant *Candida* strains.

Figure 5 presents the distribution of antifungal drug resistance patterns according to risk factors. Among the six antifungal agents used in the study, the azole antifungal group had the highest number of drug resistance (13.22%) particularly fluconazole. The results indicated that fluconazole resistance was increased in infection-related (3.0%) causes collectively (such as pneumonia, cellulitis, ascites, pleural effusions, tendonitis, and sacral ulcers), cardiovascular diseases (CVD) collectively (such as hypertension, infarct and aneurysms) (2.6%) and malignancy (1.5%). Voriconazole resistance (8.95%) showed secondly in this study and was seen in patients with malignancy (2.3%) and infection (1.5%). Micafungin resistance (6.39%) and caspofungin resistance (5.98%) were seen mostly in patients with malignancy, CVD, and infection (1.37%, average). Amphotericin B resistance (5.7%) and

flucytosine resistance (5.96%) were commonly seen in malignancy (1.9%) and infection (1.5%), respectively.

Table 1 shows the MIC of the six antifungals for the AST of the top 4 *Candida* species. Fluconazole predominantly exhibited a MIC value of 0.5 ug/mL for the (69) susceptible *C. albicans* (40.66%) and the (22) susceptible *C. parapsilosis* (10.3%). While fluconazole predominantly exhibited a minimum inhibitory concentration (MIC) value of 1.0 ug/mL for the (40) *C. tropicalis* (18.79%) identified which were four dilutions away from the breakpoint (CLSI 8 ug/mL for *C. albicans*, *C. parapsilosis* and *C. tropicalis*). Voriconazole for

Table 1. Summary distribution antifungal minimum inhibitory concentration (MIC) of isolated Top 4 *Candida* species

Antifungal Agent MIC, ug/mL	Interpretation	*C. albicans*	Percentage %	*C. glabrata*	Percentage %	*C. parapsilosis*	Percentage %	*C. tropicalis*	Percentage %
Fluconazole									
0.5	S	67	40.66%	Not reported	Not reported	17	10.3%	6	3.64%
1	S	5	3.03%	Not reported	Not reported	4	2.42%	31	18.79%
2	S	4	2.42%	Not reported	Not reported	7	4.24%	5	3.03%
4	SDD	3	1.82%	Not reported	Not reported	0	0%	1	0.61%
8	R	9	5.45%	Not reported	Not reported	1	0.61%	1	0.61%
16	R	0	0%	Not reported	Not reported	0	0%	0	0%
32	R	4	2.42%	Not reported	Not reported	0	0%	0	0%
64	R	0	0%	Not reported	Not reported	0	0%	0	0%
	Total N=165	92				29		44	
Voriconazole									
0.008	S	0	0%	Not reported	Not reported	0	0%		0%
0.12	S	77	45.83%	Not reported	Not reported	28	16.67%	46	27.38%
0.25	I	2	1.19%	Not reported	Not reported	1	0.6%	0	0%
0.5	I	2	1.19%	Not reported	Not reported	0	0%	0	0%
1	R	4	2.38%	Not reported	Not reported	0	0%	0	0%
2	R	0	0%	Not reported	Not reported	0	0%	0	0%
4	R	4	2.38%	Not reported	Not reported	0	0%	1	0.6%
8	R	3	1.78%	Not reported	Not reported	0	0%	0	0%
	Total N=168	92				29		47	
Caspofungin									
0.008	S	0	0%	0	0%	0	0%	0	0%
0.12	S	81	46.55%	6	3.45%	3	1.72%	44	25.29%
0.25	I	3	1.72%	1	0.57%	20	11.49%	0	0%
0.5	I	0	0%	1	0.57%	5	2.87%	0	0%
1	R	0	0%	0	0%	0	0%	0	0%
2	R	0	0%	0	0%	0	0%	0	0%
8	R	8	4.6%	0	0%	0	0%	2	1.15%
	Total N=174	92		8		28		46	
Micafungin									
0.008	S	0	0%	0	0%	0	0%	0	0%
0.06	S	81	46.02%	9	5.11%	1	0.57%	44	25%
0.12	S	0	0%	0	0%	1	0.57%	0	0%
0.25	S	0	0%	0	0%	0	0%	0	0%
0.5	S	1	0.57%	0	0%	21	11.93%	0	0%
1	S	0	0%	0	0%	3	1.7%	0	0%
2	R	0	0%	1	0.57%	0	0%	0	0%
4	R	2	1.14%	0	0%	0	0%	2	1.14%
8	R	8	4.54%	0	0%	1	0.57%	1	0.57%
	Total N=176	92		10		27		47	
Amphotericin B									
0.12	S	0	0%	0	0%	0	0%	0	0%
0.25	S	13	7.18%	2	1.1%	7	3.87%	26	14.36%
0.5	S	50	27.62%	8	4.42%	20	11.05%	21	11.6%
1	S	23	12.71%	2	1.1%	1	0.55%	0	0%
2	R	1	0.55%	0	0%	0	0%	0	0%
4	R	1	0.55%	0	0%	1	0.55%	0	0%
8	R	2	1.1%	0	0%	0	0%	0	0%
16	R	2	1.1%	0	0%	0	0%	1	0.55%
	Total N=181	92		12		29		48	
Flucytosine									
0.006	S	0	0%	0	0%	0	0%	0	0%
<1	S	79	73.15%	12	11.11%	27	25%	46	43.52%
2	S	3	2.78%	0	0%	0	0%	0	0%
4	S	1	0.92%	0	0%	0	0%	0	0%
8	I	2	1.85%	0	0%	0	0%	0	0%
16	R	1	0.92%	0	0%	0	0%	0	0%
32	R	0	0%	0	0%	0	0%	0	0%
64	R	6	5.55%	0	0%	1	0.92%	1	0.92%
	Total N=108	92		12		28		47	

C. albicans (45.83%), *C. tropicalis* (27.38%) and *C. parapsilosis* (16.67%) predominantly exhibited a minimum inhibitory concentration (MIC) value of 0.12 ug/mL, which is three dilutions away from its breakpoint of 1.0 ug/mL. Among the azole group, *Candida* species in this study showed greater susceptibility to fluconazole, as it required lower concentrations relative to its clinical breakpoint. Despite rising resistance rates of 13.22% for fluconazole and 8.95% for voriconazole, fluconazole remains the preferred azole due to its excellent bioavailability and more favorable safety profile. In contrast, voriconazole is being linked to a higher risk of adverse effects and significant drug–drug interactions. However, resistance to fluconazole can develop through prolonged exposure, drug efflux mechanisms, and genetic mutations. A previous study comparing fluconazole monotherapy with a combination of fluconazole and amphotericin B demonstrated that the combination therapy achieved a higher overall treatment success rate and a lower incidence of persistent bloodstream infection.[10]

For *C. glabrata*, in accordance with the updated CLSI guidelines, modifications to the fluconazole formulation in the VITEK 2 AST-YS08 card have led to the exclusion of fluconazole from routine testing.[16] Heteroresistance to fluconazole is frequently observed in *C. glabrata* and is associated with the upregulation of ABC-type drug transporters. This phenomenon can be detected and quantified using population analysis profiling, a method not routinely employed in clinical microbiology laboratories.[10] Additionally, voriconazole was not requested for reporting by clinicians. Consequently, susceptibility results for these azole antifungals are not included in this study, although both agents are known to have resistance issues with *C. glabrata*.[17] Meanwhile, MIC values and interpretations for echinocandins indicate higher susceptibility to micafungin (90%) compared to caspofungin (75%). Susceptibility to amphotericin B and flucytosine was also tested, and the epidemiological cutoff values (ECVs) for these agents suggest that *C. glabrata* does not currently harbor known antifungal resistance mechanisms against them.

Two *C. dubliniensis* were identified in this study, with similar susceptible MIC value of 0.5 ug/mL in both azole drug, while one (0.4%) was identified to be resistant to fluconazole with MIC value of 32 ug/mL based on EUCAST breakpoint of >2 ug/mL. *Candida krusei* (0.8%) identified is known to be intrinsically resistant (IR) to fluconazole and no breakpoint was reported. Most of the (79) susceptible *C. albicans*, (42) C. *tropicalis*, (22) *C. parapsilosis* and (8) *C. glabrata*, the MIC value recorded were 0.06 ug/mL for micafungin and 0.12 ug/mL for caspofungin. Micafungin shows more susceptibility because C. *albicans, C. tropicalis* and *C. parapsilosis* were four dilutions away from the CLSI breakpoint. While, in caspofungin, *C.albicans, C. tropicalis* and *C. glabrata* were only two dilutions away from the CLSI breakpoint. Seen in this is a slight increase in resistance particularly of C. albicans to both echinocardins in (8) patients (3%) with malignancy, CVD, and infection (1.1%, 1.1% and 1.5%, respectively). Amphotericin B predominantly exhibited a minimum inhibitory concentration (MIC) value of 0.5 ug/mL for the top 4 *Candida*. However, most *C. albicans* (27.62%), *C. tropicalis* (11.6%) and *C. glabrata* (4.42%) were two dilutions away from the CLSI ECV of 2 ug/mL, while *C. parapsilosis* (11.05%) was one dilution away from the ECV value of 1.0 ug/mL. So, increasing resistance was noted in the usage of Amphotericin B, especially in malignant patients infected with *C. albicans* (2.3%). The three identified rare species such as *C. dubliniensis* and *C. lusitaniae* were both susceptible to Amphotericin B with MIC range value of 0.12 ug/mL to 1.0 ug/mL. Flucytosine, a combination drug to increase effectiveness of other drugs was not suppressed from the analysis in this study to verify its effectiveness to treat severe systemic IC. Thus, it showed that the predominant susceptible MIC value was <1 ug/mL. Among these susceptible results were *C. albicans* (28.6%), *C. tropicalis* (14.7%), *C. parapsilosis* (6.4%), and *C. glabrata* (3%). Only 1.9% of *C. albicans* were identified with resistance to flucytosine especially in infection-related causes. Despite minimal clinical data, the breakpoint (>32 ug/mL) used was based on the previous CLSI M27-S3: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Third Informational Supplement, 2008. The results may outweigh the in vitro variability, and patients may benefit from this drug if proper dosing is monitored (LD_{50} = >15 gm/kg).

In addition to above findings, Appendices 6A-D show concordance with the MIC^{50}, MIC^{90} and percent of susceptible isolates of the top 4 *Candida* species according to 2022 Clinical and Laboratory Standards Institute (CLSI) Standards. Against all these top 4 *Candida* species, amphotericin B (93-100%) shows the highest susceptibility rate. *Candida* species growth is still inhibited by the lowest concentration of azole (fluconazole and voriconazole), but the MIC value (MIC^{90}) is higher than the susceptible breakpoint. *Candida parapsilosis* has the lowest susceptibility rate of 11% in caspofungin and signals the need for alternative agents in patients with persistent infection[10] due to increased intermediate growth (89%).

Moreover, Appendix 7 provides a summary of the antifungal drug resistance patterns observed among identified *Candida* species, highlighting variations in susceptibility across different antifungal agents. Candida albicans, the most prevalent species, demonstrated high susceptibility rates to antifungal drugs such as amphotericin B (30.8%), caspofungin (29.7%), and micafungin (29.7%). However, a small subset of *C. albicans* isolates exhibited resistance, notably to fluconazole (5.6%) and voriconazole (4.9%).

Candida parapsilosis, the second most common species, showed similar susceptibility patterns, with high susceptibility to amphotericin B and caspofungin but lower rates of resistance compared to *C. albicans*. Conversely, *C. glabrata* and *C. tropicalis* exhibited more concerning trends, with reduced susceptibility and occasional resistance across multiple drug classes. Notably, *C. glabrata* displayed resistance to caspofungin and fluconazole, drugs commonly used in clinical settings. Meanwhile, rare species like *C. krusei* demonstrated intrinsic resistance to fluconazole, underscoring the importance of species-level identification and tailored antifungal therapy.

Interestingly in this study, 2.63% of patients with invasive candidiasis particularly due to *C. tropicalis* (2 patients) and *C. albicans* (5 patients) were identified to be resistant to all these six antifungals. Other *Candida* species comprising

Table 2. Summary statistics of the differences in MIC of Antifungal drugs according to risk factors

Variables	Value	*p*-value
Fluconazole Risk Factor	110.93	0.005
Voriconazole Risk factor	133.62	<0.001
Caspofungin Risk factor	179.48	<0.001
Micafungin Risk factor	185.32	0.006
Amphotericin B Risk factor	176.68	<0.001
Flucytosine Risk factor	534.31	0.164

0.4% each were also identified using the VITEK 2 identification cards. These species were *C. hemolunii*, *C. lipolytica*, *C. auris*, *C. viswanath*, and *C. famanata*, however, MIC was not identified due to no reportable AST database.

Table 2 presents the statistical analysis of the differences in minimum inhibitory concentrations (MICs) of antifungal drugs according to risk factors. The results indicate significant variability in antifungal susceptibility based on patient-related factors, as shown by the p-values for fluconazole ($p = 0.005$), voriconazole ($p < 0.001$), caspofungin ($p < 0.001$), micafungin ($p = 0.006$), and amphotericin B ($p < 0.001$). These findings suggest that antifungal efficacy is influenced by specific risk factors such as underlying metabolic conditions, malignancy, and chronic kidney disease (CKD). Notably, flucytosine ($p = 0.164$) did not show statistical significance, indicating that its MIC distribution may be less affected by these clinical conditions.

These results underscore the importance of individualized antifungal treatment strategies, particularly for patients with malignancies, chronic kidney disease (CKD), and metabolic disorders, as these underlying conditions may influence antifungal susceptibility. The statistical significance observed in azole and echinocandin antifungals, especially fluconazole and caspofungin, highlights the potential for resistance development in high-risk populations. These findings highlight the critical importance of routine antifungal susceptibility testing (AFST) and tailored treatment strategies to enhance patient outcomes and curb the development of drug-resistant *Candida* infections.

DISCUSSION

The findings of this study provide significant insights into the distribution of *Candida* species, their demographic associations, and antifungal susceptibility patterns. *Candida albicans* emerged as the predominant species across all groups in this study, consistent with the meta-analysis by Yamin et al., on bloodstream infections in Southeast Asia.[18] *Candida parapsilosis* was the second most common species, notably associated with bloodstream infections and showing increased intermediate susceptibility to caspofungin. Similarly, Franconi et al., identified *C. parapsilosis* as the second leading cause of candidemia in regions including Europe, Latin America, South Africa, and Asia, while noting that echinocandin resistance remains less frequent than azole resistance. The differing susceptibility pattern observed in the present study underscores the need for molecular investigations of mutations linked to echinocandin tolerance.[19] In a trial comparing standard-dose and high-dose caspofungin regimens, the response rate for *C. parapsilosis* was lower in the standard-dose group (61%), though the difference was not statistically significant. Despite this, echinocandins remain the preferred treatment for *C. parapsilosis* infections. However, alternative agents should be considered in cases of persistent infection despite echinocandin therapy.[10]

The observed contrasting prevalence patterns underscore the need for continuous surveillance to accurately monitor the evolving burden of invasive candidiasis. A study by Yamin et al. reported prevalence rates of *C. albicans* (28.4%), *C. tropicalis* (29.2%), *C. parapsilosis* (19.1%), and *C. glabrata* (14.0%). Additionally, a multicenter study across six Southeast Asian countries identified *C. tropicalis* as the most prevalent species in hematology-oncology wards and tropical regions, likely influenced by variations in healthcare infrastructure, patient demographics, and associated risk factors.[1] These findings align with the observations of Cortes and Corrales, highlighting a global shift in *Candida* species distribution.

The detection of less common species like *C. krusei* and *C. glabrata*, though infrequent, highlights the diversity of *Candida* infections and the importance of accurate species identification for effective treatment.

Also, observed in this study, most direct wound infections occurred in outpatients, posed minimal health risks, and responded well to antifungal therapy. In contrast, hospitalized patients with risk factors and prolonged stays (>35 days) were more likely to develop systemic candidiasis and exhibit antifungal resistance. This is further complicated by the ability of *Candida* species to form biofilms, considered one of their most pathogenic traits, which can lead to treatment failure and recurrent infections. Additionally, certain species can transition into a filamentous form that enhances tissue invasion; however, this morphological shift is influenced by both environmental conditions and the specific *Candida* species involved.[6] Similarly, *Candida* isolated from respiratory and urinary specimens should be interpreted with caution, as it may represent colonization rather than true infection. In critically ill patients, concurrent candidemia originating from these sites has also been observed. Therefore, antifungal therapy should be reserved for immunocompromised patients[4,15] with clinical signs of septic shock and no alternative etiology.[20]

Similarly, Soriano et al., emphasized the importance of prompt antifungal therapy and the use of tools like MIC values for tailoring treatment strategies. Despite advances in diagnostic techniques, invasive candidiasis continues to pose a significant burden, particularly due to delayed diagnosis and the limited sensitivity of culture-based methods.

Notable resistance to azole-based treatments, including fluconazole and voriconazole, was observed. These azoles showed effectiveness but not of the lowest dose, thus,

warrant limited access for empirical therapy and somehow, becomes a challenge to patient management because this is a recommendable orally administered antifungal drug. Similar to other studies, antifungal susceptibility analysis revealed high overall effectiveness of drugs like amphotericin B and micafungin, particularly against *C. albicans* and *C. parapsilosis*. This high susceptibility shows increased chance of therapeutic concentration and effective clinical outcome. However, as amphotericin B in this study shows that MIC value (dilution away) is already near to its breakpoint. It will be for further investigation and for new treatment guidelines especially it is being recommended by Infectious Diseases Society of America (IDSA) as an alternative drug if azole or echinocandins fail to treat invasive candidiasis. Thus, this evolving resistance highlights the growing challenge of antifungal therapy, necessitating routine susceptibility testing and tailored treatment strategies like dosage adjustment based on therapeutic drug monitoring (TDM) could be needed when dealing with *Candida* strains having higher MICs near to the clinical breakpoint.[21] Furthermore, the observed variability in MICs based on risk factors underscores the complex interplay of host characteristics and fungal resistance. These findings advocate for an individualized, species-specific approach to antifungal therapy, integrating demographic and clinical factors to optimize outcomes in *Candida* infections.

CONCLUSION

This study highlights the significant prevalence of *Candida* infections and the variability in antifungal resistance patterns across different species. *Candida albicans* remains the most predominant species; however, the increasing prevalence of non-*albicans* species such as *C. parapsilosis, C. tropicalis* and *C. glabrata* underscores the need for vigilant surveillance and species-specific diagnostic approaches. The observed resistance to commonly used antifungal agents, particularly azoles like fluconazole and voriconazole, emphasizes the growing challenge of antifungal resistance, necessitating routine antifungal susceptibility testing (AFST) for effective treatment planning. Echinocandins especially micafungin is still the recommendable safest drug that shows high effectiveness against invasive candidiasis. Clinicians should consider this as their first choice of empiric treatment if needed, especially in areas where susceptibility testing is not available. Moreover, further research on amphotericin B MIC shifts is recommended due to its emerging resistance.

The findings also underscore the importance of considering patient-related factors, such as age, sex, and underlying conditions, in the management of *Candida* infections and may warrant periodic surveillance on these demographics to improve species-specific therapy. Minimum inhibitory concentration (MIC) values proved crucial in identifying resistance patterns and guiding appropriate antifungal therapy. To combat the rising threat of drug resistance, clinicians must adopt individualized, evidence-based strategies while integrating regional and local data on species distribution and antifungal susceptibility. Furthermore, the importance of knowing the MICs with different antifungal agents is to guide the infectious disease specialist, clinical pharmacologists, and pharmacists for a collaborative approach in computing for the pharmacokinetics/ pharmacodynamics (PK/PD) analysis to adjust the dosage of the antifungal drugs.

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APPENDICES

Appendix 1. Summary distribution of the proportion of *Candida* species according to age

Candida Species	Age (years)			Total (N = 266)
	0 – 18	19 – 59	≥60	
Candida albicans	1 (0.4%)	37 (13.9%)	71 (26.7%)	109 (41.0%)
Candida parapsilosis	2 (0.8%)	27 (10.2%)	33 (12.4%)	62 (23.3%)
Candida glabrata	1 (0.4%)	1 (0.4%)	18 (6.8%)	20 (7.5%)
Candida tropicalis	1 (0.4%)	15 (5.6%)	39 (14.7%)	55 (20.7%)
Candida krusei	0 (0.0%)	1 (0.4%)	2 (0.8%)	3 (1.1%)
Candida lipolytica	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)
Candida famata	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)
Candida lusitaniae	0 (0.0%)	2 (0.8%)	3 (1.1%)	5 (1.9%)
Candida orthopsilosis	0 (0.0%)	0 (0.0%)	5 (1.9%)	5 (1.9%)
Candida auris	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)
Candida dubliniensis	0 (0.0%)	1 (0.4%)	2 (0.8%)	3 (1.1%)
Candida duobushaemulonis	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.4%)

Appendix 2. Summary distribution of the proportion of *Candida* species according to sex

Candida species	Sex		Total (N = 266)
	Male	Female	
Candida albicans	52 (19.5%)	57 (21.7%)	109 (41.0%)
Candida parapsilosis	35 (13.2%)	27 (10.2%)	62 (23.3%)
Candida glabrata	14 (5.3%)	6 (2.3%)	20 (7.5%)
Candida tropicalis	19 (7.1%)	36 (13.5%)	55 (20.7%)
Candida krusei	2 (0.8%)	1 (0.4%)	3 (1.1%)
Candida lipolytica	1 (0.4%)	0 (0.0%)	1 (0.4%)
Candida famata	1 (0.4%)	0 (0.0%)	1 (0.4%)
Candida lusitaniae	3 (1.1%)	2 (0.8%)	5 (1.9%)
Candida orthopsilosis	4 (1.5%)	1 (0.4%)	5 (1.9%)
Candida auris	1 (0.4%)	0 (0.0%)	1 (0.4%)
Candida dubliniensis	1 (0.4%)	2 (0.8%)	3 (1.1%)
Candida duobushaemulonis	1 (0.4%)	0 (0.0%)	1 (0.4%)

Appendix 3. Summary distribution of the proportion of *Candida* species according to source of sample

Candida Species	Source of Sample								Total (N = 266)
	Abscess	Blood	Body Fluids	Catheter	CSF	Respiratory Specimen	Tissue	Urine	
Candida albicans	2 (0.8%)	24 (9.0%)	7 (2.7%)	0 (0.0%)	27 (10.2%)	44 (16.5%)	0 (0.0%)	3 (1.1%)	109 (41.0%)
Candida parapsilosis	0 (0.0%)	50 (18.8%)	3 (1.1%)	3 (1.1%)	2 (0.8%)	3 (1.1%)	1 (0.4%)	0 (0.0%)	62 (23.3%)
Candida glabrata	0 (0.0%)	11 (4.1%)	1 (0.4%)	0 (0.0%)	6 (2.3%)	1 (0.4%)	0 (0.0%)	1 (0.4%)	20 (7.5%)
Candida tropicalis	1 (0.4%)	10 (3.8%)	2 (0.8%)	1 (0.4%)	23 (8.6%)	18 (6.8%)	0 (0.0%)	0 (0.0%)	55 (20.7%)
Candida krusei	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.1%)
Candida lipolytica	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.4%)
Candida famata	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)
Candida lusitaniae	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (1.5%)	0 (0.0%)	0 (0.0%)	5 (1.9%)
Candida orthopsilosis	0 (0.0%)	5 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (1.9%)
Candida auris	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)
Candida dubliniensis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	3 (1.1%)
Candida duobushaemulonis	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)

Appendix 4. Summary distribution of the proportion of *Candida* species according to risk factor

Candida Species	Risk Factors											Total (N = 266)
	Age	Metabolic	Malignancy	CKD	Infection	CVD	Diabetes	Surgical Procedure	Asthma	Anemia	Auto-immune	
Candida albicans	0 (0.0)	3 (1.1)	24 (9.0)	5 (1.9)	37 (13.9)	18 (6.8)	9 (3.4)	5 (1.9)	2 (0.8)	0 (0.0)	6 (2.3)	109 (41.0)
Candida parapsilosis	7 (2.6)	1 (0.4)	16 (6.0)	1 (0.4)	19 (7.1)	12 (4.5)	3 (1.1)	2 (0.8)	1 (0.4)	0 (0.0)	0 (0.0)	62 (23.3)
Candida glabrata	0 (0.0)	0 (0.0)	5 (1.9)	3 (1.1)	9 (3.4)	1 (0.4)	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (7.5)
Candida tropicalis	0 (0.0)	3 (1.1)	12 (4.5)	5 (1.9)	15 (5.6)	10 (3.8)	6 (2.3)	3 (1.1)	1 (0.4)	0 (0.0)	0 (0.0)	55 (20.7)
Candida krusei	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)
Candida lipolytica	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Candida famata	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Candida lusitaniae	0 (0.0)	0 (0.0)	2 (0.8)	0 (0.0)	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	5 (1.9)
Candida orthopsilosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)	0 (0.0)	0 (0.0)	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	5 (1.9)
Candida auris	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Candida dubliniensis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.8)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)
Candida duobushaemulonis	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)

Appendix 5. Summary statistics of the antifungal drug resistance pattern according to risk factors

Anti-Fungal MIC Reading	Risk Factors												
	Age	Metabolic	Malignancy	CKD	Infection	CVD	Diabetes	Surgical Procedure	Asthma	Anemia	Auto-immune	X^2	*p*-value
Fluconazole													
Susceptible	0 (0.0%)	0 (0.0%)	31 (11.7%)	9 (3.4%)	36 (13.5%)	29 (10.9%)	17 (6.4%)	6 (2.3%)	2 (0.8%)	0 (0.0%)	5 (1.9%)		
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)		
Resistant	0 (0.0%)	0 (0.0%)	4 (1.5%)	1 (0.4%)	8 (3.0%)	7 (2.6%)	1 (0.4%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	73.354	<0.001
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
None	7 (2.6%)	7 (2.6%)	25 (9.4%)	4 (1.5%)	44 (16.5%)	7 (2.6%)	3 (1.1%)	4 (1.5%)	1 (0.4%)	1 (0.4%)	1 (0.4%)		
Voriconazole													
Susceptible	0 (0.0%)	0 (0.0%)	32 (12.0%)	11 (4.1%)	38 (14.3%)	31 (11.7%)	15 (5.6%)	6 (2.3%)	3 (1.1%)	0 (0.0%)	5 (1.9%)		
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Resistant	0 (0.0%)	0 (0.0%)	6 (2.3%)	0 (0.0%)	5 (1.9%)	1 (0.4%)	0 (0.0%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	70.05	0.002
Intermediate	0 (0.0%)	0 (0.0%)	2 (0.8%)	0 (0.0%)	1 (0.4%)	3 (1.1%)	2 (0.8%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
None	7 (2.6%)	7 (2.6%)	22 (8.3%)	3 (1.1%)	44 (16.5%)	7 (2.6%)	3 (1.1%)	4 (1.5%)	1 (0.4%)	1 (0.4%)	1 (0.4%)		
Caspofungin													
Susceptible	0 (0.0%)	0 (0.0%)	37 (13.9%)	12 (4.5%)	41 (15.4%)	34 (12.8%)	17 (6.4%)	8 (3.0%)	3 (1.1%)	0 (0.0%)	5 (1.9%)		
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Resistant	0 (0.0%)	0 (0.0%)	4 (1.5%)	0 (0.0%)	3 (1.1%)	3 (1.1%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	61.55	<0.001
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
None	7 (2.6%)	7 (2.6%)	21 (7.9%)	2 (0.8%)	43 (16.2%)	6 (2.3%)	2 (0.8%)	4 (1.5%)	1 (0.4%)	1 (0.4%)	1 (0.4%)		
Micafungin													
Susceptible	0 (0.0%)	0 (0.0%)	38 (14.3%)	13 (4.9%)	42 (15.8%)	33 (12.4%)	18 (6.8%)	8 (3.0%)	3 (1.1%)	0 (0.0%)	5 (1.9%)		
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Resistant	0 (0.0%)	0 (0.0%)	3 (1.1%)	0 (0.0%)	4 (1.5%)	4 (1.5%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	58.01	<0.001
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
None	7 (2.6%)	7 (2.6%)	21 (7.9%)	1 (0.4%)	42 (15.8%)	6 (2.3%)	2 (0.8%)	2 (0.8%)	1 (0.4%)	1 (0.4%)	1 (0.4%)		
Amphotericin b													
Susceptible	0 (0.0%)	0 (0.0%)	41 (15.4%)	13 (4.9%)	44 (16.5%)	35 (13.2%)	19 (7.1%)	8 (3.0%)	3 (1.1%)	0 (0.0%)	5 (1.9%)		
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Resistant	0 (0.0%)	0 (0.0%)	5 (1.9%)	0 (0.0%)	2 (0.8%)	2 (0.8%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	64.33	<0.001
Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
None	7 (2.6%)	7 (2.6%)	16 (6.0%)	1 (0.4%)	42 (15.8%)	6 (2.3%)	2 (0.8%)	4 (1.5%)	1 (0.4%)	1 (0.4%)	1 (0.4%)		
Flucytosine													
Susceptible	0 (0.0%)	0 (0.0%)	40 (15.0%)	11 (4.1%)	37 (13.9%)	32 (12.0%)	16 (6.0%)	8 (3.0%)	3 (1.1%)	0 (0.0%)	4 (1.5%)		
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Resistant	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)	4 (1.5%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	57.58	0.002
Intermediate	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
None	7 (2.6%)	7 (2.6%)	20 (7.5%)	2 (0.8%)	47 (17.7%)	9 (3.4%)	5 (19%)	4 (1.5%)	1 (0.4%)	1 (0.4%)	2 (0.8%)		

Appendix 6A. *Candida albicans*

Antifungal	Breakpoint (ug/mL)	MIC Range (ug/mL)	MIC^{50} (ug/mL)	MIC^{90} (ug/mL)	% Susceptible
Fluconazole	8	<0.5->64	0.5	8	83
Voriconazole	1	<0.008->8	0.12	1	84
Caspofungin	1	<0.008->8	0.12	0.25	88
Micafungin	1	<0.008->8	0.06	0.5	89
Amphotericin B	2 (ECV)	<0.12->16	0.5	1	93
Flucytosine	>32	<0.006->64	<1	4	90

MIC=minimum inhibitory concentration, ug/mL; MIC^{50} = MIC value at which growth was inhibited in 50% of isolates; MIC^{90} = MIC values at which growth was inhibited in 90% of isolates; According to CLSI M27 guidelines.

Appendix 6B. *Candida glabrata*

Antifungal	Breakpoint (ug/mL)	MIC Range (ug/mL)	MIC^{50} (ug/mL)	MIC^{90} (ug/mL)	%Susceptible
Fluconazole	Not reported	<0.5->64	Not reported	Not reported	Not reported
Voriconazole	Not reported	<0.008->8	Not reported	Not reported	Not reported
Caspofungin	0.5	<0.008->8	0.12	0.25	75
Micafungin	0.25	<0.008->8	0.06	0.06	90
Amphotericin B	2 (ECV)	<0.12->16	0.5	0.5	100
Flucytosine	>32	<0.006->64	<1	<1	100

MIC = minimum inhibitory concentration, ug/mL; MIC^{50} = MIC value at which growth was inhibited in 50% of isolates; MIC^{90} = MIC values at which growth was inhibited in 90% of isolates; according to CLSI M27 guidelines.

Appendix 6C. *Candida parapsilosis*

Antifungal	Breakpoint (ug/mL)	MIC Range (ug/mL)	MIC^{50} (ug/mL)	MIC^{90} (ug/mL)	%Susceptible
Fluconazole	8	<0.5->64	0.5	2	96
Voriconazole	1	<0.008->8	0.12	0.12	96
Caspofungin	8	<0.008->8	0.25	0.5	11
Micafungin	8	<0.008->8	0.5	1	96
Amphotericin B	1 (ECV)	<0.12->16	0.5	0.5	96
Flucytosine	>32	<0.006->64	<1	<1	96

MIC = minimum inhibitory concentration, ug/mL; MIC^{50} = MIC value at which growth was inhibited in 50% of isolates; MIC^{90} = MIC values at which growth was inhibited in 90% of isolates; according to CLSI M27 guidelines. **Caspofungin- 89% intermediate to Candida parapsilosis

Appendix 6D. *Candida tropicalis*

Antifungal	Breakpoint (ug/mL)	MIC Range (ug/mL)	MIC^{50} (ug/mL)	MIC^{90} (ug/mL)	%Susceptible
Fluconazole	8	<0.5->64	1	2	95
Voriconazole	0.12	<0.008->8	0.12	0.12	98
Caspofungin	1	<0.008->8	0.12	0.12	96
Micafungin	0.25	<0.008->8	0.06	0.12	94
Amphotericin B	2 (ECV)	<0.12->16	0.25	0.5	98
Flucytosine	>32	<0.006->64	<1	<1	98

MIC = minimum inhibitory concentration, ug/mL; MIC^{50} = MIC value at which growth was inhibited in 50% of isolates; MIC^{90} = MIC values at which growth was inhibited in 90% of isolates; according to CLSI M27 guidelines.

Appendix 7. Summary statistics of the antifungal drug resistance pattern of identified *Candida* species

	Fluconazole (mean ± SD)	Voriconazole (mean ± SD)	Caspofungin (mean ± SD)	Micafungin (mean ± SD)	Amphotericin B (mean ± SD)	Flucocystine (mean ± SD)
Candida albicans	2.78 ± 6.85	0.67 ± 1.83	0.79 ± 2.49	0.58 ± 2.09	0.95 ± 2.41	4.64 ± 15.0
Susceptible	69 (25.9%)	67 (25.2%)	79 (29.7%)	79 (29.7%)	82 (30.8%)	76 (28.6%)
Susceptible dose dependent	3 (1.1%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	15 (5.6%)	13 (4.9%)	8 (3.0%)	8 (3.0%)	6 (2.3%)	5 (1.9%)
Intermediate/susceptible increase exposure	0 (0.0%)	6 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.1%)
None	22 (8.3%)	21 (7.9%)	22 (8.3%)	22 (8.3%)	21 (7.9%)	25 (9.4%)
Candida parapsilosis	0.43 ± 1.11	0.47 ± 0.62	0.11 ± 0.16	0.31 ± 1.03	0.22 ± 0.54	1.30 ± 8.11
Susceptible	22 (8.3%)	67 (25.2%)	79 (29.7%)	79 (29.7%)	82 (30.8%)	76 (28.6%)
Susceptible dose dependent	0 (0.0%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	1 (0.4%)	13 (4.9%)	8 (3.0%)	8 (3.0%)	6 (2.3%)	5 (1.9%)
Intermediate/susceptible increase exposure	0 (0.0%)	6 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.1%)
None	39 (14.7%)	21 (7.9%)	22 (8.3%)	22 (8.3%)	21 (7.9%)	25 (9.4%)
Candida glabrata	0.80 ± 3.57	0.50 ± 0.15	0.05 ± 0.07	0.13 ± 0.44	0.28 ± 0.33	0.35 ± 0.45
Susceptible	0 (0.0%)	1 (0.4%)	6 (2.3%)	8 (3.0%)	10 (3.8%)	8 (3.0%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	1 (0.4%)	0 (0.0%)	1 (0.4%)	2 (0.8%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	19 (7.1%)	18 (6.8%)	12 (4.5%)	10 (3.8%)	10 (3.8%)	12 (4.5%)
Candida tropicalis	2.12 ± 5.11	0.17 ± 0.53	0.38 ± 1.49	0.26 ± 1.18	0.57 ± 2.13	1.79 ± 8.55
Susceptible	40 (15.0%)	44 (16.5%)	43 (16.2%)	43 (16.2%)	44 (16.5%)	39 (14.7%)
Susceptible dose dependent	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	4 (1.5%)	1 (0.4%)	2 (0.8%)	2 (0.8%)	1 (0.4%)	1 (0.4%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	10 (3.8%)	10 (3.8%)	10 (3.8%)	10 (3.8%)	10 (3.8%)	15 (5.6%)
Candida krusei	0.00 ± 0.00	0.08 ± 0.69	0.25 ± 0.25	0.08 ± 0.07	1.00 ± 1.00	23.3 ± 40.4
Susceptible	0 (0.0%)	44 (16.5%)	43 (16.2%)	43 (16.2%)	1 (0.4%)	1 (0.4%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)
Resistant	0 (0.0%)	1 (0.4%)	2 (0.8%)	2 (0.8%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	3 (1.1%)	10 (3.8%)	10 (3.8%)	10 (3.8%)	0 (0.0%)	2 (0.8%)
Candida lipolytica	8.00 ± 0.00	0.25 ± 0.00	0.12 ± 0.00	0.06 ± 0.00	0.25 ± 0.00	0.99 ± 0.00
Susceptible	0 (0.0%)	0 (0.0%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Candida famata	0.50 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.06 ± 0.00	0.50 ± 0.00	0.48 ± 0.00
Susceptible	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Candida lusitaniae	0.20 ± 0.45	0.24 ± 0.54	1.60 ± 3.58	0.01 ± 0.03	0.45 ± 0.37	0.80 ± 0.45
Susceptible	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	1 (0.4%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (1.5%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (1.5%)	1 (0.4%)	4 (1.5%)
None	4 (1.5%)	4 (1.5%)	4 (1.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Candida orthopsilosis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	5 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	5 (1.9%)	5 (1.9%)	0 (0.0%)	5 (1.9%)	5 (1.9%)	5 (1.9%)
Candida auris	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)
Candida dubliniensis	11.17 ± 18.04	0.08 ± 0.06	0.08 ± 0.06	0.04 ± 0.03	0.46 ± 0.48	0.65 ± 0.51
Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)
Candida duobushaemulonii	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00
Susceptible	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)
Susceptible dose dependent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.4%)	0 (0.0%)
Intermediate/susceptible increase exposure	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
None	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	0 (0.0%)	0 (0.0%)

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Mismatch Repair (MMR) Status among Colorectal Cancer Patients in a Philippine Tertiary Hospital: A 4-Year Review

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ABSTRACT

Background. Approximately 15% of colorectal cancers exhibit deficient mismatch repair (dMMR) status, and these cases have a better prognosis and are less prone to metastasis. Moreover, dMMR is associated with an improved response to immune checkpoint inhibitors. Currently, local data on the MMR status of colorectal cancer patients remains scant.

Objective. The proponents aimed to determine the MMR status among colorectal cancer patients in a Philippine tertiary hospital.

Methodology. This is a descriptive cross-sectional study that included 42 patients with colorectal cancer seen at the Chinese General Hospital and Medical Center (CGHMC) from January 2021 to June 2024. Data was collected via retrospective review of histopathologic reports.

Results. Forty-two (42) patients were included in the study. The mean age of included patients was 61.8 years, and most were males. Half had well-differentiated tumor grade, and the most common tumor locations were rectum (38%) and sigmoid (36%). Three patients (7.14%; 95% CI:1.50-19.48%) were considered deficient. Tumor locations in dMMR patients were the cecum, descending colon, and rectum. Compared to MMR-proficient, dMMR patients had a lower mean age (63.1 vs. 45.7 years). Also, a higher proportion of males (13%) were dMMR than females (0%).

Conclusion. dMMR is uncommon among the colorectal cancer cases in this study, and was only seen at the cecum, descending colon, and rectum. Descriptive analysis revealed that patients with dMMR were younger than MMR-proficient patients. Moreover, a higher proportion of males were dMMR than females. Larger, multicenter studies are warranted to validate these preliminary findings and guide future clinical decision-making.

Key words: biomarkers, colorectal neoplasms, dMMR, DNA mismatch repair

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INTRODUCTION

According to the World Health Organization (WHO), colorectal cancer is the fourth leading cause of cancer-related death worldwide and accounts for 608,000 deaths, mainly affecting individuals over 50 years of age. There has been increasing incidence reported in young adults in Australia, Canada, and the USA. In the Philippines, colorectal cancer ranks fourth among the cancer-related deaths of Filipinos. According to the Philippine Cancer Society, Inc., almost 75 percent of the individuals affected were aged 50 and above, while only about three percent were children aged 14 years and below.[1]

Approximately 15% of colorectal cancers exhibit deficient DNA mismatch repair (dMMR), resulting in microsatellite instability.[2] These tumors are hypermutated, with an abundance of mutation-derived neoantigens that trigger a robust immune response within the tumor microenvironment. Phenotypically, dMMR colorectal cancers are characterized by a right-sided predominance, a tendency for poor differentiation, and a better prognosis in the absence of distant metastasis.[3,4] However, a worse prognosis was observed in dMMR patients with advanced stages of colorectal cancer, including those with metastasis. dMMR occurs in a small subset of advanced colorectal

cancers, leading to a high mutational burden, and the resulting neoantigens are recognized by the patient's immune system.[5] Furthermore, dMMR colorectal cancers are characterized by prominent lymphocyte infiltration, indicating an active immune response.

At the Chinese General Hospital and Medical Center (CGHMC), the chosen study setting, more than 100 colorectal cancer patients are seen annually. In 2021, mismatch repair (MMR) testing became available at CGHMC, which opened more treatment modalities for cancer patients. Currently, in the Philippines, there is limited data on the MMR status of colorectal cancer patients. One such study that touched on this topic is by Sacdalan et al., in which they showed that 12% of young-onset colorectal cancer patients are dMMR. This study, therefore, aims to determine the MMR status among colorectal cancer patients seen in a Philippine tertiary hospital from January 2021 to June 2024. Specifically, the study aims to determine the prevalence of colorectal cancer patients with dMMR. Moreover, the study aims to describe the MMR status of colorectal cancer patients by demographics, histologic grade, and tumor location.

Determining the MMR status in colorectal cancer will have significant implications for patients, clinicians, hospitals, and researchers. For patients, this information can guide treatment selection, as dMMR are more likely to respond to immune checkpoint inhibitors. Clinicians will benefit from this knowledge to make more informed treatment decisions and individual patient characteristics. Hospitals will need to establish robust testing and analysis pipelines to routinely assess MMR status in colorectal cancer patients, which can improve patient stratification and guide the use of targeted therapies. Researchers may continue to investigate the complex interplay between MMR, tumor mutational burden, and the immune landscape in colorectal cancer, to develop more effective and personalized treatment strategies.

METHODOLOGY

This is a descriptive cross-sectional study which included all colorectal cancer patients with available data on MMR status seen in CGHMC from January 2021 to June 2024. CGHMC is a tertiary private hospital located in Manila City, Philippines. In CGHMC, MMR immunohistochemistry involved the use of 4 μm formalin-fixed, paraffin-embedded tissue sections that were stained for MMR proteins using the OptiView DAB IHC Detection Kit (Roche Diagnostics, Basel, Switzerland) on a Ventana Benchmark ULTRA automated stainer (Tissue Diagnostics, ADRIAMED Ltd., Skopje, Macedonia). The slides were counterstained with hematoxylin II and a bluing reagent. The following monoclonal antibodies (Ventana Medical Systems, Tucson, AZ, USA) were used as part of the Ventana MMR IHC Panel: MLH1 (clone M1), PMS2 (clone A16-4), MSH2 (clone G219-1129), and MSH6 (clone SP93). The internal positive controls which include non-neoplastic epithelial cells, stromal cells, and lymphocytes, were evaluated for each slide. In contrast, the external positive controls consisted of normal colonic mucosa with intact nuclear expression of all four MMR proteins. The interpretation criteria were as follows: Intact expression: unequivocal nuclear staining in viable tumor cells, in the presence of internal positive controls (nuclear staining in lymphocytes, fibroblasts, or normal colonic epithelium in the vicinity of the tumor; loss of expression – unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls. If an unequivocal nuclear stain is absent in internal positive controls and/or background staining interferes with interpretation, the test should be considered unacceptable and repeated.

The researchers included patients of all age groups, admitted as either pay or charity cases, with histopathologically-confirmed diagnosis of colorectal adenocarcinoma, and underwent biopsy and/or colorectal surgery for tumor excision, with specimens submitted to the CGHMC Institute of Pathology for MMR immunohistochemistry. Excluded were those with unavailable results for MMR status.

OpenEpi sample size calculator was used to calculate the minimum sample size requirement. A minimum of 37 patients is required, given a prevalence of dMMR of 21%,[6] a maximum tolerable error of 5%, an alpha level of 5%, and a finite population correction of 43%. Although a minimum sample size calculation was presented, the researcher opted to conduct a total enumeration, wherein all eligible cases would be included in the study. The list of colorectal cancer patients with specimens submitted for immunohistochemistry was obtained from the CGHMC Section of Histopathology.

The researcher performed data gathering from October 1 to 31, 2024. The following data were obtained from the histopathologic reports and recorded in a standardized data collection form: age, sex, tumor location, tumor histologic grade, MMR status. Data obtained from MMR results indicated intact or absent nuclear expressions for MLH1, PMS2, MSH2, and MSH6.

Data was encoded in MS Excel by the researcher. Stata MP version 17 software was used for data processing and analysis. Continuous variable (i.e., age) was presented as mean (standard deviation/SD) due to the normal data distribution based on the Shapiro-Wilk test. Categorical variables were expressed as frequencies and percentages. Missing data was neither replaced nor estimated.

Ethics approval was granted by the CGHMC Research Ethics Review Board (CGHMC RERB Protocol No. 2024-R-80).

RESULTS

A total of 42 patients with colorectal cancer were included in the study. Table 1 presents the characteristics of patients. The mean age was 61.9 years (range, 29-88 years), with the majority being males. Half had well-differentiated tumor grade, and the most common tumor locations were rectum (38%) and sigmoid (36%). Two patients had multiple tumor locations. One had tumors in both the transverse and descending colon, and the other patient had tumors in the descending colon and rectum.

Of the 42 patients with data on MMR status, 3 had dMMR, corresponding to a prevalence of 7.14 (95% CI: 1.50-19.48). Table 2 shows the MMR status by tumor location. Of the three patients with dMMR, tumors were in the cecum, descending colon, and rectum. Only one patient

Table 1. Characteristics of colorectal cancer patients (n = 42)

Characteristics	n (%); Mean ± SD
Age (in years), mean	61.8 ± 12.5
Sex	
Female	18 (43)
Male	24 (57)
Histologic grade [n=18]	
Poorly differentiated	1 (6)
Moderately differentiated	8 (44)
Well differentiated	9 (50)
Tumor location	
Cecum	1 (2)
Ascending colon	1 (2)
Transverse colon	3 (7)
Descending colon	6 (14)
Sigmoid	15 (36)
Rectum	14 (38)
Multiple location	2 (4)

Table 2. MMR status by tumor location among colorectal cancer patients (n=42) [a]

Tumor location	N	dMMR, n (%)	MMR-proficient, n (%)
Cecum	1	1 (100)	0
Ascending colon	1	0	1 (100)
Transverse colon	4	0	4 (100)
Descending colon	8	1 (13)	7 (87)
Sigmoid	15	0	15 (100)
Rectum	15	1 (7)	14 (93)

[a] Two patients had tumor in two locations; dMMR: deficient mismatch repair; MMR: mismatch repair; % presented are row percentages

Table 3. MMR status by age, sex, and histologic grade (n=42)

	N	dMMR n (%); Mean ± SD	MMR-proficient n (%); Mean ± SD
Age (in years), mean	42	45.7 ± 17.0	63.1 ± 11.5
Sex			
Female	18	0 (0)	18 (100)
Male	24	3 (13)	21 (88)
Histologic grade [n=18]			
Poorly differentiated	1	1 (100)	0 (0)
Moderately differentiated	8	0 (0)	8 (100)
Well differentiated	9	0 (0)	9 (100)

dMMR: deficient mismatch repair; MMR: mismatch repair; % presented are row percentages

had a tumor located in the cecum, which was classified as dMMR. Meanwhile, dMMR was noted in 13% of tumors in the descending colon and 7% in the rectum. MMR status by age, sex, and histologic grade by were also explored (Table 3). The mean age of dMMR cases was lower than that of MMR-proficient cases. In terms of sex, none of the females were dMMR, compared to 13% of males. The lone patient with a poorly differentiated histologic grade was classified as dMMR. While all patients with well- and moderately differentiated histologic grades were MMR-proficient.

DISCUSSION

While MMR status is increasingly utilized in the management of colorectal cancer, most of the data available are derived from Western or East Asian populations. In the Philippines and other Southeast Asian countries, data on these biomarkers remain limited. This study aimed to determine the prevalence as well as clinicopathologic associations in a cohort of 42 Filipino colorectal cancer patients who were tested for MMR.

The dMMR rate of 7.14% in this study is lower than the rates reported in Western cohorts, where the prevalence ranges from 10% in early-stage colorectal cancer and to approximately 4-5% in metastatic cases.[7] In large, pooled studies, the overall prevalence is estimated at 11.7%.[8] This discrepancy may be attributed to the predominance of left-sided tumors in our sample and a potentially lower burden of hereditary syndromes such as Lynch syndrome in Filipinos. In clinical practice, the low rate of dMMR implies that immunotherapy based on this biomarker may apply only to a small population of Filipino colorectal cancer patients. This necessitates the use of broader biomarker panels, such as BRAF and KRAS, to identify additional candidates for immune checkpoint inhibitors.

Consistent with findings in the literature, the 3 dMMR in this study was identified in tumors located in the cecum, descending colon, and rectum. No cases were observed in the sigmoid, transverse, or ascending colon. This supports the established pattern that dMMR and MSI-H are more common in right-sided tumors due to unique molecular pathways involving hypermethylation and BRAF mutations.[9,10] The low prevalence of dMMR in our study may also be due to the small sample size for right-sided cases, since only two patients in the cohort have cecal or ascending tumors.

All three dMMR cases are seen among male patients, and dMMR patients were relatively younger than MMR-proficient cases (mean age 45.7 vs. 63.1 years). This is consistent with the data showing that dMMR is more common in early-onset colorectal cancer and that male sex may be associated with certain MSI-H subtypes.[11] The only poorly differentiated tumor in our cohort was also dMMR, which is consistent with studies that exhibit strong associations between MSI-H and poorly differentiated histology, mucinous features, and increased immune cell infiltration.[12–14] Although histologic data were limited, the trend supports the use of histomorphologic features as a basis for MMR testing in low-resource settings.

The study has some limitations. First, the characteristics of patients included in this study may differ from those presenting in other institutions, which limits the generalizability of the results. Moreover, not all colorectal cancer cases seen in our institution undergo MMR testing since this was based on the physician's discretion and the patient's decision. Due to the high cost of MMR testing, not all patients can afford this procedure, which may potentially introduce selection bias. Second, due to the low sample size, the prevalence of dMMR is imprecise as evidenced by the wide 95% CI. Third, due to the retrospective nature of this study, completeness of information cannot be ascertained, leading to information bias. In this study, not all patients had data on histologic grade, particularly those who underwent biopsy only. Fourth, right-sided tumors were underrepresented, possibly underestimating the true prevalence of dMMR.

Despite these limitations, the study represents one of the few Filipino colorectal cancer cohorts with biomarker data and lays the foundation for future multicenter studies with expanded molecular testing.

CONCLUSION

This study provides one of the first local analyses of MMR status in colorectal cancer patients in the Philippines. The local data shows that dMMR is relatively uncommon in this population, and with a predominance of left-sided tumors. This implies potential benefit from immunotherapy even in MMR-proficient cases. Tumor location and histologic grade are the key features that influence the expression of these biomarkers. These findings further highlight the need for broader molecular profiling and biomarker testing as part of routine care, especially in low- to middle-income settings where funds and treatment access may be limited. Larger, multicenter studies are warranted to validate these preliminary findings and guide future clinical decision-making.

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Both authors certified fulfillment of ICMJE authorship criteria.

DATA AVAILABILITY STATEMENT

No datasets were generated or analyzed for this study.

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OPEN ACCESS – REVIEW ARTICLE

Revolutionizing Pathology in the Philippines: Artificial Intelligence in Digital Image Analysis

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ABSTRACT

Artificial Intelligence (AI) is transforming the landscape of pathology, particularly in resource-constrained settings like the Philippines. This narrative review explores the applications, challenges and future potential of AI in digital image analysis for pathology practices. By synthesizing peer-reviewed literature from 2019 to 2025, the review highlights the role of machine learning (ML) and deep learning (DL) algorithms in enhancing diagnostic accuracy, workflow efficiency and clinical decision-making. AI-driven tools such as convolutional neural networks (CNNs) and transfer learning models have demonstrated significant success in tumor detection, biomarker evaluation and predictive analytics, paving the way for personalized medicine. However, barriers such as limited annotated datasets, privacy concerns and model interpretability hinder widespread adoption. The review emphasizes the need for ethical frameworks, workforce training and infrastructure development to ensure equitable and effective integration of AI into pathology practices. By addressing these challenges, AI has the potential to improve diagnostic precision, expand access to healthcare and modernize pathology services in the Philippines.

Key words: artificial intelligence, pathology, digital image analysis, Philippines, deep learning, machine learning, diagnostic assistance

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INTRODUCTION

The field of pathology research is changing rapidly because of artificial intelligence (AI), especially in terms of research approaches, diagnostic procedures and educational instruction. AI-enhanced computers can now do tasks that once required cumbersome manual methods; for example, they can recognize images, analyze data and speed up diagnostics in pathology and arguably surpass traditional methods in certain workflows.[1]

The COVID-19 pandemic has fast-tracked the development and use of digital pathology by offering the opportunity for remote, end-to-end digitization of histopathological workflows. The design of a new remote workflow illustrates the importance of AI in the delivery of clinical services to safeguard their continuity, resilience and to support the longer-term development of telepathology in the face of extraordinary circumstances.[1]

Machine learning (ML) and deep learning (DL), which are core components of AI, enable systems to improve their output through feedback mechanisms and iteration without the need for a rule-based system that is explicitly programmed. DL employs multilayered artificial neural networks to extract and distinguish complex, nonlinear, and/or high dimension data patterns. These methods have been extremely successful in histopathology in terms of extracting features from whole-slide images, detecting abnormalities, and automating aspects of image classification, allowing for consistent, rapid, reliable, and reproducible results that improve the sensitivity and specificity of diagnostic interpretations.[2]

AI algorithms have achieved impressive results in a wide range of clinical domains, including diagnosing ophthalmic

diseases, detecting oncogenic gene fusions in lung cancers, and identifying disease-specific biomarkers for precision medicine. Furthermore, one branch of AI known as computer vision allows for automated whole-slide image analysis, which significantly reduces turnaround time for pathology cases while also assisting pathologists with interpretative and diagnostic decision-making.[3]

Apprehensions by pathologists are expected but understandable and need to be addressed appropriately. To facilitate successful technology acceptance, it must be emphasized that pathologists will not be replaced through AI implementation, as its primary purpose is to enhance and not substitute for their professional functions.[4] The combination of artificial intelligence pattern recognition abilities and human professional knowledge, the so-called "human in the loop" strategy, results in improved diagnostic accuracy, especially during challenging or uncommon treatment scenarios.[5,6] This paradigm and the broader concept of "group intelligence" model, creates exciting opportunities for health care innovation.[7]

As the world of artificial intelligence develops, its acceptance and use in healthcare delivery and biomedical education and training are vital. Preparing the practitioners of the future with competency in the use of artificial intelligence tools will support the routine implementation of artificial intelligence in pathology practices.[8,9] Together, advances in artificial intelligence technologies and expert human judgment will shape the future of the pathology practice environment, enhancing diagnostic accuracy and ultimately, patient outcomes.

Although there has been a lot of progress in this area, the real-world use of artificial intelligence in pathology is still a major hurdle. The main worries are various factors, such as the performance differences of convolutional neural networks (CNNs) at different hospitals and among different patient groups, and the very existence of imaging protocols that are not standardized, as well as the risk of using flawed or non-inclusive training datasets, all of which are pointed out in recent studies regarding CNN-based classification of brain tumors.[10] Xie et al., concluded that numerous top-notch models depend on small, well-defined datasets and thus are prone to issues of non-generality, non-interpretation, and non-reproducibility when applied to real-world clinical settings. Ethical issues like openness, model interpretability, and accountability to the public make it even harder to lay down a process, while medical personnel's reluctance about relying on machines continues to be a roadblock to the embracing of automated systems. These difficulties bring into sharp focus the need for a hybrid, clinician-centered approach that effectively marries technology's latest breakthroughs with the everyday practicalities of clinical pathology.[10]

The principal aim of this manuscript is to explore the transformative role of artificial intelligence in pathology while critically exploring the barriers to widespread integration, in particular, foreshadowing how AI supports research, diagnosis and education in pathology, to discuss its role in the COVID-19 pandemic and to suggest best practice strategies for uptake including the use of a "human in the loop" approach. The manuscript aims to clarify the opportunities and constraints in this domain with the aim of informing practice, policy and future research in digital pathology.

METHODOLOGY

Literature search strategy

This narrative review compiled peer-reviewed literature on artificial intelligence (AI) in a digital image analysis seen in some pathology practices in the Philippines. A systematic electronic search was carried out using PubMed, Scopus, IEEE Xplore and Google Scholar, from January 2019 until August 2025. The search strategy consisted of combinations of keywords and Boolean operators, ("artificial intelligence" OR "machine learning" OR "deep learning" OR "digital pathology") AND ("Philippines" OR "Southeast Asia") AND ("image analysis" OR "histopathology"). Title/abstract title screening and full-text review were completed by two independent reviewers. These reviewers reconciled discrepancies by consensus or with a third reviewer. The reviewers limited their considerations to studies published in English that focused on studying diagnostic performance (e.g., sensitivity, specificity, accuracy) of AI models used in pathology.

Inclusion and exclusion criteria

Studies were included if they focused on the application of artificial intelligence (AI) in pathology, particularly in digital image analysis, clinical diagnostics, or machine learning (ML) methods for histopathology. Eligible studies emphasize diagnostic accuracy, workflow efficiency and/or contributions to personalized medicine.

Studies were excluded if they (a) examined AI applications outside the field of pathology, (b) discussed general medical AI without a focus on digital imaging or histopathology, (c) employed outdated methodologies or lacked sufficient methodological rigor, or (d) overlapped with inclusion criteria but did not report measurable outcomes related to diagnostic accuracy, clinical utility, or workflow improvements. Studies with insufficient data, non-peer-reviewed reports, or conference abstracts without full-text availability were also excluded.

Risk of bias assessment

The QUADAS-2 tool was employed to assess the methodological quality of the included studies on diagnostic accuracy. The evaluation was performed based on four major domains, namely, patient selection, index test, reference standard, and flow and timing. In general, a good number of studies showed a low risk of bias in their index test and reference standard domains, especially those that used deep learning architectures for cancer prognosis, tumor detection, and image classification, which is in line with the methodological strengths recognized in RNA-seq-based survival prediction models by Huang et al.[11] On the other hand, there were issues in the selection of patients, since some of the studies depended on data from single centers or retrospectives datasets which made the results less generalizable. Furthermore, flow and timing bias were noted in the studies that did not clarify whether index test interpretations were not influenced by the reference standards, thus raising the risk of information leakage. Several models reported high diagnostic and prognostic

Table 1. Comparison of Machine Learning and Deep Learning AI

Aspect	Machine Learning (ML)	Deep Learning (DL)
Model types	Decision Trees, SVMs, Ensemble Methods	CNNs, RNNs, Transformers
Feature extraction	Manual Feature Engineering	Automatic Feature Extraction
Data requirements	Smaller datasets can be effective	Requires large datasets for training
Interpretability	Generally more interpretable	Often considered a "black box"
Applications	Classification of structured data	Image classification, segmentation, detection

performance levels, comparable to that of expert assessment, however, their reliance on curated, homogeneous datasets and lack of external validation raised applicability issues. These phenomena similar to the limitations pointed out by Huang et al.,[11] which call for the employment of more varied datasets and rigorous validation as a means to boost confidence in AI-assisted diagnostic tools.

Taken together, while most included studies reported promising accuracy, efficiency, and workflow improvements, the risk of bias analysis highlights the need for larger, multi-institutional studies with transparent reporting, external validation and improved dataset diversity. These measures would reduce bias and strengthen confidence in the clinical translation of AI applications in pathology.

Data extraction and thematic analysis

The following information were extracted from each of the studies: author, year, AI model used, dataset size, pathology domain, outcome metrics and key findings. Studies were categorized and synthesized by AI technique: traditional machine learning (ML), deep learning (DL) and explainable AI (XAI).

Given methodological heterogeneity, including variations in algorithm types, model architectures, data sources and clinical endpoint, results were summarized narratively rather than pooled quantitatively. ML models such as support vector machines (SVM) and decision trees were contrasted with convolutional neural networks (CNNs) and transfer learning strategies in DL. Similarly, XAI approaches like LIME and SHAP were explored for their roles in enhancing model interpretability. These groupings allowed for thematic synthesis based on functionality, assumptions and applicability in resource-constrained healthcare systems such as those in the Philippines.

Key themes analyzed

The following key themes were analyzed:

1. AI techniques and algorithms in pathology
2. AI applications in pathology
3. Workflow optimization
4. Challenges and limitations
5. Validation and clinical implementation
6. Future directions in AI-driven pathology

Critical evaluation and discussion

Qualitative synthesis identified patterns, knowledge gaps and comparative insights. AI's impact on diagnostic accuracy and efficiency was assessed through global comparisons and potential applications in the Philippines. Ethical concerns, implementation barriers and future possibilities were examined.

RESULTS AND DICUSSION

The following section presents findings clustered according to thematic discussions. While structured searches of the literature informed this synthesis, it does not meet the methodological standards of a systematic review as defined by PRISMA 2020 guidelines. As such, this review should be interpreted as an integrative narrative overview rather than a formal systematic analysis.

Foundations of AI in pathology: ML, DL and XAI

Comparative performance of ML and DL techniques

Machine learning (ML) and deep learning (DL) continue to refine pathology diagnostics, with advancements in transfer learning and explainable AI (XAI) further enhancing precision and efficiency. As shown in Table 1, ML and DL differ in model architecture, feature extraction strategies, data requirements and interpretability, yet both support increasingly sophisticated diagnostic applications. ML enables systems to learn from data patterns and make predictions without explicit programming. DL, a specialized branch of ML, uses layered neural networks to analyze complex data such as medical images.

ML algorithms like decision trees and support vector machines (SVMs) support clinical decisions by organizing data into structured decision pathways and identifying patterns in high-dimensional space. Ensemble models like random forests and gradient boosting combine algorithms to produce more stable and precise diagnostic tools.[11]

DL models, particularly convolutional neural networks (CNNs), have transformed medical image analysis by extracting image features automatically. CNNs have demonstrated high accuracy in brain tumor detection using MRIs.[12] Recurrent neural networks (RNNs) manage sequential clinical data to predict disease progression,[13] while transformer architectures, originally developed for language processing, now enhance image analysis by learning long-range relationships.[14]

Transfer learning using pre-trained models such as VGG and ResNet improves training efficiency and performance, especially with limited datasets.[15] These methods have proven successful in melanoma diagnostics.[16] Tools like LIME and SHAP contribute to XAI by making AI model decisions interpretable, supporting clinician understanding and building trust.[17]

Applications in Diagnostics and Clinical Workflows

Applications and technical enhancements of AI in pathology

AI enhances digital pathology through applications in diagnostic support, tumor detection, image classification, biomarker quantification and predictive analytics.[18] AI improves the analysis of whole slide images (WSIs), supporting faster and more accurate diagnoses.[19] In urologic pathology and oncology, AI assists in histopathological analysis, helping tailor treatment strategies.[20-22]

In prostate cancer diagnosis, AI systems have demonstrated expert-level grading accuracy, thereby supporting standardized histological classification.[23,24] For instance, Trabelsi et al.,[25] demonstrated that AI-driven classification frameworks applied to PET/CT imaging improved the precision and consistency of tumor staging in lung cancer, reducing variability that often arises from manual interpretation. Similarly, Han[26] highlighted how AI systems in brain tumor recognition can enhance diagnostic reliability by minimizing human error and addressing inconsistencies in expert assessments. Together, these findings underscore how AI not only strengthens diagnostic accuracy but also improves workflow efficiency by supporting faster, more standardized review processes across large volumes of clinical imaging data.

Beyond prostate pathology, AI applications have also been extended to pediatrics and other malignancies. Kamp et al.,[27] evaluated AI-based histopathology models in Wilms tumor diagnosis, demonstrating comparable accuracy to human experts in differentiating tumor subtypes. However, the study noted that performance was influenced by the size of the training dataset, pointing to a limitation in generalizability when data are scarce. Similarly, AI-driven methods for quantifying protein expression in immunohistochemistry (IHC) have been shown to improve reproducibility in biomarker assessment, supporting more consistent treatment planning.[28] These workflow enhancements reduce inter-observer variability and shorten turnaround time, which are critical to clinical efficiency.

Huang et al.,[29] extended this application to MRI-based monitoring of disease progression, demonstrating how AI can aid longitudinal tracking of tumor dynamics. While promising, these radiology-focused studies often rely on retrospective datasets, raising questions about real-world applicability in diverse patient populations.[30]

Predictive modeling also enhances the functionality of AI in clinical decision-making. Keim-Malpass et al.,[30] and Rana and Shuford[31] examined outcome prediction models in the context of oncology and demonstrated improved capability in predicting disease trajectories with timely intervention strategies. These predictive abilities, when merged with pathology workflows, can serve to connect diagnostics knowledge with individualized treatment plans.

Enhancing workflow efficiency and quality control

AI enables workflow efficiency and quality control in pathology by improving aspects such as image segmentation, slide quality assessment and automatic specimen worry; just to name a few. Convolutional neural networks (CNN) have been applied in histopathology (or other pathology applications) for anatomical segmentation with great accuracy.[32] In one study, CNNs that achieved a Dice coefficient of 73%, when segmenting slides for pancreatic ductal adenocarcinoma, meaning a substantial overlap of automatic segmentation with expert annotations. The study utilized digitized pathology images from many clinical cases and showed that automated segmentation saves pathologists time and effort, while providing fixed and reproducible edges. The authors did admit to shortcomings in external validity, attempting to use a model with slides from institutions with different stain protocols had potentially reduced accuracy as it portrayed a challenge to cross-site generalizability.[33]

AI systems are also being utilized to improve quality control in pathology workflows. Automated algorithms can screen and flag low-quality or artifact-heavy slides that could compromise diagnostic interpretation.[34] This contributes to efficiency by ensuring that pathologists focus only on diagnostically usable material and minimizes delays caused by repeat slide preparation. Such quality control mechanisms support laboratory standardization, reduce variability across technicians and create a more robust diagnostic pipeline.

Another critical factor in developing effective AI models is the availability of annotated datasets. Since manual annotation by expert pathologists is labor-intensive, researchers have explored alternative approaches. Selnes et al.,[35] for instance, evaluated a semi-supervised learning model for gastrointestinal polyp detection in endoscopic images. Their study demonstrated that the model achieved high sensitivity and specificity even when trained on a relatively small manually labeled dataset, thereby reducing the annotation burden. While promising, the study acknowledged that performance may vary when applied to rare or atypical polyp morphologies, suggesting that broader validation is required before clinical deployment.

Finally, AI-driven triaging systems have shown potential in optimizing hospital resource allocation and prioritizing urgent cases.[36,37] By automatically categorizing specimens according to risk profiles, these systems accelerate review of high-priority slides, enabling earlier interventions and improving patient care efficiency. Although current evidence is largely based on retrospective validation studies, the results suggest that AI-based triaging can significantly streamline diagnostic workflows, reduce turnaround times and improve alignment of resources with patient needs.

Ethical, technical and implementation challenges

Challenges and barriers to AI adoption in pathology

AI adoption in pathology encounters several significant challenges spanning data, ethics and practical implementation.[38] A primary technical barrier is the scarcity of large, diverse and well-annotated datasets critical for effective AI training.[39] Developing these datasets involves substantial financial investment, raises patient privacy concerns and requires considerable pathologist time for manual annotation.[40] Eloy et al.,[41] conducted a systems-based review of data management

in pathology laboratories, demonstrating that structured digital archiving and efficient data pipelines can improve processing speed without sacrificing diagnostic quality. However, they highlighted that most datasets remain limited in scope, typically sourced from single institutions or specific populations, which restricts the external validity of AI models. Supporting these findings, Berbís et al.,[42] identified a "translation gap" in their multicenter analysis showing that models trained on narrowly defined datasets performed well locally but exhibited reduced accuracy when applied to data from different geographic or clinical settings. Their study underscored the promise of federated learning and international data-sharing initiatives to enhance generalizability, despite ongoing legal and ethical hurdles.

Clinical integration and workforce readiness

Ethical difficulties also have a heavy burden on the role of AI in Pathology. Interpretability of AI models has been identified as an important issue regarding clinician acceptance. Clinicians do not favor trusting "black box" systems that do not make clear how predictions were made, which can foster distrust of AI models. Montezuma et al.,[43] carried out a survey of practicing pathologists working in academic institutions, with over 60% of respondents unwilling to rely on AI tools that did not provide a defined decision pathway. They made a call for the development of explainable AI systems, but they recognized that there are trade-offs in explaining how the AI model made a particular prediction versus computational power. Dow et al.,[44] examined existing guidelines from institutions in North America and Europe and summarized that ethical scrutiny and regulatory guidance related to pathology AI model use were considerably behind other areas of technology. The absence of policies means a lack of accountability and limits the potential rate of adoption of AI in clinical practice, especially in low-resource contexts.

Benchmarking AI tools through comparative metrics

Integrating AI into pathology workflows raises additional issues related to validation, clinician acceptance and workforce readiness. For example, Hanna et al.,[45] performed a validation study using cross-validation and external datasets ultimately finding high diagnostic reliability when trained on a diverse dataset with diverse training variants; however, accuracy decreased markedly for rare tumor subtypes, highlighting the importance of continual validation across pathology domains. Zarella et al.,[46] studied experiences from pilots for AI implementations in clinical pathways, highlighting the positive impact on turnaround time and improved accuracy across AI-based pathology systems across clinical pathology. However, many clinicians were cautious and indicated that they would wait for large prospective studies before adopting the technology.

Scalability and evidence-based implementation in the Philippine context

To provide further context to these findings, Huo et al.,[47] systematically examined the integration of AI in a hospital network and concluded that AI adoption was determined by AI model quality but more importantly by sufficiently training staff and changing their workflows. In their mixed methods study, Huo et al., reported that not being adequately trained was a major barrier to integration, although results were limited to a single region. It appears that in addition to the studies previously presented, all these studies suggest that effective AI adoption in pathology relies on strong validation, reasonable integration plans and workforce development.

AI implementation in pathology flow diagram

The flow chart (Figure 1) provides a framework for understanding the process of AI implementation in pathology. At the center is an AI model that can be implemented in three contexts: challenges and barriers, clinical implementation and validation or benchmarking. The challenges and barriers, namely ethical, technical and practical issues, need to be accounted for in the pathway to adoption. The bottom of the flow chart displays scalability in the Philippine context as the final output but only supported by the former issues and the workflow characteristics. The arrows emphasize that the emergence from these barriers, clinician readiness and validation of AI tools are interdependent processes that will determine whether AI can be successfully scaled and sustainably implemented into local pathology workflows.

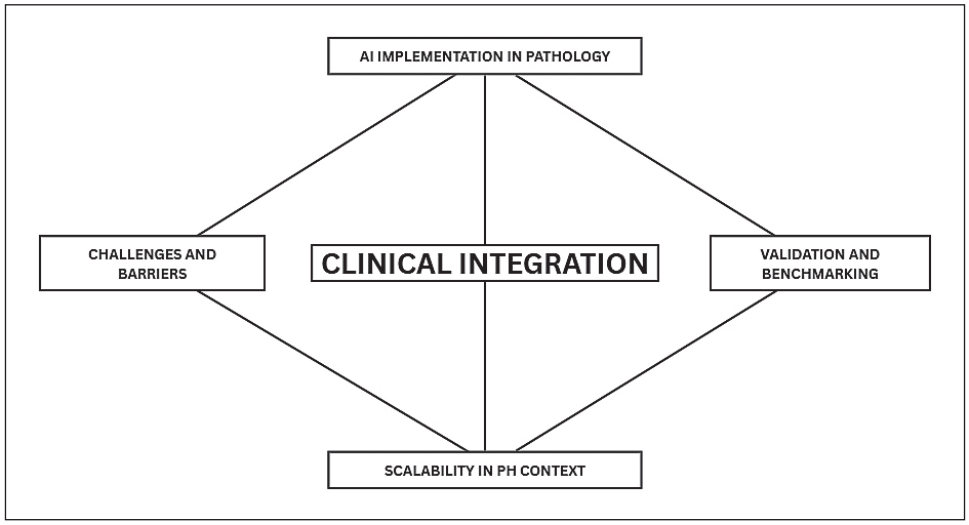

Figure 1. Framework for clinical integration of AI in pathology.

Future directions and innovations

AI in pathology must remain a supporting technology that supplements the capabilities of pathologists with its diagnostic capabilities, not displace them as the final decision-maker. An effective AI could improve both the speed and accuracy of diagnosis, while providing a human expert with a valuable tool to manage complex image analysis. In this way, we maintain human decision making but improve human oversight in decision-making, improving our ability to serve clinical responsibility and ultimately the patient. The regulatory oversight from the Department of Health (DOH) and Food & Drug Administration (FDA) on the AI development process put the care back into healthcare and ensure we can safely develop and utilize these technologies in a healthcare context.[48]

As we move forward, there is hope for new technological innovations with generative AI and self-supervised learning to solve the problem of limited labeled data in medical imaging. Generative AI will allow advances in the field without the notable burden of needing annotated datasets, which can be timely and expensive to put together, ultimately allowing safe and rapid development of a more robust AI model. For example, Yao et al.,[49] have been able to create new sophisticated algorithms that can detect subtle features of an image that an observer (a human) may miss or not even see, thus allowing enough insight for more individualized and specific management for the patient. These technologies are just a couple examples of the ways in which AI can augment healthcare solutions and importantly create unique and meaningful diagnostic insight.

In addition, using AI technology live during clinical work has the potential to provide pathologists with immediate diagnostic assistance, improving the decisions they can make in real time. Andreychenko et al.,[50] describe real-time AI image analysis using objective diagnostic support that is relevant to workflows and could increase diagnostic productivity in busy clinical practice. Certainly, there are promising opportunities for AI to improve equity in healthcare through diagnostics in settings with fewer resources. Chatrian et al.,[51] provided one example of connecting rural healthcare providers via AI-powered digital platform to receive expert-level pathology diagnosis remotely, which could facilitate high-quality diagnostic services to places with poorer access to specialist care.

These possibilities and directions offered by AI suggest a continued emergence of advanced computational approaches alongside clinical practice. We hope to use these together to improve clinical accuracy, enable smarter workflows and supply more equitable access to care, all with the supervision of a human pathologist.

Comparative performance of ML and DL techniques

Conventional machine learning (ML) methods such as decision trees and support vector machines (SVMs) have become popular due to their efficiency and an emphasis on human interpretability. In contrast, deep learning (DL)/convolutional neural networks (CNNs) methods, for example, VGG and ResNet, are more adept at processing nonlinear problems on high-dimensional and complex data such as medical images.

In the end, it is up to the user or researcher to determine the best technique to utilize. Interpretability is still a major factor in the decision-making. Machine Learning (ML) methods can be made interpretable by means of the employment of specialized software tools like LIME and SHAP which reveal the involvement of different model features throughout the modeling and prediction processes (thus creating some visibility and transparency on the influence of certain features on the model predictions). Deep Learning (DL) models have the issue of non-interpretability; nonetheless, there have been recent efforts applying LIME and SHAP to convolutional neural network (CNN) outputs that are still far from being on par with ML methods as far as explanation is concerned and have not been developed for the health sciences and medical imaging areas.

Ultimately, the decision to use ML or DL techniques depends on multiple factors: data availability, task complexity and resource constraints. For simpler tasks with structured data and limited samples, traditional ML approaches provide a reliable and interpretable solution. However, for more complex tasks involving large volumes of unstructured image data, DL methods exhibit superior performance despite higher computational demands. This trade-off is exemplified in the reviewed studies, which consistently show ML's advantage in speed and straightforward interpretation and DL's strength in accuracy and handling data complexity.

Applications and technical enhancements of AI in pathology

AI applications within pathology can be broadly categorized into predictive analytics, tumor detection, digital image analysis and immunohistochemical quantification. Advances in the use of deep learning data-driven algorithms for evaluating biomarkers have refined each stage of clinical decision-making in a personalized way. AI-based predictive models help in forecasting disease progression and treatment response, increasing the overall ability to deliver more proactive and personalized healthcare to patients. AI helps to minimize human error in diagnostics and thus optimizes workflow for diagnostic pathology to improve the practice of precision medicine and clinical outcomes.

Another area of AI use seeks to improve automation of tasks related to slide segmentation, quality control and case prioritization. Accurate segmentation and removal of low-quality suboptimal slides can maximize the reliability and speed of diagnosis. Automated data annotation can expedite the model training process, while AI-based case triage can direct clinician attention to those cases that require urgent or immediate management.

Challenges and barriers to AI adoption in pathology

However, even with those considerations, there are several challenges to consider regarding AI adoption in pathology. The challenges are to acquire sufficient high-quality labeled data sets, data privacy considerations and labeling and generalizability issues. Low data quality diminishes the reliability of the diagnostic, while privacy issues introduce ethical issues particularly when dealing with patient cases that are sensitive. In addition, when there are often very diverse demographic populations accommodating them, it

Table 2. Summary of AI applications in digital pathology (2019-2025)

Study (year)	Disease area / task	Sample size / dataset	AI model type	Performance metrics	Validation method
McGenity et al. (2024)	Various diseases (WSI-based diagnosis)	152,000+ WSIs across 100 studies	Various AI models (systematic review)	Sensitivity: 96.3% (95% CI: 94.1–97.7); Specificity: 93.3% (95% CI: 90.5–95.4)	Meta-analysis of 48 studies
Vorontsov et al. (2019)	Pan-cancer detection across 17 types	1.5 million WSIs	Vision Transformer (Virchow)	AUC: 0.949 (overall); AUC: 0.937 (7 rare cancers)	Internal and external validation
Talo (2019)	Histopathology image classification	Public datasets (24 categories)	DenseNet-161, ResNet-50	Accuracy: 97.89% (DenseNet-161); 98.87% (ResNet-50)	Cross-validation
Alom et al. (2023)	Multiple tasks (e.g., IDC detection, segmentation)	Public datasets (various tasks)	Advanced DCNNs (e.g., IRRCNN, DCRCN)	High performance across tasks (e.g., sensitivity, specificity, F1-score)	Task-specific evaluations
Allen et al. (2025)	HER2 status evaluation in breast cancer	~1,100 samples	10 AI tools compared	High agreement with expert pathologists; variability at low expression levels	Comparative study across tools

WSI: Whole Slide Image
AUC: Area Under the Receiver Operating Characteristic Curve
DCNN: Deep Convolutional Neural Network
IRRCNN: Inception Residual Recurrent Convolutional Neural Network
DCRCN: Densely Connected Recurrent Convolution Network
IDC: Invasive Ductal Carcinoma

is often challenging to generalize through AI modelling, thereby making it hard to deliver standard of care.

The interpretability of AI models also represents an essential challenge that matters to trust levels perceived by pathological and clinical professionals. In the absence of exploration of ethical and timely explanations of model behavior and outputs clinicians may be unwilling to trust AI generated suggestions. Additionally, lack of regulatory certainty regarding the utilization of AI tools in the clinical environment causes additional hesitation to transfer to practice. These limitations are minimal by adopting data-centric approach, as well as external auditing and ethical oversight will play vital roles in creating fair and successful AI models and methods in pathology practice.

Clinical integration and workforce readiness

To make AI tools clinically applicable, it is necessary that they are well-validated and materialize into being aligned with the current clinical workflow. Although pilot studies report enhanced quality of diagnosis with the help of AI, there are doubts about it among healthcare professionals. The solution to this barrier does not exist in the technical aspect of integration alone but a workforce development strategy of upskilling and confidence training over AI-enabled systems.

The readiness of the future includes incorporating the training on AI in education in pathology, developing the collaboration with other areas of medicine, as well as data scientists and engineers and the development of the guideline on the application of AI in diagnostics and easy access to it. Participation in such programs is of special importance to low-resource countries, like the Philippines, where the availability of specialists trained at the level, as well as infrastructure in form of laboratories is scarce.

Benchmarking AI tools through comparative metrics

The recent rise of artificial intelligence (AI) in the digital pathology domain has undoubtedly altered the landscape of histopathological diagnostics. As in low-resource settings such as the Philippines, the ability to compare the characteristics of AI model performance is important, including feature counts, model architecture, sensitivity, specificity and area under the curve (AUC). Ultimately, a tabular representation of these performance metrics can help facilitate informed decision-making.

As an example, McGenity et al.,[52] mentioned in their paper that employing AI models based on large whole-slide image datasets enabled them to achieve 96.3% sensitivity and 93.3% specificity as the diagnostic accuracies. This showed that AI systems are not only capable of mimicking but also at times even outdoing the performance of the human pathologists. In a similar vein, the works of Talo and Alom et al.,[53,54] have proven steady performance of the convolutional neural networks and transfer learning methods across various histopathological tasks, allowing the reinforcement of the strength of these architectures even though different diseases are involved. The recent launch of a foundation model like Virchow, trained on more than a million digital pathology slides, has not only confirmed the generalization power of AI across different tissue types and diagnostic settings but has also increased it as reported by Vorontsov et al.[55] To these developments, Allen[56] mentioned the formation of new frameworks for the systematic comparison of AI-based digital pathology tools which ensure the availability of practical metrics for the evaluation of the model performance under real-world conditions. The healthcare stakeholders will be able to tell which AI model best fits their current evidence-based practice and local clinical priorities by having these findings presented in comparative tables (Table 2).[52-56]

Scalability and evidence-based implementation in the Philippine context

Such advanced models like Virchow, trained on more than 1.5 M digital slides, have demonstrated an AUC above 0.95 in detecting common and rare cancers, rendering them highly valuable in settings where diagnostic capacity is limited. Besides these, validation protocols using cross-validation and external datasets as pointed out in many recent reviews serve to buttress these AI tools' strength and generalizability.

For the case in the Philippines, given the problems with delayed diagnostics and limited specialists, validation becomes even more important. In addition to providing clarity with respect to AI model performance, comparative tables allow decision-makers and technology adopters to prioritize tools that are proven to work in realistic environments. This structured process promotes transparency, enhances clinician trust and fosters the modernization of pathology services through data-driven scalable AI integration.

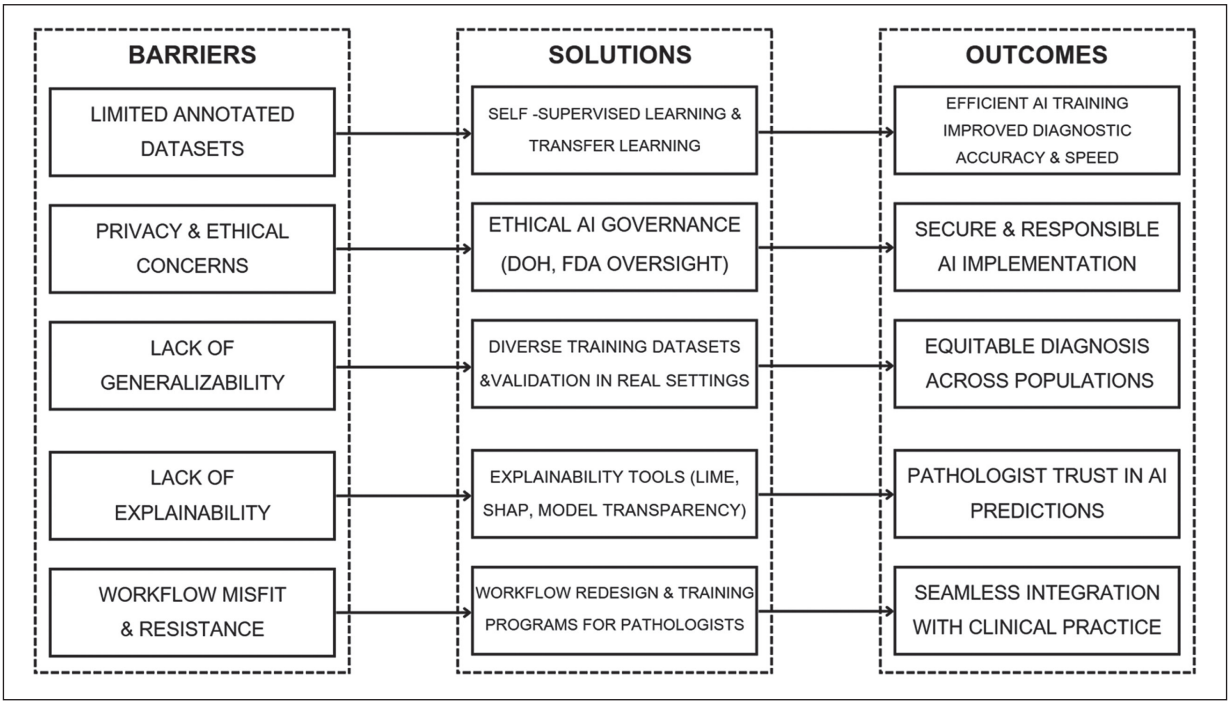

Figure 2. Key barriers in the implementation of AI in pathology.

AI implementation in pathology flow diagram

The flow diagram (Figure 2) illustrates the key barriers to implementing artificial intelligence (AI) in pathology and maps them to corresponding enablers or solutions that address these challenges, ultimately leading to improved clinical outcomes. For instance, the lack of annotated datasets can be mitigated by using self-supervised and transfer learning techniques, enabling efficient AI model training. Privacy and ethical concerns are countered through robust AI governance frameworks and oversight by institutions like the DOH and FDA. Generalizability issues are resolved by using diverse datasets and validating AI tools in real-world settings to ensure equitable diagnostics across different populations. The barrier of AI explainability is addressed through tools like LIME and SHAP, which increase transparency and build trust among pathologists. Lastly, resistance to AI integration due to workflow misalignment is overcome by redesigning clinical workflows and providing training programs, promoting smooth adoption and use of AI in daily pathology practice.

CONCLUSION

Artificial Intelligence (AI) is revolutionizing the field of pathology, particularly in resource-constrained settings like the Philippines. By leveraging machine learning (ML) and deep learning (DL) techniques, AI enhances diagnostic accuracy, speeds up workflows and supports clinical decision-making. Tools such as convolutional neural networks (CNNs) and transfer learning models like ResNet and VGG have demonstrated remarkable success in tumor detection, biomarker evaluation and predictive analytics. These advancements not only improve diagnostic precision but also enable personalized medicine, addressing the challenges of delayed diagnostics and limited access to specialized care in underserved regions. However, AI is not a replacement for pathologists; instead, it complements their expertise, fostering a collaborative intelligence model that combines human judgment with AI's ability to identify complex patterns.

Despite its transformative potential, the integration of AI into pathology faces significant barriers, including the lack of annotated datasets, privacy concerns and limited generalizability of models. Ethical and regulatory frameworks, along with tools like LIME and SHAP, are essential to build trust and transparency among clinicians. Additionally, workforce readiness through structured training programs is critical to ensure smooth adoption of AI technologies. For the Philippines, addressing these challenges requires investments in infrastructure, national support for digital innovation and collaboration between hospitals, universities and technology providers. While AI alone cannot solve systemic healthcare issues, its prudent implementation can fill critical gaps in diagnostics and remote access, improving patient outcomes and advancing the country's healthcare system.

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All authors certified fulfillment of ICMJE authorship criteria.

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SMARCB1 (INI-1)-deficient Sinonasal Carcinoma: A Case Report and its Clinical Implications on Diagnosis and Management

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ABSTRACT

SMARCB1 (INI-1)-deficient sinonasal carcinoma is a rare, poorly differentiated, and locally aggressive neoplasm. Frequently, this disease entity mimics benign head and neck diseases hence it poses a challenge to diagnose and manage such cases. Herein, we have documented a case of a 66-year-old female who presented with a right nasal mass on endoscopy. On microscopy, well-defined nests of plasmacytoid tumor cells infiltrating a desmoplastic stroma with areas of necrosis with focal hemorrhages were noted. Based on the histomorphology and immunohistochemistry studies, this case was signed out as SMARCB1 (INI-1)-deficient (sinonasal) carcinoma. This is the first reported case in the Philippines based on a search of local journal databases. Recent advancements in therapeutics point out the value of providing molecular characterization of these tumors.

Key words: SMARCB1-deficient Tumors, SMARCB1 (INI-1) gene, sinonasal carcinoma, poorly differentiated sinonasal carcinoma, undifferentiated sinonasal carcinoma

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INTRODUCTION

SMARCB1 (INI-1)-deficient sinonasal carcinoma is a rare, poorly differentiated, and locally aggressive neoplasm. It is often diagnosed at an advanced stage, as it initially mimics benign conditions like epistaxis, allergic rhinitis, sinusitis, or presence of nasal polyps. In advanced stages, it can invade the orbits or cranium, causing ophthalmologic and neurologic symptoms, distant metastases, and eventual death.[1] With the advent of immunohistochemistry and molecular studies, morphologically poorly differentiated sinonasal carcinomas can now be classified further, providing insights on their diagnosis, prognosis and management with the use of novel therapeutic agents.[2]

Currently, there are no reported cases published in the Philippines (HERDIN and Philippine E-Journal). Hence, it is important to document the findings of a SMARCB1 (INI-1)-deficient sinonasal carcinoma and describe its key histomorphologic and immunohistochemistry findings.

CASE

The patient is a 66-year-old female, non-smoker and a former factory worker, with a one-month history of a right nasal cavity mass accompanied by congestion, facial pain, rhinorrhea, proptosis, and diplopia. The CT scan showed a large hyperdense mass arising from the right ethmoid sinus and invading the right maxillary sinus and sphenoid sinus with extension towards the right orbit (Figure 1). The initial impression was that of an esthesioneuroblastoma and a biopsy was subsequently performed.

Gross examination showed multiple cream-tan to dark brown irregularly shaped soft tissues measuring from 0.4 up to 1.3 cm in widest diameter with a soft tan-brown cut surface. Microsections show irregular nests of plasmacytoid tumor cells infiltrating a desmoplastic stroma with areas

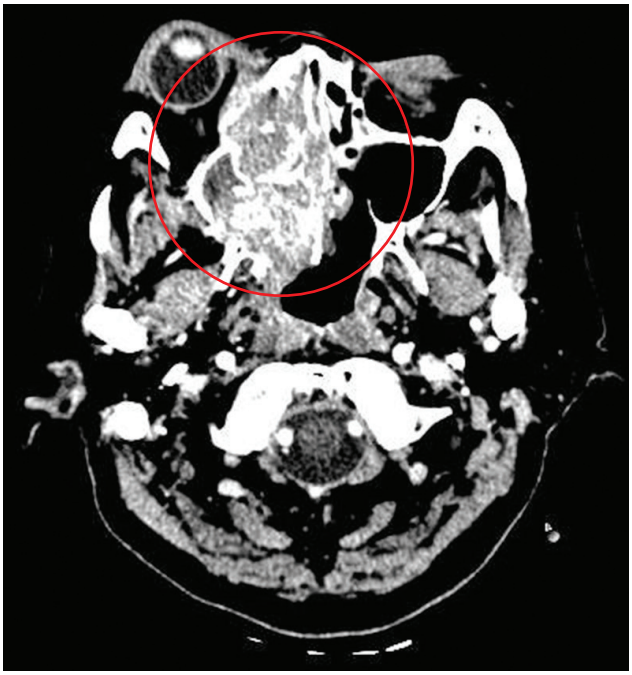

Figure 1. Head CT scan (axial view, brain window) shows a large hyperdense mass *(red circle)* arising from the right ethmoid sinus, invading the right maxillary sinus and sphenoid sinus, and extending to the right orbit.

of necrosis and hemorrhage. The tumor cells exhibit eccentric, ovoid, and hyperchromatic nuclei, some with prominent nucleoli, and ample eosinophilic cytoplasm. No gland formation was observed (Figures 2 and 3).

The case was initially signed out as a round cell neoplasm. Immunohistochemistry studies showed tumor cells that were positive for EMA, pancytokeratin, p63, and p40, and negative for synaptophysin, chromogranin, CD56, S100, desmin, SMA, and PR. INI-1 showed complete loss of nuclear expression in the cells of interest (Figure 4).

Given the morphologic and immunohistochemical profile of the mass, this case was signed out as SMARCB1 (INI-1)-deficient (sinonasal) carcinoma.

DISCUSSION

The SWI/SNF family of chromatin remodeling complexes are key regulators of nucleosome positioning and are composed of large, complicated macromolecules with various subunits attached. Their nomenclature, established by the Human Genome Organization, remains problematic due to the presence of multiple synonymous terms.[3] SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (SMARCB1), also known as integrase interactor 1 (INI-1), is a tumor suppressor gene found at chromosome 22q11.2 which encodes a protein subunit of SWI/SNF nucleosome remodeling complex. SMARCB1 is normally expressed in all cells.[4,5]

The loss of nuclear expression of the *SMARCB1* gene is often caused by biallelic inactivation through homozygous deletion, intragenic deletions, frameshift or nonsense mutations, or chromosomal loss (monosomy 22). It is central to the pathogenesis of various pediatric and adult sarcomas which include atypical teratoid rhabdoid tumors of the central nervous system, malignant rhabdoid tumors of the kidney and soft tissue, and SMARCB1 (INI-1)-deficient sinonasal carcinoma to name a few entities.[6] Interestingly, these tumors are reported to be genomically stable with the SMARCB1 gene being the only one altered. This is a finding that contrasts with the genetic instability that is a hallmark of most cancers.[7] Currently, it is still unknown how SMARCB1-deficient cancers arise in adults. In recent studies, rhabdoid tumors arise from the neural crest cells that lose SMARCB1 during development suggesting that SMARCB1-deficient tumors are often seen more in children than in adults.[8]

Histomorphologically, several studies have reported that the most common presentation of this neoplasm is an undifferentiated basaloid or "blue cell" tumor forming solid well-demarcated nests and sheets infiltrating a desmoplastic stroma. In the basaloid variant, the tumor cells have occasional palisading of the nucleus and high nucleus:cytoplasm ratios. Occasional singly scattered rhabdoid or plasmacytoid cells can also be identified. Squamous differentiation is not appreciated in all reported cases of this type. The second most common type would

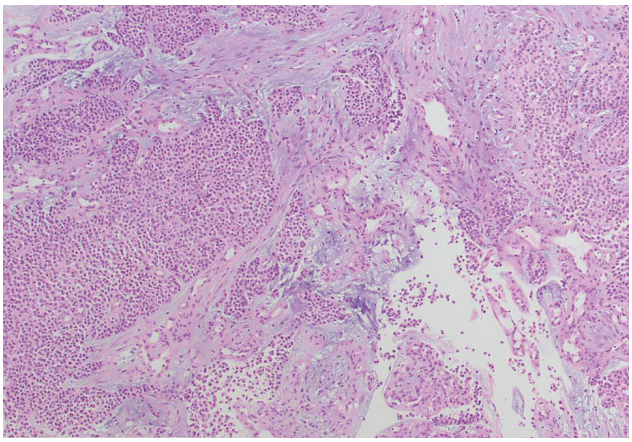

Figure 2. Biopsy of the mass shows irregular nests and sheets of plasmacytoid tumor cells infiltrating the stroma (H&E, 100x).

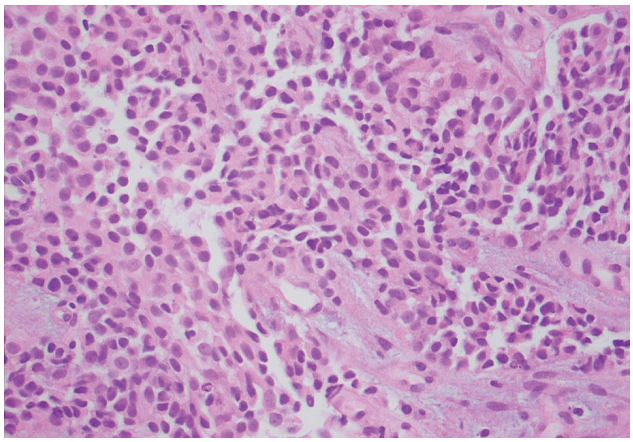

Figure 3. The biopsy shows tumor cells with eccentric, ovoid and hyperchromatic nuclei, some with prominent nucleoli, and ample eosinophilic cytoplasm (H&E, 400x).

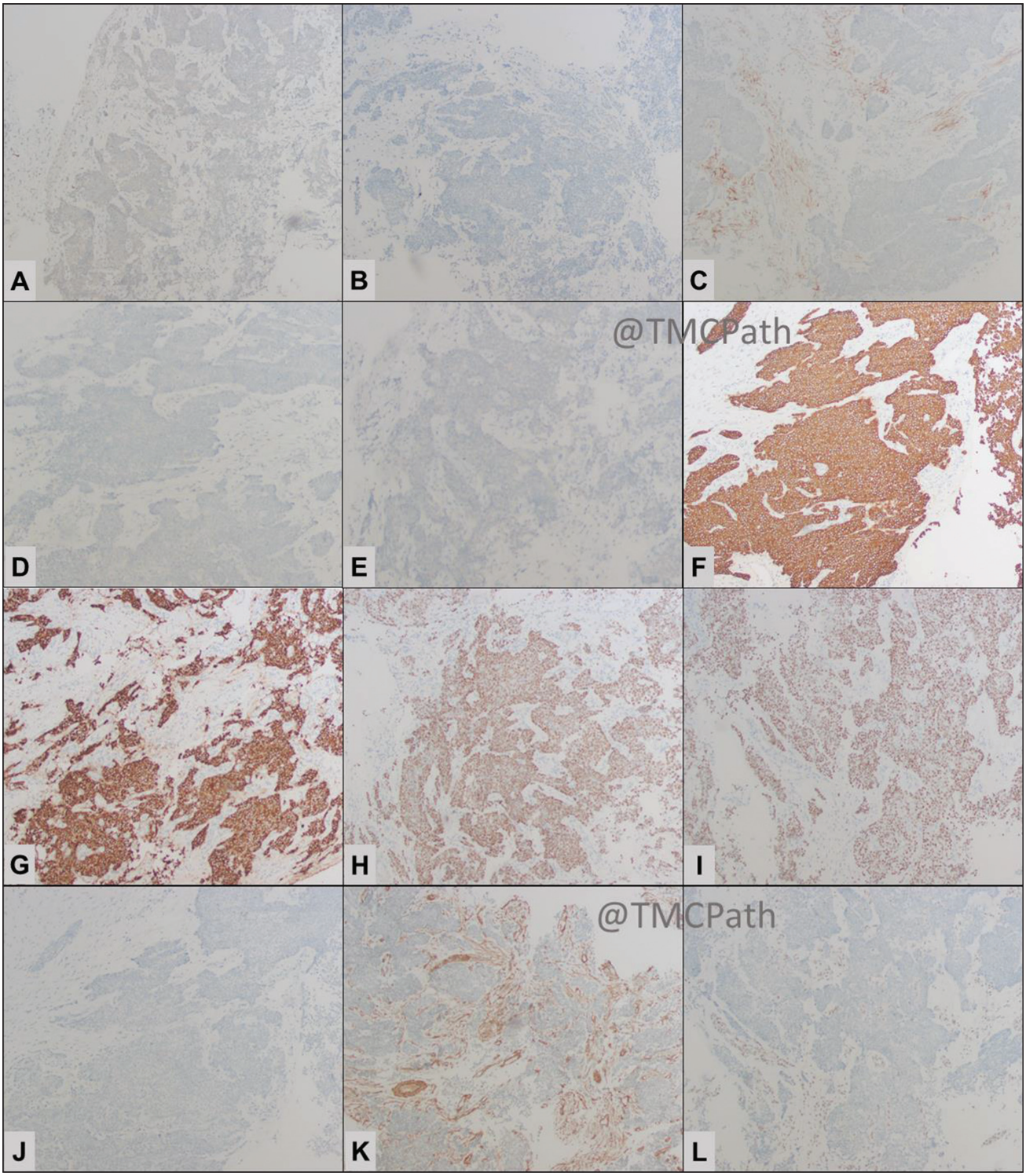

Figure 4. The immunohistochemistry panel of the biopsied mass (Immunohistochemistry stain, horseradish peroxidase method, 100x). The tumor cells stained negative for S100 **(A)**, desmin **(B)**, CD56 **(C)**, synaptophysin **(D)**, chromogranin **(E)**, PR **(J)**, and SMA **(K)** while stained positive for pancytokeratin **(F)**, EMA **(G)**, p63 **(H)**, and p40 **(I)**. INI-1 **(L)** exhibits loss of nuclear expression in the cells of interest).

be the plasmacytoid/rhabdoid or "pink cell tumor" variant which is described as nests and sheets of predominantly plasmacytoid cells. The tumor cells exhibit large oncocytic squamoid cells with acantholytic-like arrangement similar to oncocytic adenocarcinoma of the salivary glands. Other histomorphological features have been described in other reported cases such as pure sarcomatoid carcinoma types, plasmacytoid tumors with glandular differentiation, and mixed types.[9,10] In this case, the biopsied mass exhibited irregular nests of numerous tumor cells that are plasmacytoid in morphology.

Distinguishing between various poorly differentiated sinonasal carcinomas through morphology alone is difficult hence, immunohistochemistry stains are often requested. Common histomorphologic differentials for SMARCB1-deficient sinonasal carcinoma include basaloid squamous cell carcinoma, neuroendocrine tumors, and NUT carcinoma. Squamous cell carcinomas would typically express cytokeratins such as CK5/6 and pancytokeratins, while neuroendocrine tumors would stain positive for chromogranin, synaptophysin, and CD56 but lack cytokeratin expression. NUT carcinomas are distinctly positive for NUT protein. SMARCB1-deficient tumors may show variable expression of squamous markers and neuroendocrine markers but are negative for NUT protein. The most definitive immunohistochemical finding of this type of tumor is the loss of SMARCB1 (INI-1) expression in tumor cells, a feature typically retained in other sinonasal malignancies.[9,10] As mentioned previously, the loss of INI-1 is the hallmark of many rare and aggressive teratoid/rhabdoid tumors. The INI-1 immunohistochemistry stain has a high specificity and sensitivity in these types of tumors including SMARCB1-deficient sinonasal carcinomas. In most cases, immunohistochemistry is sufficient for diagnosis, and molecular testing is not clinically indicated, except in difficult or complex cases where loss of SMARCB1 expression cannot be confirmed.[10,11]

SMARCB1-deficient carcinomas are associated with a high recurrence rate and a worse prognosis compared to the other types of poorly differentiated sinonasal carcinomas with patients dying of the disease from zero to two (0-2) years from diagnosis.[12] Aggressive treatment, with surgical resection followed by adjuvant radiotherapy and concurrent chemotherapy, is often indicated.[13] With the advent of immunotherapy and targeted treatment, one promising target being studied is the enhancer-of-zeste-homolog-2 (EZH2) enzyme. Under normal conditions, SMARCB1 within the SWI/SNF complex suppresses PRC2–EZH2 activity, leading to the activation of tumor suppressor genes and repression of cell cycle–promoting genes. In contrast, the loss of SMARCB1 enhances PRC2–EZH2 activity. EZH2 inhibitors, such as tazemetostat, target the PRC-EZH2 complex hence preventing the upregulation of the oncogenic pathways such as *myc*, *sonic hedgehog*, and *WNT-β-catenin* similar to how SMARCB1 functions. Phase I and Phase II clinical trials for tazemetostat are reported to be ongoing.[14] In a case study done by Zhao and colleagues, PDL-1 inhibitors such as pembrolizumab were incorporated in the treatment regimen of three patients with SMARCB1-deficient sinonasal tumors. Two patients achieved a complete response, while the third, despite experiencing recurrence following treatment interruption, demonstrated sustained disease control with PDL-1 inhibition in later stages. This implies that PDL1 inhibitors may improve clinical outcomes of these patients.[15] Other targeted therapies such as histone deacetylase inhibitors, aurora-A-kinase inhibitors, and CDK4 inhibitors are being investigated in other carcinomas and sarcomas with SMARCB1-deficiency.[16] Recent discoveries and advancements in therapeutics point out the value of providing molecular characterization of these aggressive tumors.

CONCLUSION

The case presented in this paper highlights the importance of recognizing SMARCB1 (INI-1)-deficient sinonasal carcinoma as among the differential diagnosis of a poorly differentiated sinonasal carcinoma, the challenges in clinically diagnosing the entity, and its histomorphologic and immunohistochemistry findings. Advancements in the molecular characterization of these aggressive carcinomas may provide key insights on their pathogenesis, diagnosis, and optimal management. Awareness and early recognition of the entity may help positively impact the overall survival of affected patients.

ACKNOWLEDGMENTS

The authors extend their special thanks to Dr. Lester Thompson for his expert insights and constructive input regarding SMARCB1-deficient tumors of the head and neck, which greatly enhanced the clinical and pathological understanding presented in this case.

ETHICAL CONSIDERATION

Despite multiple efforts to obtain informed consent, the patient could not be reached. Due diligence was exercised in attempting to locate the family (absence of a recorded address, lack of responses, and no available cellphone or landline as verified by the Records Section), and the patient was ultimately lost to follow-up.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

DATA AVAILABILITY STATEMENT

No datasets were generated or analyzed for this study.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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None.

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OPEN ACCESS – CASE REPORT

Lipomatous Angiomyofibroblastoma in the Vulva: A Case Report with Review of Literature

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ABSTRACT

This is a case of a 55-year-old female, surgical menopause for nine years, not on hormone therapy with one year history of a progressively enlarging left vulvar mass, who sought consult and subsequently underwent excision of mass. Microscopic findings showed alternating hypo- and hypercellular areas composed of plump spindle to epithelioid cells distributed in an edematous stroma with varying amount of collagen. Tumor cells have bland chromatin, inconspicuous nucleoli, and absent mitoses. The cells surround small to medium-sized thin-walled, hyalinized vessels found in hypercellular areas. An adipocytic differentiation of more than fifty percent of tumor is observed further classifying this neoplasm as lipomatous angiomyofibroblastoma, making this an even rarer type of benign mesenchymal tumor of the vulva. Immunohistochemistry stains performed showed positive staining for vimentin, smooth muscle actin, BCL-2, ER, PR, and negative staining for desmin which supports the diagnosis. The clinical presentation, operative findings, histopathologic features and the various considerations are discussed. Literature review of vulvar angiomyofibroblastoma is also presented.

Key words: vulva, angiomyofibroblastoma, lipomatous angiomyofibroblastoma

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INTRODUCTION

Lipomatous angiomyofibroblastoma (LAMF) is a rare, benign soft tissue tumor with a prominent well-differentiated adipocytic component. Only 10% of angiomyofibroblastomas are classified as LAMF.[1] These tumors commonly present as painless vulvar masses. Histologically, they are composed of spindle cells and epithelioid cells surrounding vascular network in a background of loose and edematous stroma. Mature adipocytes comprise a significant portion of the tumor. LAMFs have a good prognosis, with conservative local excision as curative treatment, and uncommon local recurrence.[2]

To date, no known studies in the Philippines have reported cases of vulvar LAMF. This report aims to provide clinical and histopathologic data of this uncommon benign soft tissue tumor in the vulva in the local setting.

CASE

Patient is a 55-year-old female, G3P3 (3-0-0-3) previous normal spontaneous delivery, surgical menopause for nine years, status post total abdominal hysterectomy in 2019 for removal of uterine myomas in a private hospital, not on hormone therapy and consulted for left vulvar mass. One year prior, patient noted a 2.0 cm soft cystic mass on the left vulva, which was non-tender, and non-erythematous, with no other associated symptoms. She did not take any medications nor sought consult at this time. In the interim, patient noted the mass to be increasing in size but still with no medications nor consults done.

One month prior, patient observed that the mass was approximately 5.0 cm. Persistent progression in size of the mass prompted her to consult with an obstetrician-

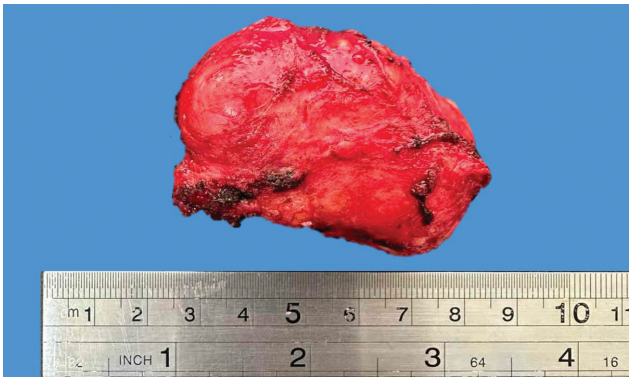


Figure 1. External surface of the mass, post-operatively.

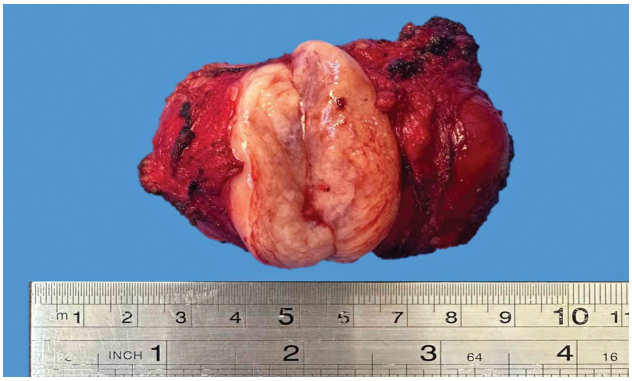


Figure 2. Cut surface of the mass, post-operatively.

gynecologist, with physical examination findings of a 5.0 cm cystic left vulvar mass. Transvaginal ultrasound showed surgically absent uterus, small ovaries, left adnexa, and a thin- walled cyst measuring 2.02 x 1.45 x 1.74 cm, volume of 2.68 ml. with no color on flow mapping suggestive of para-ovarian cyst.

Patient sought another consult which revealed a 6.0 x 5.0 cm cystic mass, non-tender and non-erythematous, located at the 5 o'clock position of the left vulvar area. Speculum exam and internal examination was deferred. She was advised excision of mass. Intra-operatively, a fluctuant vulvar mass admixed with blood, measuring 6.0 x 5.0 cm, was obtained (Figures 1 and 2). Patient tolerated the procedure without undue complications and was discharged several hours after recuperating from anesthesia. Follow-up consult two weeks after surgery was uneventful.

Submitted specimen at the histopathology laboratory showed a circumscribed, irregularly ovoid, lobulated, rubbery mass measuring 6.0 x 4.0 x 2.5 cm with a gray-pink external surface covered with focal fibrous tags. It has been previously opened along one side, and further sectioning revealed a pink gray, glistening soft cut surface.

Microscopic examination showed alternating hypo- and hypercellular areas composed of spindle to epithelioid myofibroblastic cells distributed in a loose and fibrous stroma (Figure 3). There was no involvement of the surgical margins. The cells surrounded small to medium-sized thin-walled, as well as hyalinized vessels, that were irregularly distributed (Figures 4 and 5). More than 50%

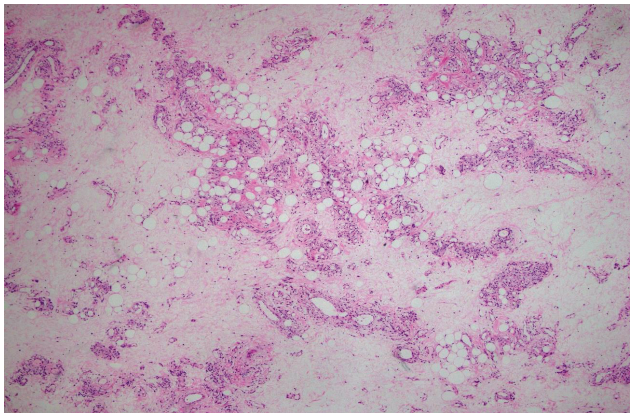
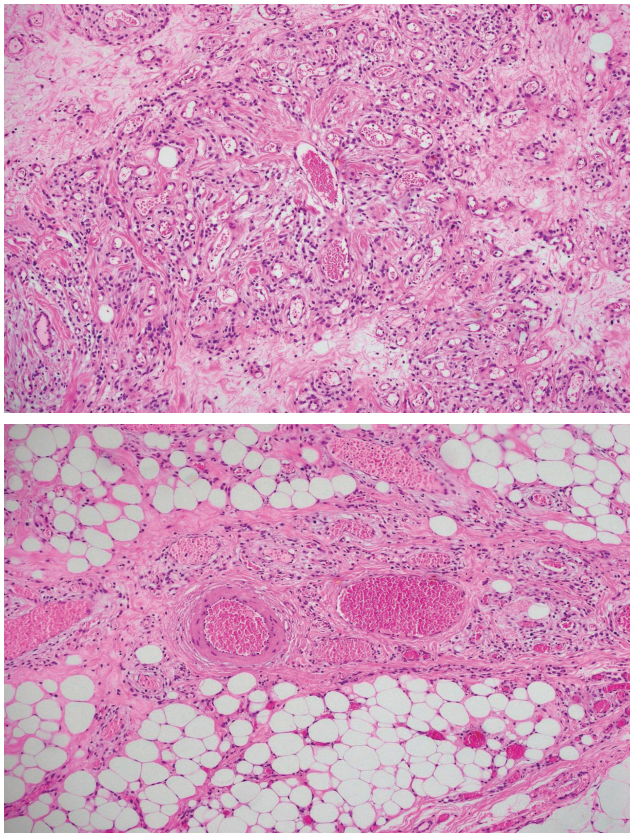


Figure 3. Alternating hypo- and hyper-cellular areas (H&E, 40x).



Figures 4 and 5. Spindle to epithelioid cells surrounding small to medium-sized, thin-walled, as well as hyalinized vessels (H&E, 100x).

of the tumor consisted of mature fat cells (Figure 6). The tumor cells were monomorphic with bland chromatin and inconspicuous nucleoli, and absent mitoses and necrosis (Figures 7 and 8). Immunohistochemistry stains performed showed positive staining for vimentin, smooth muscle actin, BCL-2, ER, PR, and negative staining for desmin (Figure 9 A-F).

DISCUSSION

An angiomyofibroblastoma is a benign, well-circumscribed myofibroblastic neoplasm, usually arising in the pelviperineal region, especially the vulva, and composed

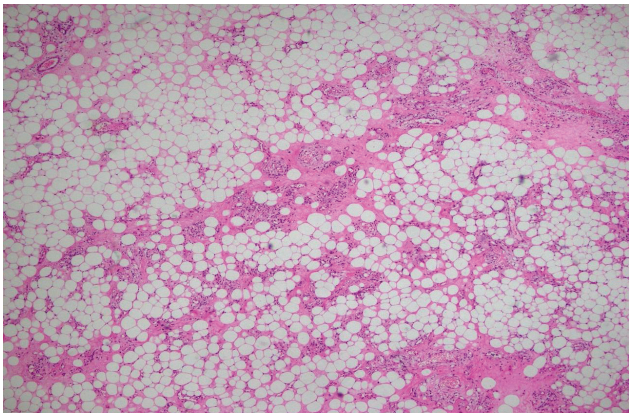
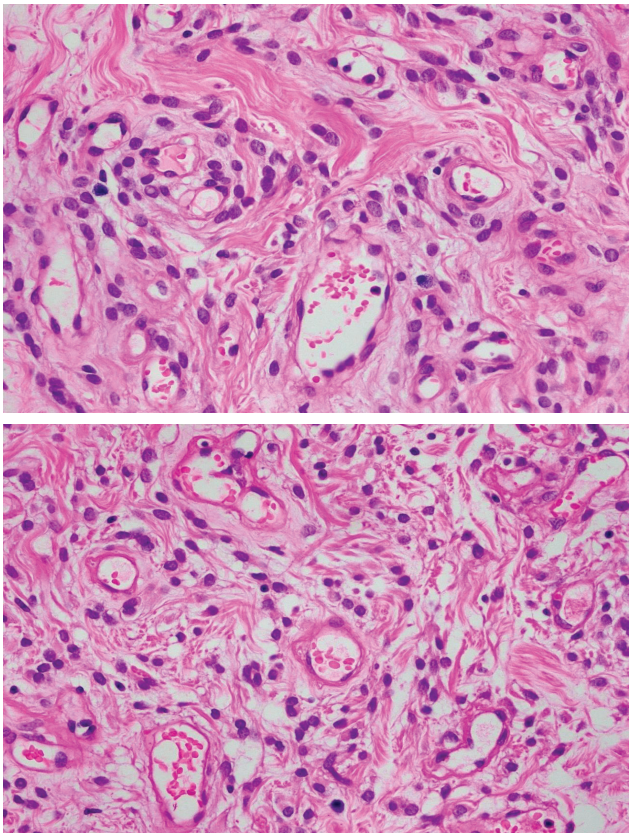


Figure 6. Numerous fat cells within the tumor (H&E, 40x).



Figures 7 and 8. Monomorphic spindle to epithelioid cells with bland chromatin, inconspicuous nucleoli, and absent mitoses and necrosis surrounding small to medium-sized dilated vessels (H&E, 400x).

of stromal cells distinctive of this anatomical region.[1] Angiomyofibroblastoma is uncommon, having an incidence comparable to that of deep (aggressive) angiomyxoma, which is unknown due to its rarity. These tumours arise predominantly in adult females, between menarche and menopause. About 10% of patients are postmenopausal. Convincing examples have not been described before puberty, and rare cases occur in males.

Pathophysiology is uncertain but may arise from subepithelial stroma or a perivascular stem cell.[1] In tumors with a significant adipocytic component, said components can possibly be caused by adipocytic metaplasia or fatty differentiation of tumor cells.[3] It has also been postulated that genital angiomyofibroblastomas may arise from precursor cells of hormonally responsive stroma, capable of multidirectional mesenchymal differentiation, which could be either be fibroblastic, myofibroblastic, or lipomatous.[2]

Common clinical features include a slowly enlarging, painless, circumscribed mass. It may be present for weeks to years before diagnosis, and multifocal cases are rare. The most frequent preoperative diagnosis is Bartholin gland cyst. Grossly, lesions are unencapsulated but well circumscribed, with a tan/pink cut surface and a soft consistency. Necrosis is not seen. Most cases measure <5 cm in maximum diameter, although rare examples as large as 10 cm have been recognized. The clinical presentation of this case is a one-year history of a slowly enlarging, non-tender mass which was initially observed to be 2.0 cm, with no other associated symptoms.

Histologically, tumor cells are round to spindle-shaped with eosinophilic cytoplasm, concentrated around vessels. Binucleated and multinucleated tumor cells are common. Mitoses are rarely seen. In postmenopausal women, stroma is more fibrous rather than edematous, with hyalinization of vessel walls. Essential criteria include the following: well-circumscribed, prominent stromal vessels, and round to spindle-shaped cells (often multinucleated) in a perivascular distribution.[1] Ten percent of cases have a prominent well-differentiated adipocytic component,[1] which are classified as lipomatous variants of angiomyofibroblastomas (LAMF) in review of literature.[3,4] While angiomyofibroblastomas are rare, LAMFs are even rarer, with no reported cases in the local setting.

In a 20-year literature review and case report done in 2015, there were a total of ten reported cases of angiomyofibroblastoma, four of which were the lipomatous variant.[4] Age at time of diagnosis ranged from 23 to 50 years old, and reported symptom was a painless mass or lesion. All LAMFs occurred in the vulva, and size varied from 1.1 to 11 cm with an average of 5.5 cm. In six of the cases, mature adipocytes comprised 30% to 80% of the mass. In all ten cases, LAMFs were histologically similar, being described as having hypercellular and hypocellular areas composed of spindle and epithelioid cells, in a background of loose and edematous material. Condensed spindle cells surrounding vascular networks were dispersed throughout the tumor, with mature adipocytes comprising a significant portion of the tumor volume. Features of malignancy like high mitotic rate, cytologic atypia, and necrosis were absent.

Another literature review and case report were done in 2016.[2] They reported 16 cases of LAMF, wherein the fatty component comprised 30% to >90% of the tumor. In this study, they state that LAMF may be used when fat cells constitute more than or equal to 30% of the tumor. Tumor size ranged from 1.8 to 11.0 cm, while patient age ranged from 23 to 69 years of age. Histologically, the case report revealed an abundance of fat cells involving around 85% of the tumor, with numerous medium and small-sized vessels, and multifocal fibrous areas and pseudoangiomatous spaces. Similar to the study done in 2015, there was a

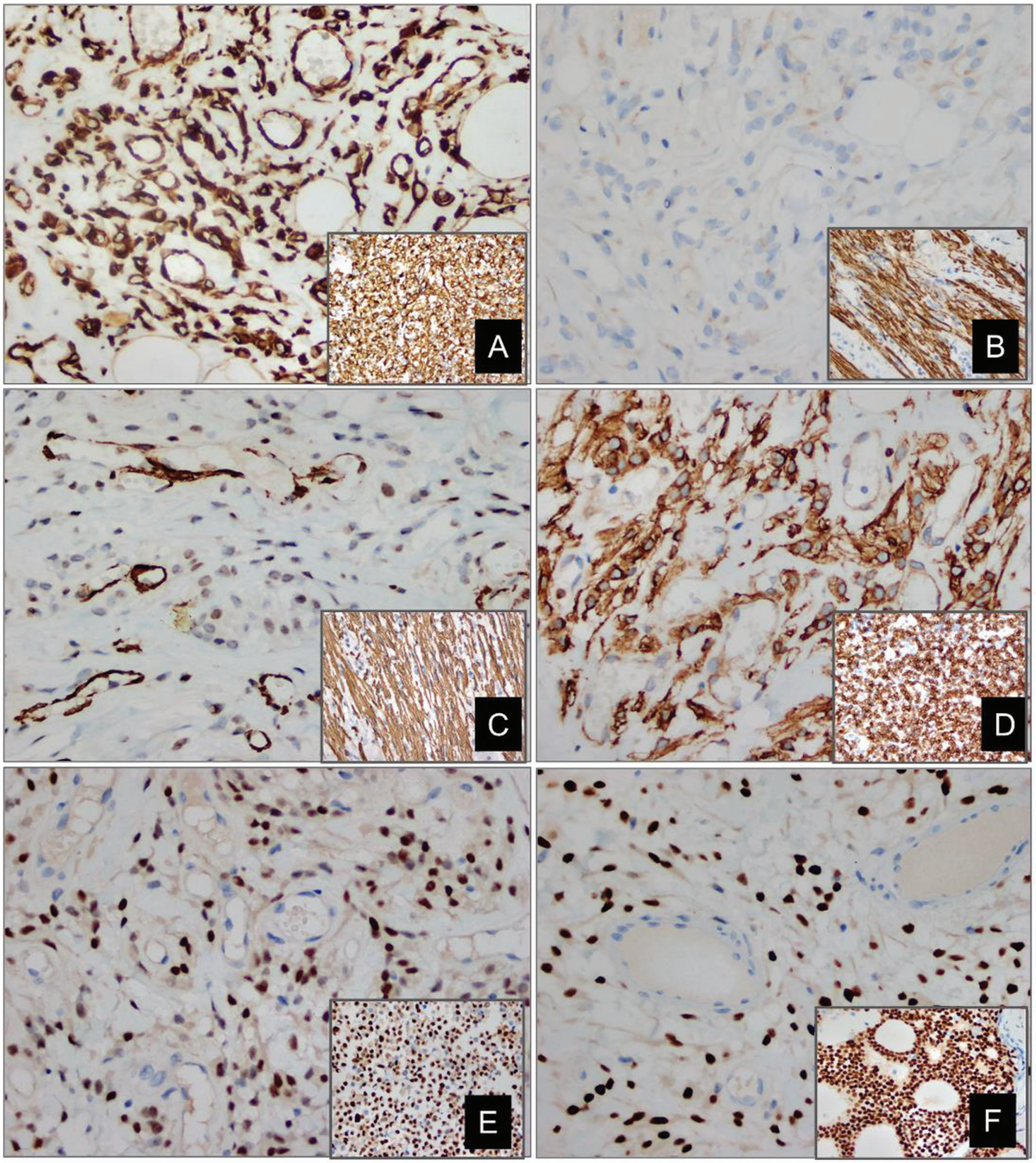


Figure 9. Immunohistochemistry stains (Horseradish peroxidase method, 400x) with respective positive controls *(inset)*: **(A)** Vimentin, **(B)** Desmin, **(C)** SMA, **(D)** BCL-2, **(E)** ER and **(F)** PR.

proliferation of spindle-shaped, rounded or epithelioid tumor cells in small nests and cords within perivascular fibrous tissue located in both fatty and fibrous areas. No mitotic figures were seen.[4]

Immunohistochemical studies of the tumor cells based on the aforementioned studies would show positive staining for vimentin, BCL-2, estrogen and progesterone receptors. Occasionally, tumor cells would be positive in CD34, desmin, CD10, CD99, muscle-specific actin, smooth muscle actin, and S100. Negative staining is seen for cytokeratin, EMA, c-kit, HMB45, GFAP, CD68, and factor VIII.[2,4] This is in comparison to the WHO Classification of Female Genital Tumors, which states that majority of cases would show strong and diffuse positive staining for desmin, and focal positivity for SMA or pan-muscle actin. However, it was also acknowledged that desmin staining may be reduced or absent in postmenopausal cases.[1]

Differential diagnoses for LAMF include benign and malignant mesenchymal neoplasms of the female genital tract. Benign mesenchymal neoplasms include superficial and aggressive angiomyxoma, solitary fibrous tumor, cellular angiofibroma, and spindle cell lipoma. Liposarcomas fall under the category of malignant mesenchymal neoplasms.

Superficial angiomyxomas present as multinodular dermal or subcutaneous myxoid lesions that consist of bland spindle cells. They are seen on the vulva, but they are more commonly seen on the lower limbs, head, and neck.[5] They occur more frequently in males, but in females, they usually present at reproductive age. Compared to LAMF, they have no perivascular congregation of cells, with prominent stromal mucin and conspicuous stromal neutrophils, with the last feature not seen in LAMF. Immunohistochemical studies are not useful in distinguishing between the two.[4] Superficial angiomyxoma was ruled out due to patient's age and the histomorphologic features of the tumor, which had the presence of perivascular congregation of said cells and lack of stromal mucin and conspicuous stromal neutrophils.

Aggressive angiomyxomas (AAM) occur in deep soft tissues of the perineum, pelvis, and genitalia of women of reproductive age. They are typically large, with most tumors measuring more than 10 cm, and have a more uniformly hypocellular myxoid stroma. Tumor cells are more uniformly spindled, with infiltrative borders and no perivascular congregation of spindle cells. Despite the word "aggressive" in its name, the term mainly refers to the tumor's predilection for local recurrence and is considered a benign tumor. They have a high local recurrence rate. AAM was ruled out as well, due to the patient's age, the histomorphologic features of the patient's tumor, which presented with perivascular congregation of tumor cells and no presence of infiltrative borders. Immunohistochemical studies overlap among LAMF and AAM,[3] but it has been noted that tumor cells of AAMs have a positive staining for HMGA2.[6] Despite this, HMGA2 is non-specific for AAM as positive staining can also be seen in a subset of leiomyomas. Correlation between immunohistochemical and histomorphologic features are therefore essential.

Solitary fibrous tumors are grossly well-circumscribed masses, usually unencapsulated, that consist of ovoid to fusiform spindle cells with indistinct cell borders arranged haphazardly or in short, ill-defined fascicles. Compared to LAMF, they are more commonly seen in extragenital sites and have prominent staghorn vessels with thick keloid-like stromal collagen bands. CD34 and STAT6 are positive on immunohistochemical stain, but like superficial angiomyxomas, these studies are not useful in distinguishing between the two. Solitary fibrous tumors were ruled out due to the site of predilection of these tumors being extra-genital, as well as the patient's tumor not exhibiting prominent staghorn vessels with thick stromal collagen bands.

Cellular angiofibromas are also superficial soft tissue tumors, but compared to LAMF, the tumor is more highly cellular, with an abundance of vessels and the cells that proliferate are primarily spindled. Stroma is also more collagenous and/or hyalinized. In terms of immunohistochemistry stains, cellular angiofibromas are more frequently positive for Desmin, CD34, ER, and PR.[3,4] Cellular angiofibromas were ruled out as the patient's tumor also had presence of hypocellular areas, and the cells were not exclusively spindle-shaped, as it also included epithelioid cells.

Spindle cell lipomas are similar to LAMFs in that there are proliferating spindle cells, however these are the only cells that the tumor consists of, and they do not form nests. They are also usually seen as a component of leiomyomas and tend to be located within the myometrium instead of the vulva.[4] ER positivity has also not been reported in spindle cell lipoma.[3] Spindle cell lipomas were ruled out due to the site of predilection, as well as the presence of epithelioid cells and formation of nests of cells in the patient's tumor.

Malignant mesenchymal neoplasms include liposarcoma. Liposarcomas can also arise in the soft tissue of the vulva in middle-aged women, with tumor cells exhibiting significant nuclear atypia.[7] In cases where there is minimal nuclear atypia, as seen in well-differentiated liposarcoma, the presence of lipoblasts would favor liposarcoma over LAMF. Local recurrence in liposarcomas of the vulva have been reported. In the case of the patient's tumor, no nuclear atypia, no mitoses and no lipoblasts were seen.

The clinical presentation of a slowly growing vulvar mass over a course of one year not associated with any other symptoms, the gross appearance of a circumscribed, lobulated, soft to rubbery mass with defined borders, the microscopic features composed of edematous stroma having hypo- and hypercellular areas containing spindle to epithelioid cells with scattered thin-walled small to medium sized vessels amongst a background of abundant mature adipocytes, absence of nuclear atypia, necrosis, and mitoses are features compatible with lipomatous angiomyofibroblastoma. The positive stains for vimentin, smooth muscle actin, BCL-2, ER, and PR although non-specific also support our histologic diagnosis.

While there are multiple differential considerations for LAMF, immunohistochemical studies are not useful in distinguishing between the multiple soft tissue tumors as most have similar staining profiles.[8] Ultimately, differentiation among the tumors is based on clinical history and histomorphologic characteristics. In terms of treatment, conservative local excision is considered curative, and local recurrence is rare.[2]

CONCLUSION

Lipomatous angiomyofibroblastoma (LAMF) is a rare phenomenon, with few reported cases on literature review and none in the local setting. While immunohistochemistry studies are similar among the soft tissue tumors located in the vulva, diagnosis of LAMF is mainly based on clinical history, morphologic and histopathologic characteristics. This matters since differential diagnoses include a whole gamut of benign mesenchymal as well as aggressive angiomyxomas and liposarcomas of the lower genital tract.

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ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

DATA AVAILABILITY STATEMENT

No datasets were generated or analyzed for this study.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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PHILIPPINE JOURNAL OF PATHOLOGY
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World Wide Web

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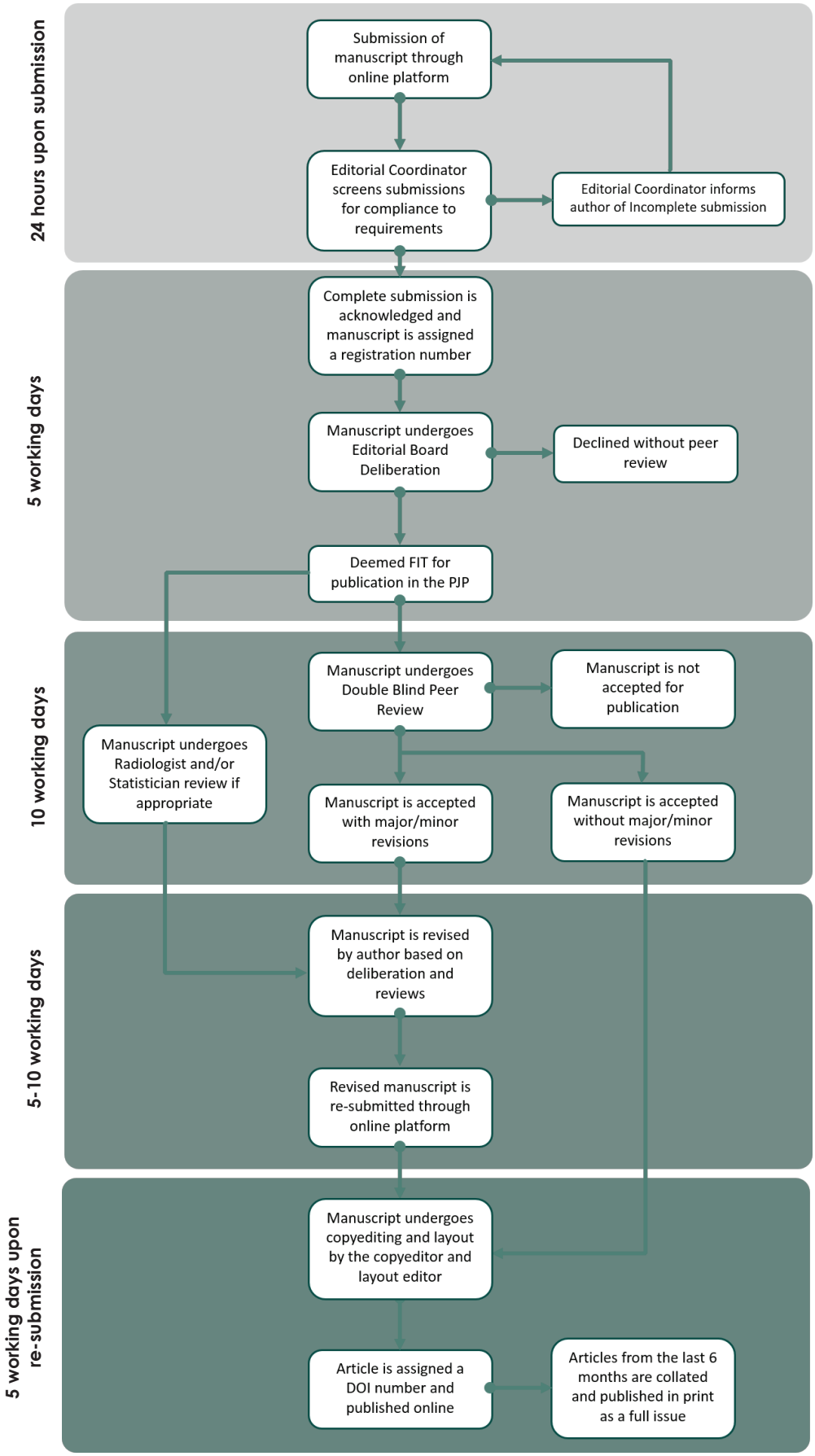

Figure 1. Editorial Process Flow.

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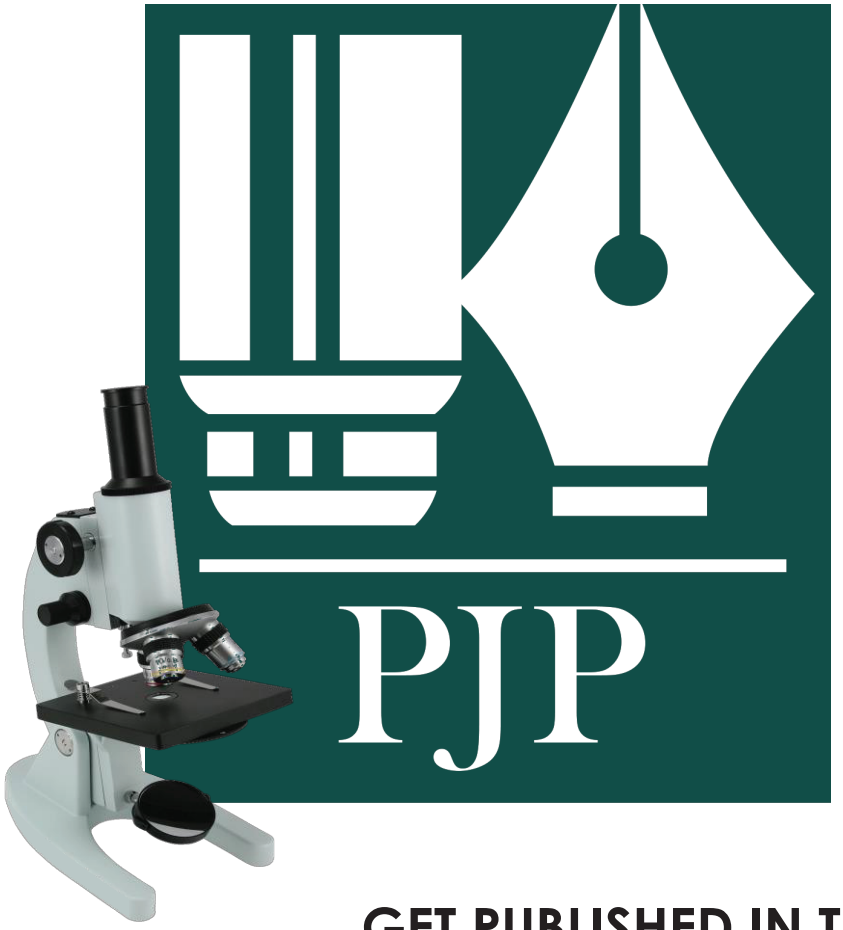

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