Note

Emergence of *Vanrija humicola* as a pathogen of urinary tract infections in Korea

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Abstract

Vanrija humicola, a yeast belonging to *Trichosporonaceae*, is rarely pathogenic. All cases of isolation of *V. humicola* were retrospectively reviewed from 2021 to 2023. A total of four *V. humicola* were isolated from urine samples. Organisms cultured for 5 days at 25°C produced yellow, dry and cerebriform colonies, and were successfully identified as *V. humicola* using Bruker Biotyper MALDI-TOF. Two recent isolates were resistant to fluconazole, echinocandins, and flucytosine. In all 4 cases, *V. humicola* was sporadically isolated more than 14 days after admission. One case was presumed to be colonized. Of the other three cases that developed a urinary tract infection (UTI), only one with pancytopenia was treated for UTI by *V. humicola* with caspofungin, but expired 4 days later. *V. humicola* has emerged as a drug-resistant fungal pathogen of hospital-acquired UTI. Species identification and antifungal susceptibility testing of this organism are required for critical patients.

Keywords: *Vanrija humicola,* urine, culture, infection, antifungal susceptibility

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The genus *Vanrija* was initially characterized by Moore with *Vanrija humicola* designated as the type species [1,2]. Comprising *basidiomycete* fungi within the *Trichosporonaceae* family, *Vanrija* is widely distributed in the environment but an oppotunistic pathogen in severely debilitated hosts [3]. Among the nine recognized *Vanrija* species [4], *V. humicola* has previously been referred to as *Cryptococcus humicola, Candida humicola, Cryptococcus humicolus, Torula humicola, Apiotrichum huicola, Azymoprocandida humicola, Mycotorula humicola, or Asterotremella humicola* [5]. There is fewer than 10 cases published [2,3,6-9]. Systemic infections of *V. humicola* occur in immunocompromized hosts, such as central nervous system infections in human immunodeficiency virus (HIV) infected patients or cancer patients in literatures [9]. Until now, the only two reports regarding on urinary isolates were reported available in English [6,10], and there is still no report on a human infection in Korea. At our hospital, a tertiary care hospital in Seoul, Korea, *V. humicola* has been infrequently detected since it first appeared in 2021. This study therefore aims to elucidate the clinical significance of *V. humicola* isolated at our hospital.

Since 2016, a total of four cases of *V. humicola* identified to species-level using MALDI Biotyper (Bruker Daltonics, Bremen, Germany) were recovered from urine culture. The clinical characteristics of the patients with *V. humicola* were retrospectively reviewed through electronic medical records. Clinical relevance of *V. humicola* were evaluated with pathogenicity, infection types, risk factors of acquisition and epidemiological linkage between the cases. This study was approved by the Institutional Review Board of Asan Medical

Center (No. 2024-0049) and the requirement for informed consent was waived. Two recent isolates were subcultured on blood agar plate (BAP), potato dextrose agar, and Sabouraud dextrose agar (SDA) plate and colonial and microscopic growth was observed with incubation at both 25°C and 37°C. Phenotypic tests were performed using the VITEK2 YST (bioMérieux), API 20C AUX (bioMérieux) and urease test tubes. Sequence analysis was carried for the internal transcribed spacer and D1/D2 regions of the 26S ribosomal DNA. Antifungal susceptibility test was performed using the Sensititre YeastOne YO10 AST (Thermo Fisher Scientific) with visual reading after 48 hours of incubation at 25°C.

All four cases recovered *V. humicola* from urine cultures after hospitalization for 14-100 days due to serious underlying and comorbid diseases. None of them did not cluster during hospitalization (Table 1). All except one showed symptoms or signs of cystitis as well as urine cultures produced pure culture of *V. humicola* > 100,000 CFU/mL. A man with a traumatic esophageal rupture had 3,000 CFU/mL of *V. humicola* isolated from his urine, and the urine analysis was normal, suggesting that he was colonized with *V. humicola*. The other three were immunocompromized, of whom two were with cancer chemotherapy and one with pancytopenia and prednisolone treatment. Two indwelled foley catheter for 24 and 45 days, respectively, and developed hematuria. Although one patient received voriconazole due to *Candida krusei* isolated from mediastinitis sites, the only patient to receive antifungal treatment targeting *V. humicola* infection was an 86-year-old man with pancytopenia who received 60 mg prednisolone daily for 25 days. The patient was empirically treated with 50 mg caspofungin for three days, but died 4 days after isolation of *V. humicola*, before the species identification was reported. Three remaining patients were discharged with resolution of chief complaints without any treatment targeted for *V. humicola* (Table 1).

All four isolates were identified to *V. humicola* first with a score of 1.37 - 1.85 by Biotyper, and urease-positive when tested using the colonies on BAP. Culture growth of two recent isolates was better at 25°C than 37°C. With an incubation at 25°C, it presented yellow, dry and cerebriform colonies with a fimbriate margin on day 5. Microscopic examination revealed budding yeasts of oval or ellipsoidal cell with pseudohyphae (Fig. 1). The VITEK2 YST card failed to identify species due to no active reaction, and API 20C AUX identified *V. humicola* with the first priority but not differentiated from *Trichosporon mucoides*. The colonies on SDA were better identified with the score values of 1.94 and 2.04, respectively, while those on BAP showed the score values of 1.41 and 1.37, respectively as a score value of Biotyper. Sequence analysis of both isolates yielded excellent identification of *V. humicola* (Table 2). Antifungal susceptibility of these two isolates showed high minimum inhibitory concentrations (MIC) to fluconazole, caspofungin, micafungin, anidulafungin and 5-flucytosine (Table 2).

Table 1. Clinical presentation of the four patients with *Vanrija humicola* isolated in urine samples

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Age (yr) / sex	46/F	49/M	71/M	86/M
Underlying diseases	Cervical cancer, past breast cancer 1 year ago	Esophageal rupture with multiple repair surgery	Non-small cell lung cancer, diabetes mellitus	Unknown carcinoma on neck
Urine cultures positive for *Vanrija humicola*				
Specimendates (HD)	Sep 2023 (14)	Nov 2023 (100)	Feb 2021 (45)	May 2021 (28)
Quantity (CFU/mL)	> 10,000	3,000	> 100,000	> 100,000
Other culture	No growth from blood cultures	No growth from blood and sputum cultures	No growth from stool and sputum cultures	No growth from blood cultures
Fever (°C)	38.6	37.7	No	38.7
Urinary catheter use (days)	Yes (24)	No	Yes (45)	No
Comorbid conditions	Ileostomy, Percutaneous nephrostomy	Med iastinitis	Herpes simplex virus meningoen-cephalitis	Toxic epidermal necrolysis
Prior immunosuppressant use (days)	Pembrolizumab (12)	No	Osimertinib (4)	Prednisolone (25)
Prior antimicrobial use (days)	Meropenem (7)	Voriconazole (4), Vancomycin (15), Meropenem/ Ertapenem (15)	No	Meropenem (4), Vancomycin (4)
Laboratory findings				
WBC (/μL), neutrophil%	17,600, 89.0%	6,300, 64.1%	11,300, 84.1%	900, 57.6%
CRP (mg/dL)	9.56	0.78	6.48	5.54
Creatinine (mg/dL)	0.73	3	0.46	0.84
Serum β-D-glucan (pg/mL)	Not tested	Not tested	Not tested	600.4 pg/mL
Urinalysis				
Hemoglobin	4+	-	4+	-
Nitrite	-	-	-	-
Leukocyte	3+	-	-	-
Bact eria/yeast	Not tested	Not tested	Yeast, many	Not tested
Antifungal therapy (days)	None	Voriconazole (29)	None	Caspofungin (3)
Clinical outcome	Discharge	Discharge	Discharge	Expired

Abbreviations: F, female; M, male; HD, hospitalization days; CFU, colony-forming unit; WBC, peripheral white blood cell count (×1000/μL).

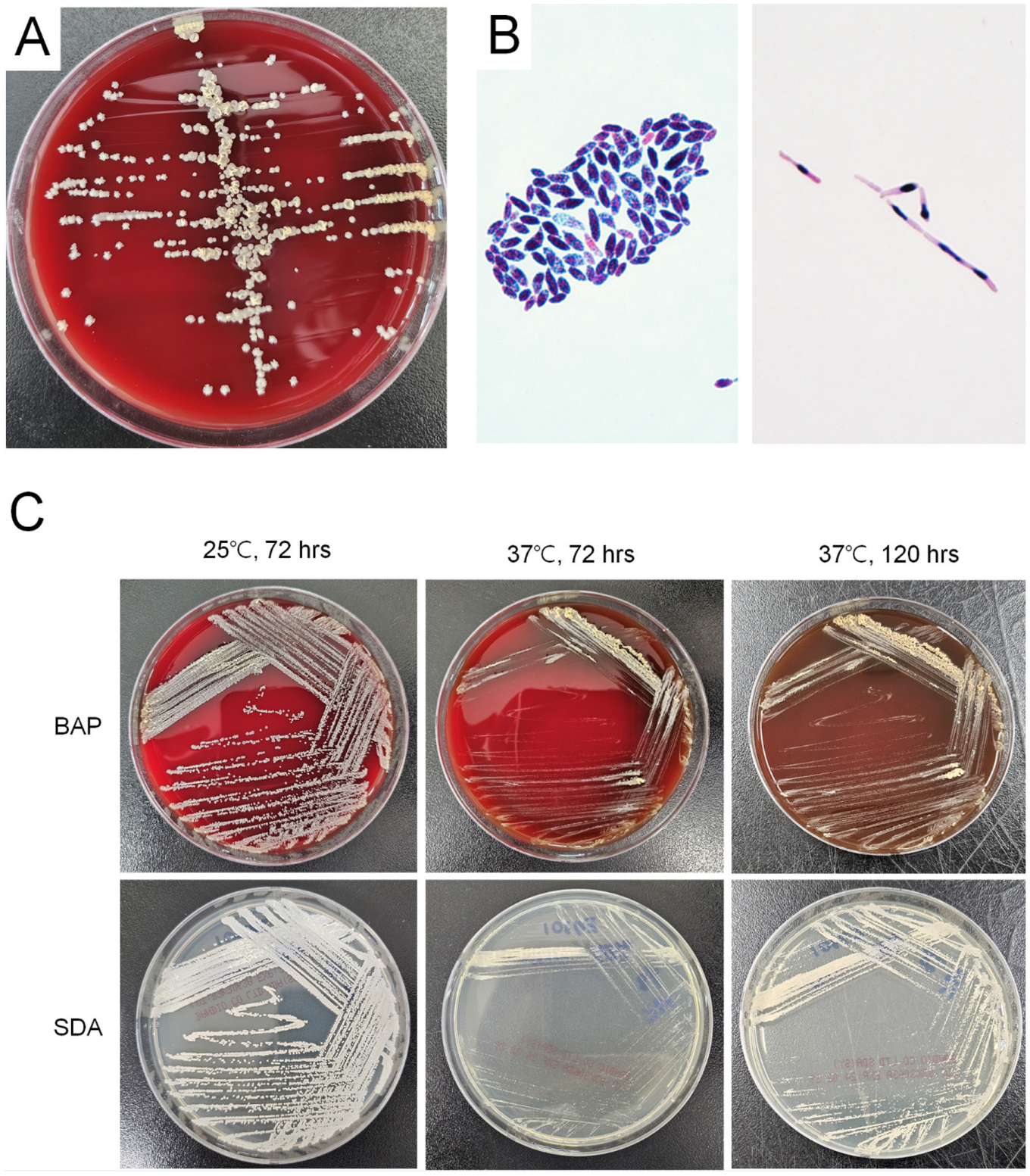

Fig. 1. Culture findings of *Vanrija humicola* in this study. (A) After 5 days of growth on BAP, colonies are of yellow, dry and cerebriform with a fimbriate margin. (B) Microscopic examination showed budding yeasts of oval to ellipsoidal cells (2-8 × 3-12 μm) (Gram stain, ×1,000) (left side) and pseudohyphae (10-15 μm) (PAS stain, ×1,000) (right side). (C) Colonies on BAP and SDA grew more vigorously at 25°C, than at 37°C. BAP, blood agar plate; SDA, Sabouraud dextrose agar.

Table 2. Microbiological characteristics of two recent isolates of *Vanrija humicola*

Characteristics		Isolate from patient 1	Isolate from patient 2
Species identification			
MALDI Biotyper (Score)			
Colonies on blood agar plate		*Vanrija humicola* (1.37)	*Vanrija humicola* (1.41)
Colonies on Sabouraud dextrose agar		*Vanrija humicola* (2.04)	*Vanrija humicola* (1.94)
VITEK YST		Unidentified due to growth failure	Unidentified due to growth failure
API 20C AUX kit (% identification)		*Cryptococcus humicola* (50.5)/ *Trichosporon mucoides* (47.2)	*Cryptococcu humicola* (51.4)/ *Trichosporon mucoides* (48.1)
Sequence analysis			
ITS region,	Accession number*	PP178147.1	PP188532.1
	First match (%identity)	*Vanrija humicola* FJ515176 (100)	*Vanrija humicola* FJ515176 (100)
D1/D2 region,	Accession number*	PP218158.1	PP218156.1
	First match (%identity)	*Vanrija humicola* KY110010 (99.5)	*Vanrija humicola* KY110010 (99.5)
Antifungal susceptibility, minimum inhibitory concentrations (μg/mL)			
Fluconazole		128	64
Itraconazole		0.5	0.25
Voriconazole		2	2
Posaconazole		1	0.5
Caspofungin		8	8
Anidulafungin		8	8
Micafungin		8	8
Amphotericin B		0.25	0.25
5-flucytosine		64	16

Abbreviation: ITS, internal transcribed spacer.
*Accession numbers assigned by GenBank (https://www.ncbi.nlm.nih.gov/genbank/) for the two strains in this study.

V. humicola was first described in 1975 in patients with conjunctivitis and ophthalmopathy [7]. Only two reports on urinary isolates have been published [2,10]. In a case of systemic infection, *V. humicola* is isolated from the blood, bone marrow, liver biopsy, lymph node, and urine [10]. In this study, all but one funguria of *V. humicola* were presumed to be a nosocomial urinary tract infection (UTI). Neither of VITEK2 YST or API 20C identified *V. humicola* correctly, unlike previous reports where *Candida humicola* or *Cryptococcus humicola* were identified using the API 20C or ID 32C systems [2,10]. The biochemical profile of API 20C AUX showed that the two highest priority species, *V. humicola* and *T. mucoides*, were indistinguishable, which is not surprising given the fact that both species are closely related urease-positive yeasts [11]. Before introduction of MALDI-TOF in routine identification of yeast, this *Candida*-like yeast is probably underdetected due to relatively slow growth, although colony morphology is pathognomonic when it matured to yellow, dry and cerebriform colonies. Both mass spectrometric analysis and sequence analysis of 26S rRNA showed reliable identification. It is noteworthy that species identification by mass spectrometry was more successful for colonies grown on SDA than those grown on BAP, which is consistent with the previous study on culture yield of fungal keratitis [12]. Therefore, SDA-cultured colonies are preferred for appropriate identification of *V. humicola* by mass spectrometry.

Though three were compatible with UTI, only one of them treated with antifungal therapy and the other two were uneventful without treatment. Therefore, clinical outcome of *V. humicola* UTI may not be dependent on appropriate antifungal therapy. This is plausible considering that antifungal therapy is not always warranted for *Candida* UTI [13]. However, an elderly patient with pancytopenia receiving caspofungin could be fatal. Considering that both isolates tested for antifungal susceptibility were resistant to all echinocandins, it was likely that this case was inadequately treated. Only three reports detailing the antifungal susceptibilities of *V. humicola* have been published to date [6,8,14]. It is susceptible to amphotericin B and itraconazole [6], but consistently resistant to echinocandin and 5-flucytosine [14], like as two closely related genera, *Cryptococcus* and *Trichosporon* species, are intrinsically resistant to echinocandin [3]. Of a few fatal cases in previous reports, the isolate from a case with pulmonitis and meningitis showed high MIC across all tested antifungals, including fluconazole, itraconazole, caspofungin, and amphotericin B [8], and a case of meningitis in an HIV patient is fatal despite fluconazole therapy over a 4-week period [9,10]. Therefore, antifungal susceptibility testing is required for appropriate therapy for the *V. humicola* infection of critically ill patient.

In conclusion, *V. humicola* emerged as a pathogen of UTI in Korea. This UTI presented as a nosocomial infection in hospitalized patients with immunocompromised conditions. For funguria of *V. humicola*, timely species identification based on MALDI-TOF mass spectrometry and antifungal susceptibility testing should be available in a clinical microbiology laboratory.

Ethics statement

This study was approved by the Institutional Review Board of Asan Medical Center (No. 2024-0049) and the requirement for informed consent was waived.

Conflicts of interest

Eun Jeong Won has been an associate editor of the *Annals of Clinical Microbiology* since January 2024. However, she was not involved in the review process of this article. No other potential conflict of interest relevant to this article was reported.

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