



Case Report

# Discordant Automated and Conventional Identification of Invasive Trichosporonosis: A Case for Enhanced Laboratory Practices



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## Abstract

**Background and objectives:** Accurate identification of invasive fungal pathogens is crucial for appropriate antifungal therapy. The Department of Clinical Laboratory at Indus Hospital & Health Network, Karachi, Pakistan, reported two cases of invasive fungal infections between 1<sup>st</sup> January and 31<sup>st</sup> March 2024 in which conventional identification methods and automated systems produced discordant results, highlighting critical diagnostic challenges. **Case presentation:** Two invasive yeast isolates initially showing budding yeast cells without pseudohyphae on Gram stain were subjected to conventional identification using cornmeal-Tween 80 agar, chrome agar, and BiGGY agar, followed by automated identification using the VITEK 2 ID-YST system and confirmatory API 20C AUX testing. Both isolates demonstrated typical soft, wrinkled, cream-colored colonies on Sabouraud dextrose agar, which on chrome agar appeared as dry, blue colonies and on BiGGY agar as dry, brown colonies. Characteristic arthroconidia and blastoconidia formation on cornmeal-Tween 80 agar were observed, consistent with *Trichosporon* species. However, the VITEK 2 ID-YST system identified both isolates as *Cryptococcus laurentii* with good confidence levels. India ink staining was negative for both isolates. Confirmatory API 20C AUX testing correctly identified both isolates as *Trichosporon asahii* (identification profile 3740734). **Conclusions:** This discordance between automated and conventional methods underscores the continued importance of conventional identification techniques and highlights potential limitations of automated systems for certain uncommon yeasts. Laboratories should maintain proficiency in conventional methods and consider confirmatory testing when automated results conflict with morphological findings. The clinical implications of misidentification include inappropriate antifungal selection, given the different susceptibility patterns between these species.

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## Introduction

Invasive infections caused by non-*Candida* yeasts have emerged as significant clinical challenges, particularly in immunocompromised patients.<sup>1</sup> Among these, *Trichosporon asahii* (*T. asahii*) represents an important opportunistic pathogen capable of causing both superficial and invasive infections, with mortality rates exceeding 80% in disseminated disease.<sup>1,2</sup> Accurate species-level identification is paramount for optimal patient management, as different yeast species exhibit distinct antifungal susceptibility patterns and clinical behaviors.

*T. asahii*, historically known as the causative agent of white piedra, has gained recognition as an emerging cause of bloodstream infections in immunocompromised hosts.<sup>2</sup> The organism is characterized by its ability to form arthroconidia, blastoconidia, and pseudohyphae, distinguishing it from other yeasts. In contrast, *C. laurentii* is considered a saprophytic yeast that rarely causes human infections, typically presenting as an opportunistic pathogen in severely immunocompromised patients.<sup>3</sup>

The diagnostic landscape for yeast identification has evolved significantly with the introduction of automated modalities such as the VITEK 2 automated system. While these systems offer rapid turnaround times and standardized protocols, concerns persist regarding their accuracy for uncommon species. Several studies have documented misidentification rates ranging from 1.7% to 21% for various automated platforms, with particular challenges noted for non-*Candida* yeasts.<sup>4,5</sup>

Conventional identification methods, including morphological assessment on specialized media and biochemical testing through systems such as API 20C AUX, remain an established reference method in many clinical laboratories.<sup>6</sup> These methods rely on phenotypic characteristics that have been validated over decades of clinical use. However, the interpretation of conventional methods requires specialized expertise

**Keywords:** *Trichosporon asahii*; *Cryptococcus laurentii*; VITEK 2 automated system; Fungal pathogens; Invasive infection.

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**Table 1. Characteristics, antifungal susceptibility, and clinical implications of *Trichosporon asahii* and *Cryptococcus laurentii***

Characteristic	<i>Trichosporon asahii</i>	<i>Cryptococcus laurentii</i>
Usual clinical setting	Immunocompromised hosts; invasive disease	Rare; typically in severely immunocompromised
Colony morphology	Dry, wrinkled, cream-colored; leathery	Mucoid, smooth, cream-colored
Key microscopy features	Arthroconidia, blastoconidia, pseudohyphae	Usually round yeast cells, variable capsule
India ink stain	Negative for capsule	May show thin or absent capsule
Echinocandin susceptibility	Resistant	Variable; often susceptible
Azole susceptibility	Susceptible (voriconazole preferred)	Variable; fluconazole usually effective
Therapeutic implication	Avoid echinocandins; use azoles	Fluconazole or amphotericin B
Potential for misidentification	High with automated systems	High with automated systems
Clinical consequence of error	Inappropriate therapy, increased mortality	Risk of undertreatment or overtreatment

that may be declining in many clinical laboratories.

The clinical implications of yeast misidentification extend beyond academic interest (Table 1). *Trichosporon* species demonstrate notable resistance to echinocandins; most isolates retain susceptibility to azole antifungals, and voriconazole is frequently active, but reduced susceptibility or resistance to voriconazole has been reported in sporadic cases.<sup>7</sup> Conversely, *C. laurentii* exhibits different susceptibility patterns, with variable azole resistance reported in environmental isolates.<sup>8</sup> Misidentification could therefore lead to inappropriate empirical therapy, treatment delays, and adverse patient outcomes.

Cross-reactivity between *Trichosporon* and *Cryptococcus* antigens has been well documented, with *T. asahii* possessing cell wall components that cross-react with *C. neoformans* capsular polysaccharide.<sup>9</sup> This immunological similarity may contribute to diagnostic confusion and potentially explains some automated system misidentifications.

We report two cases of invasive fungal infections in which conventional identification methods correctly identified *Trichosporon* spp. (API-identified as *T. asahii*), while the VITEK 2 automated system consistently misidentified the organisms as *C. laurentii*. This discordance highlights the continued importance of conventional identification techniques and raises important questions about reliance on automated systems for definitive yeast identification.

### Case presentation

Two patients with suspected invasive fungal infections were identified during routine clinical microbiology surveillance at the Section of Microbiology, Department of Clinical Laboratory, Indus Hospital & Health Network, Karachi, Pakistan, between 1<sup>st</sup> January and 31<sup>st</sup> March 2024. The first case involved a three-year-old female with pelvic rhabdomyosarcoma and a right percutaneous nephrostomy tube, admitted with fever and presumptive urosepsis. The second case was a 43-year-old female with hypertension and chronic kidney disease stage 4, admitted with volume overload and suspected sepsis. Both patients were started on a 14-day course of oral voriconazole based on the preliminary identification. Following treatment, repeat cultures showed no growth, and both patients demonstrated clinical improvement and were subsequently discharged.

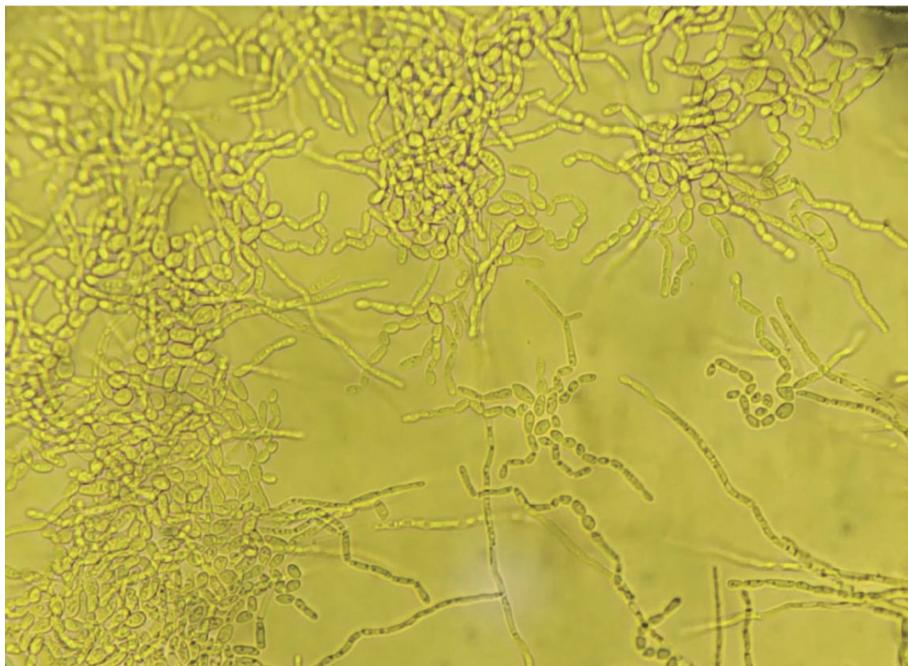
During their hospital stay, blood cultures were collected

as part of the diagnostic workup, according to standard protocols, using the BD BACTEC blood culture system (Becton, Dickinson and Company). Positive blood culture bottles were detected through automated flagging and subsequently processed for gram stain, fungal isolation and identification.

Primary isolation was performed by subculturing positive blood culture bottles onto 2 Sabouraud dextrose agars, which were incubated at 25 °C and 37 °C for 48–72 h. Initial morphological assessment was conducted using standard Gram staining techniques to evaluate cellular morphology, budding patterns, and the presence of pseudohyphae. For conventional identification, isolates were subcultured onto cornmeal-Tween 80 (CMT) agar to assess pseudohyphae, arthroconidia, and blastoconidia formation, following standard protocols with incubation at 25 °C for 48–72 h. Chrome agar (CHROMagar Candida, CHROMagar, Paris, France) was used for colony color differentiation, with incubation at 37 °C for 24–48 h. BiGGY (Bismuth Glucose Glycine Yeast) agar was employed for phenotypic characterization, with incubation at 25 °C for 48–72 h. India ink staining was performed using standard techniques to assess capsule formation. Automated identification was conducted using the VITEK 2 automated system (bioMérieux) with ID-YST cards according to the manufacturer's specifications, utilizing 47 biochemical reactions with fluorometric detection for yeast identification. Confirmatory identification was performed using API 20C AUX (bioMérieux), consisting of 20 microtubes containing dehydrated substrates for carbohydrate assimilation testing, with results interpreted after 48–72 h of incubation at 30 °C according to manufacturer guidelines. Standard quality control procedures were implemented using reference strains including *Candida albicans* ATCC 10231 and *Cryptococcus neoformans* ATCC 208821, with all media tested for sterility and growth promotion prior to use.

Blood cultures became positive after 24–48 h of incubation, with subsequent subcultures yielding pure yeast isolates that demonstrated consistent morphological and biochemical characteristics. Initial Gram staining of both isolates revealed oval to round budding yeast cells measuring approximately 3–5 µm in diameter. Notably, pseudohyphae were not observed in the initial Gram stain preparations. Upon subculture to CMT agar, both isolates demonstrated characteristic *Trichosporon* morphology, including the formation of arthroconidia, blastoconidia, and pseudohyphae (Fig. 1).

Colony morphology on routine Sabouraud dextrose agar

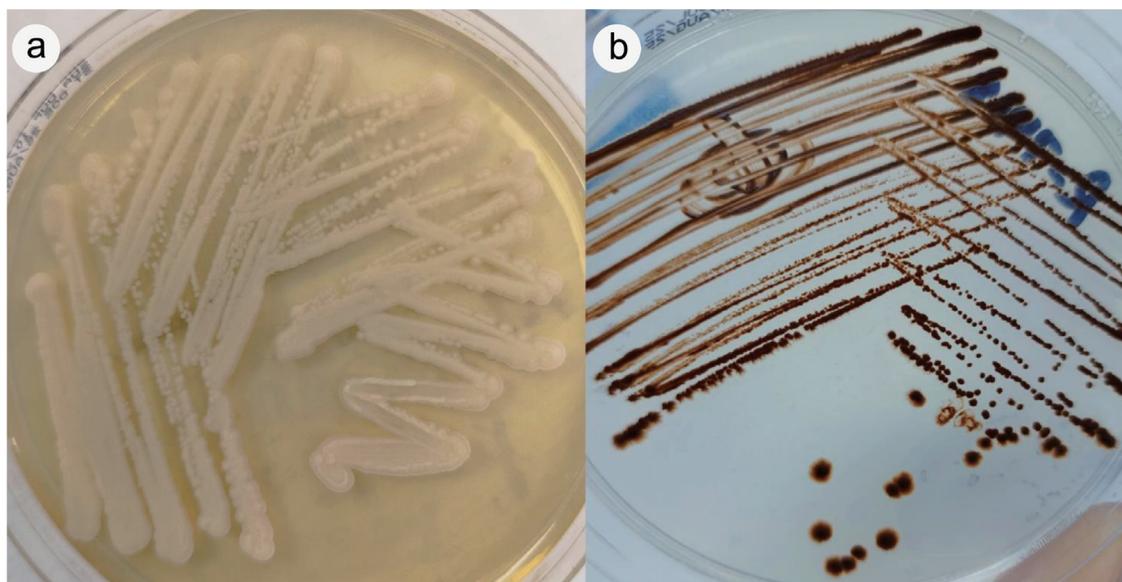


**Fig. 1.** Microscopic appearance of *Trichosporon asahii* colonies cultured on cornmeal-Tween 80 agar (Magnification: 40×).

showed dry, wrinkled, cream-colored colonies typical of *Trichosporon* spp., with a characteristic leathery texture that distinguished them from typical *Candida* isolates (Fig. 2a). Chrome agar revealed distinctive dry, blue colonies, while BiGGY agar produced dry, brown colonies, both characteristic findings for *T. asahii* (Fig. 2b). These morphological characteristics, combined with the specialized media results, provided strong phenotypic evidence for *Trichosporon* identification. However, the VITEK 2 ID-YST system identified both isolates as *C. laurentii*, exhibiting positivity for urease, phosphatase, and the assimilation of numerous carbohydrates, including D-glucose, D-galactose, and sucrose, whereas

amygdalin assimilation, esculin hydrolysis, and the enzymatic test  $\beta$ -N-acetyl-glucosaminidase (NAGA1) were negative. This combination of reactions allowed the VITEK 2 YST system to identify *C. laurentii* with a high confidence level of 85%–90%, which was consistently reproduced upon repeat testing, suggesting systematic rather than random error and raising concerns about the reliability of automated systems for this particular species.

API 20C AUX testing confirmed the conventional identification, correctly identifying both isolates as *T. asahii* based on carbohydrate assimilation patterns. The identification profile 3740734 corresponded to *T. asahii* with an identification



**Fig. 2.** Colony morphology of *Trichosporon asahii* on (a) Sabouraud dextrose agar and (b) Bismuth glucose glycine yeast agar.

probability of 99.9%, as reported in the APIWEB™ database (bioMérieux). The assimilated substrates included glucose, glycerol, 2-keto-gluconate, adonitol, xylose, galactose, inositol, lactose, maltose, sucrose, trehalose, and raffinose, while the organism was negative for assimilation of erythritol, arabinol, gluconate, dulcitol, cellobiose, melezitose, and soluble starch. This confirmatory testing validated the conventional identification methods and provided additional confidence in the final species determination.

## Discussion

The discordance between automated and conventional methods raises important questions about the reliability of automated systems for uncommon yeasts. Previous studies have documented similar challenges with VITEK 2 identification of *Trichosporon* spp., with accuracy rates varying significantly depending on the species and database version used.<sup>10,11</sup> The automated system's misidentification likely stems from similarities in certain biochemical reactions between *T. asahii* and *C. laurentii*, particularly in carbohydrate utilization patterns that may overlap under automated testing conditions. This highlights a fundamental limitation of automated systems when dealing with species that share similar metabolic profiles but differ significantly in morphological characteristics. The cross-reactivity between *Trichosporon* and *Cryptococcus* antigens, well documented in the literature, may contribute to this diagnostic confusion, as *T. asahii* possesses cell wall components that cross-react with *C. neoformans* capsular polysaccharide.<sup>9</sup>

The API 20C AUX system's accuracy in these cases demonstrates the continued value of comprehensive biochemical testing for challenging yeast identifications, particularly when automated systems provide conflicting results. The API system's superior performance likely reflects its more comprehensive substrate panel and longer incubation period, allowing for better phenotypic differentiation between morphologically similar species.

Both isolates were negative for India ink staining, excluding the presence of a significant capsule. This finding was inconsistent with a *C. laurentii* identification, as many *Cryptococcus* spp. demonstrate variable capsule formation, although *C. laurentii* is known to have a thin or absent capsule.<sup>12</sup> The negative India ink result, combined with the characteristic morphological findings on specialized media, provided additional evidence against the automated system's identification and supported the conventional identification as *Trichosporon* spp.

The misidentification of *T. asahii* as *C. laurentii* carries significant clinical implications that extend beyond laboratory accuracy to patient care outcomes (Table 1). *Trichosporon* spp. demonstrate intrinsic resistance to echinocandin antifungals, including anidulafungin, micafungin, and caspofungin, which are often used as first-line therapy for invasive candidiasis.<sup>7</sup> This resistance pattern is due to differences in cell wall composition, particularly the absence of  $\beta$ -1,3-glucan, the target of echinocandin antifungals. In contrast, azole antifungals, particularly voriconazole, demonstrate excellent activity against most *Trichosporon* spp. and represent the preferred therapeutic option for invasive trichosporonosis.<sup>13</sup> *C. laurentii*, while rare as a human pathogen, typically responds to fluconazole therapy, although resistance has been reported in some environmental isolates.<sup>8</sup> The different therapeutic approaches required for these organisms underscore the critical importance of accurate identification for optimal patient management and highlight the potential consequences of diagnostic errors.

The successful treatment of both patients with voriconazole and their subsequent clinical and microbiological recovery provides compelling therapeutic confirmation of the correct identification as *T. asahii* rather than *C. laurentii*. This clinical outcome is significant because the two pathogens have distinct antifungal susceptibility profiles. *Trichosporon* spp. exhibit intrinsic resistance to echinocandins but are typically susceptible to azoles, particularly voriconazole, which is recommended as first-line therapy for invasive trichosporonosis. In contrast, while *C. laurentii* may respond to fluconazole, its susceptibility to voriconazole is less predictable and not considered a standard first-line therapeutic option. The rapid resolution of fungemia and clinical improvement following voriconazole monotherapy aligns with the expected response for *Trichosporon* infection and contradicts the likely suboptimal outcome had the misidentification as *C. laurentii* been reported without confirmatory testing. This underscores that clinical response can serve as an adjunctive validation of microbiological identification, especially in cases of diagnostic discrepancy.

The reliability of conventional identification methods for *Trichosporon* spp. without molecular confirmation warrants consideration. While API 20C AUX and morphological characteristics are well-established tools with documented accuracy for many yeasts, their precision for the *Trichosporon* genus can be variable due to phenotypic similarities among closely related species. In our cases, the identification of *T. asahii* was supported by a consistent phenotypic profile across conventional culture media, including characteristic arthroconidia formation on CMT agar and distinctive colony morphology on chrome and BiGGY agars, all of which aligned with the API 20C AUX result. This concordance strengthened the validity of our identification. Nevertheless, we acknowledge that definitive species-level resolution within this complex genus often requires molecular techniques, such as sequencing of the internal transcribed spacer region. The absence of such confirmation is a limitation of our study, and future work would benefit from integrating molecular analysis to unequivocally validate conventional identifications.

This report highlights several important diagnostic considerations that have broader implications for clinical mycology practice. First, the morphological variability observed in these cases, particularly the absence of pseudohyphae in initial Gram stains despite subsequent characteristic features on specialized media, can be misleading and emphasizes that morphological assessment must extend beyond single methodologies. This variability may result from different growth conditions, inoculum size, or the physiological state of organisms in blood culture bottles, requiring interpretation within a broader diagnostic context. Second, while automated identification systems offer significant efficiency and standardization advantages, they create potential vulnerabilities when encountering uncommon species or those presenting with atypical phenotypic profiles. The known limitations of automated systems for certain species categories, particularly non-*Candida* yeasts that share biochemical similarities with common pathogens, require explicit recognition and implementation of mitigation strategies. Third, the cross-reactivity between *Trichosporon* and *Cryptococcus* spp. extends beyond morphological similarities to encompass antigenic cross-reactions that can confuse both automated identification systems and serological assays, reflecting an underlying immunological similarity that continues to present diagnostic challenges in clinical practice.<sup>9</sup> Fourth, database limitations in automated systems represent a fundamental constraint, as these platforms rely on comprehensive phenotypic databases that may have insufficient representation of species that are less commonly encountered, particularly

those causing rare but clinically significant infections. These considerations collectively underscore that accurate identification of uncommon invasive fungal pathogens requires a multifaceted approach integrating morphological expertise, recognition of system limitations, and appropriate confirmatory strategies.

Based on the findings, several recommendations are proposed. First, clinical laboratories should maintain expertise in conventional identification methods, ensuring ongoing training and quality assurance to sustain competency in morphological assessment and specialized media interpretation. Second, verification protocols should be implemented for discordant results between automated systems and morphological findings, with clear algorithms guiding when confirmatory testing is warranted. Third, molecular methods such as DNA sequencing should be considered for definitive identification, especially for clinically significant isolates or those with conflicting identification results. Fourth, educational initiatives in clinical mycology should be intensified to address interpretation skills amidst increasing reliance on automation. Lastly, automated system manufacturers should enhance their databases to include comprehensive phenotypic data of uncommon species, improving diagnostic accuracy.

Consideration of molecular methods for definitive identification represents an important advancement in clinical mycology, particularly for isolates with clinical significance or those showing discordant identification results. DNA sequencing, particularly of the internal transcribed spacer region, provides definitive species identification and can resolve cases where conventional and automated methods disagree.<sup>14</sup> However, the implementation of molecular methods requires significant investment in equipment, training, and quality assurance, making them more suitable for reference laboratories or specialized centers. Educational initiatives in clinical mycology remain essential for maintaining diagnostic accuracy in an era of increasing automation, as the interpretation of morphological findings and the recognition of discordant results require specialized expertise that may be declining in many clinical laboratories.

The antifungal considerations arising from accurate identification cannot be overstated, particularly given the high mortality rates associated with invasive trichosporonosis. *Trichosporon* bloodstream infections carry mortality rates exceeding 80% in some series, particularly when inappropriate therapy is initiated or when diagnosis is delayed.<sup>1,2</sup> Early recognition and appropriate antifungal selection are crucial for patient survival, with current guidelines recommending voriconazole as the preferred agent for invasive trichosporonosis based on its excellent *in vitro* activity and favorable clinical outcomes.<sup>15</sup> Alternative agents include posaconazole and itraconazole, while amphotericin B demonstrates variable activity depending on the species and strain.<sup>7,13,15,16</sup> The therapeutic landscape for trichosporonosis continues to evolve, with new antifungal agents under development and combination therapy strategies being explored for severe cases.

Our study had a few limitations that should be acknowledged, including the small number of cases and the single-center experience, which may limit the generalizability of our findings. Additionally, molecular confirmation was not performed, relying instead on conventional methods and API 20C AUX results for definitive identification. However, the consistent findings across multiple identification platforms and the characteristic morphological features provide strong evidence for the correct identification and support the conclusions drawn from this case series.

The emergence of *Trichosporon* as a significant invasive

fungal pathogen, combined with the challenges of accurate identification, demands continued vigilance and methodological improvement in clinical mycology. Our cases demonstrate that while automated systems provide valuable diagnostic support and have revolutionized clinical microbiology practice, they cannot replace the clinical microbiologist's expertise in recognizing and resolving discordant results. The continued evolution of the fungal pathogen landscape, driven by increasing immunosuppression and antimicrobial pressure, requires adaptive diagnostic strategies that can accommodate both common and uncommon species. Conventional methods, despite being labor-intensive and requiring specialized expertise, remain indispensable for accurate identification of uncommon yeasts and serve as a critical quality control measure for automated systems. The integration of multiple identification approaches, supported by appropriate training and quality assurance programs, represents the optimal strategy for maintaining diagnostic excellence in clinical mycology.

## Conclusions

These cases demonstrate that while automated identification systems provide valuable diagnostic support, they cannot replace expert interpretation in resolving discordant results. Accurate species-level identification of uncommon invasive yeasts remains essential for optimal patient management. Clinical laboratories must maintain proficiency in conventional identification methods and implement confirmatory testing strategies when automated results conflict with morphological findings. The integration of morphological expertise, recognition of system limitations, and appropriate verification approaches is fundamental to maintaining diagnostic excellence in clinical mycology.

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## Conflict of interest

The authors declare no conflicts of interest.

## Author contributions

Conceptualization (MAK), data curation (MA, SBF), formal analysis (MAK, MA), investigation (MAK, MA, SBF), supervision (MAK), validation (MAK, MA), visualization (MAK), writing – original draft (MAK), and writing – review & editing (MA, SBF, SZ). All authors have approved the final version and publication of the manuscript.

## Ethical statement

This study was performed following the ethical standards of the institutions to which the authors are affiliated and in accordance with the Declaration of Helsinki (as revised in 2024). The protocol was approved by the Institutional Review Board (IHNN-IRB#1 Biomedical/Clinical) of the Indus Hospital & Health Network (Ref # IHNN\_IRB\_2024\_09\_004). Written informed consent was obtained from the legally authorized guardian in the first case and from the patient in the second case for publication of this case report.

## Data sharing statement

All data generated or analyzed during the course of this study are included in this article.

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