ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1997, p. 1375–1376 Vol. 41, No. 6
0066-4804/97/$04.00+0

NOTES

Candida norvegensis: a Fluconazole-Resistant Species

PER SANDVEN,[1]* KARI NILSEN,[1] ASBJØRN DIGRANES,[2] TRYGVE TJADE,[3] AND JØRGEN LASSEN[4]

Department of Bacteriology, National Institute of Public Health, 0462 Oslo,[1] Department of Microbiology and Immunology, Haukeland Hospital, 5021 Bergen,[2] Department of Microbiology, Akershus Central Hospital, 1474 Nordbyhagen,[3] and Norwegian Radium Hospital, 0310 Oslo,[4] Norway

Received 16 December 1996/Returned for modification 31 January 1997/Accepted 24 March 1997

***Candida norvegensis* has been an unusual cause of infections in humans. In Norway this species was isolated from eight patients from 1990 to 1996 and was of probable pathogenic significance in four of them. All isolates were resistant to fluconazole. The same was true for two *C. norvegensis* isolates from before 1940, and it is therefore assumed that the fluconazole resistance is inherent.**

Candida norvegensis has been an unusual cause of infections in humans. It was first isolated in Norway from the sputum of three patients with asthma nearly 60 years ago (2, 3). The first report of a verified clinical infection appeared in 1990, when a case of invasive disease in an immunosuppressed renal transplant patient on continuous ambulatory peritoneal dialysis was described (6). Two new reports on *C. norvegensis* infections from a total of seven patients appeared in 1996 (4, 5).

During the last few years *C. norvegensis* has been found in clinical specimens from eight patients in Norway. The aim of this study has been to obtain clinical information on these patients and to study the susceptibilities of *C. norvegensis* isolates to amphotericin B, flucytosine, fluconazole, and itraconazole.

Patients. Clinical information regarding our patients is summarized in Table 1. Patients 1 and 2 belong to the group of three patients from whom Dietrichson (2, 3) isolated *C. norvegensis* for the first time nearly 60 years ago. Among the remaining eight patients, *C. norvegensis* was isolated from cultures of blood from three patients, all of whom had underlying malignant diseases. The yeast was also isolated from urine from one of these patients. *C. norvegensis* was isolated from drainage fluid from the abdomen from three other patients, two of whom had peritonitis after perforated gastric and duodenal ulcers, respectively. The third patient had an underlying malignant disease. The yeast was isolated from urine and sputum from the last two patients, respectively. Both patients had an underlying malignant disease.

It is difficult to evaluate the pathogenic significance of *C. norvegensis* in these patients since all had severe underlying diseases, but it was considered that it was of probable pathogenic significance in four of the eight patients (Table 1). The eight patients reported previously all had severe underlying conditions (4–6). *C. norvegensis* was considered to be of pathogenic significance in all cases reported from Denmark (5, 6) and of possible clinical significance in two of four cases from Manchester, United Kingdom (4). *C. norvegensis* should therefore be included among the non-*Candida albicans* species capable of causing infections in immunocompromised patients.

It is interesting that this species seems to be isolated more frequently from Denmark and Norway than elsewhere. Two of the three previous reports on clinical infections caused by *C. norvegensis* were from Denmark (5, 6), and in addition, 35 other isolates have been identified as *C. norvegensis* in Denmark during a 25-year period. These latter isolates were mainly recovered from specimens from the respiratory tract, oropharynx, or vagina (5). In a recent prospective study in Norway on yeast infections in patients with peritonitis, this species was isolated from 2 of 117 patients (8).

TABLE 1. Characteristics and probable clinical significance of *C. norvegensis* for 10 patients from whom the yeast was isolated

Patient no.	Age (yr)	Source	Underlying disease	History	Antifungal treatment	Probable significance
1	?	Throat	Asthma	Unknown	None	Colonization
2	?	Throat	Asthma	Unknown	None	Colonization
3	60	Blood, urine	Ovarian carcinoma	Pneumonia, urinary tract infection; fatal outcome	Fluconazole	Pathogenic
4	60	Sputum	Cancer of the esophagus	No sign of infection	None	Colonization
5	51	Urine	Uterine sarcoma	Recovered slowly on fluconazole	Fluconazole	Pathogenic
6	69	Abdominal drainage	Adenocarcinoma of the bladder	Became afebrile on no treatment	None	Unknown
7	75	Abdominal drainage	Peritonitis	Complicated febrile course with fatal outcome	Fluconazole	Colonization
8	76	Abdomen	Peritonitis	Complicated postoperative course and fatal outcome	None	Pathogenic
9	83	Blood	Myelomatosis, renal failure	Neutropenic with fatal outcome	Fluconazole	Pathogenic
10	37	Blood	Acute myelogenous leukemia	Febrile, neutropenic, fatal outcome	Unknown	Unknown

* Corresponding author. Mailing address: Department of Bacteriology, National Institute of Public Health, P.O. Box 4404 Torshov, N-0403 Oslo, Norway. Phone: 47 22 04 22 00. Fax: 47 22 04 25 18.

TABLE 2. Antifungal susceptibility of *C. norvegensis* isolates determined by a colorimetric broth microdilution method and E-test

Patient no.	MIC (μg/ml)						
	Amphotericin B		Fluconazole		Flucytosine		Itraconazole, E-test[a]
	Microdilution	E-test	Microdilution	E-test	Microdilution	E-test	
1	0.25	0.094	32	128	8	≥32	0.19
2	0.25	0.25	32	≥256	2	0.75	0.012
3	0.5	0.19	≥64	128	16	≥32	1
	1	0.75	≥64	≥256	16	≥32	0.5
4	0.5	0.5	≥64	64	8	≥32	0.5
5	1	0.19	≥64	128	16	≥32	0.5
6	0.5	0.25	32	≥256	16	32	1
	0.5	0.75	32	≥256	16	≥32	1
7	1	0.19	≥64	128	16	≥32	1
	1	0.38	≥64	128	16	≥32	0.5
8	0.5	0.19	≥64	≥256	16	≥32	1
9	0.5	0.5	≥64	128	16	≥32	0.38
10	0.25	0.25	≥64	≥256	2	3	1

[a] Microdilution test results are not available for itraconazole.

Identification. Thirteen *C. norvegensis* isolates from 10 patients were studied (Table 2). All isolates formed yellowish white, glistening colonies on Sabouraud agar, and they also had a characteristic sweet smell. The germ tube test was negative. On cornmeal–Tween 80 agar, the isolates produced pseudohyphae and blastoconidia. All isolates were able to grow at 43°C. The results of the conventional biochemical tests were similar for all isolates. There was no fermentation of any of the six carbohydrates tested, and of 12 carbohydrates tested, only glucose was assimilated. Because of this lack of reactivity, identification by conventional test systems may be problematic. We first misidentified one isolate as *Candida lipolytica*, and another of our isolates (isolate 2/96), sent to the Centraalbureau voor Schimmelcultures for confirmation, was first misidentified by investigators there as *Pichia cactophila*. By using the ATB 32C system (bioMérieux, Marcy l'Etoile, France), 12 of the isolates gave the same profile number (0200010005) and all isolates were correctly identified as *C. norvegensis*. This test system is, however, quite expensive as well as labor-intensive, and it is therefore probably used to a limited extent.

One reason for the lack of reports on *C. norvegensis* isolates could therefore be that this species is misidentified or possibly that isolates are not identified to the species level. Four of our patients were hospitalized at the Norwegian Radium Hospital, where all yeast isolates thought to be of clinical importance are identified to the species level. Two other patients were enrolled in a clinical trial in which all yeasts isolated were identified to the species level. It is therefore likely that more *C. norvegensis* isolates would be recognized if a higher proportion of isolates of clinical importance were identified.

Antifungal susceptibility. A colorimetric broth microdilution method was used for amphotericin B, flucytosine, and fluconazole susceptibility testing, and the broth microdilution test was performed as described by Pfaller and Barry (7). Each isolate was also tested for susceptibility to amphotericin B, fluconazole, flucytosine, and itraconazole by the E-test method (AB Biodisk).

The results of the susceptibility tests are presented in Table 2. All isolates were resistant to fluconazole (MICs, ≥32 μg/ml), and 11 isolates were also relatively resistant to flucytosine (MICs, ≥8 μg/ml). The four isolates reported by Hood et al. (4) were fluconazole resistant but flucytosine susceptible. The fluconazole resistance is probably an inherent trait since the two preserved isolates from before 1940 were also resistant to this drug and since only two of the eight recent patients had been treated with fluconazole before *C. norvegensis* was isolated. It is therefore unlikely that fluconazole resistance in *C. norvegensis* isolates is caused by fluconazole usage. It is not known whether the in vitro resistance correlates with in vivo resistance, but the results of studies with other *Candida* spp. indicate that this might be the case (1). *C. norvegensis* was recovered from cultures of blood from two of our patients while they were on fluconazole treatment.

The results of this study underline the clinical importance of identification and susceptibility testing of clinically significant yeast isolates.

REFERENCES

1. **Cormican, M. G., and M. A. Pfaller.** 1996. Standardization of antifungal susceptibility testing. J. Antimicrob. Chemother. **38:**561–578.
2. **Dietrichson, E.** 1954. Etude d'une collection norvégienne de levures. Ann. Parasitol. **24:**272–288.
3. **Dietrichson, E.** 1954. Etude d'une collection norvégienne de levures. Ann. Parasitol. **24:**460–498.
4. **Hood, S. V., C. B. Moore, and D. W. Denning.** 1996. Isolation of *Candida norvegensis* from clinical specimens: four case reports. Clin. Infect. Dis. **23:** 1185–1187.
5. **Nielsen, H., and J. Stenderup.** 1996. Invasive Candida norvegensis infection in immunocompromised patients. Scand. J. Infect. Dis. **28:**311–312.
6. **Nielsen, H., J. Stenderup, B. Bruun, and J. Ladefoged.** 1990. *Candida norvegensis* peritonitis and invasive disease in a patient on continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. **28:**1664–1665.
7. **Pfaller, M. A., and A. L. Barry.** 1994. Evaluation of a novel colorimetric broth microdilution method for antifungal susceptibility testing of yeast isolates. J. Clin. Microbiol. **32:**1992–1996.
8. **Sandven, P., H. Qvist, E. Lingaas, and K. E. Giercksky.** 1996. Significance of yeasts recovered from intra-abdominal specimens in patients with gastrointestinal perforations, abstr. J147, p. 246. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.