ACTA MYCOLOGICA
Vol. 39 (1): 7-12
2004

Candida dubliniensis Sullivan et al., a new species in the human respiratory system

ANNA BIEDUNKIEWICZ-ZIOMEK and MARIA DYNOWSKA

Departament of Mycology, University of Warmia and Mazury in Olsztyn
Oczapowskiego 1A, PL-10-719 Olsztyn-Kortowo

Biedunkiewicz-Ziomek A., Dynowska M.: *Candida dubliniensis Sullivan et al., a new species in the human respiratory system*. Acta Mycol. 39 (1): 7-12, 2004.

Long-term observations of broadly defined mycological features of various human ontocoenoses show that the ontocoenosis of the respiratory system is characterised by rapid changes of the dynamics and biodiversity of the fungi. The continuity of studies on the subject and a great biological diversification of the clinical material, collected mostly from individuals suffering from chronic diseases of the respiratory system and from oncological patients, contribute to the detection of many interesting and important species. In the last few years, the studies have been extended to include healthy individuals. Special attention is paid to the age and the place of residence of the subjects.

The group analysed in this project comprised randomly chosen students from whom biological material was collected from primary infection routes. The material collected was treated in keeping with generally accepted recommendations for diagnostic mycological laboratories. *Candida dubliniensis*, a species not recorded in Poland previously, was found among the 7 fungi identified in the reconnaissance studies. The fungus is an opportunistic pathogen, strictly related to *Candida albicans*, however, different from it epidemiologically.

Candida dubliniensis was isolated from the oral cavity and the throat. Its growth was poor or medium on Sabouraud agar; the colonies were creamy-coloured, soft and smooth. On Nickerson medium, it produced pseudomycelium with characteristic thick and inflated pseudohyphae on which grape-like blastoconidia, and big, darkly pigmented terminal chlamydospores, appearing characteristically between 1 and 3, formed. Chlamydospore formation under the inflated terminal cell is also characteristic of this species.

The isolation of *Candida dubliniensis* from the respiratory system strictly corresponds to the studies by Dynowska (1993) on the blurring of physiological and ecological boundaries between trophic groups of potentially pathogenic fungi and corroborates her hypotheses on the continuous occurrence of new species in organ ontocoenoses.

Key words: *Candida dubliniensis*, respiratory system, healthy individuals

INTRODUCTION

Long-term observations of broadly defined mycological features of various human ontocoenoses show that the ontocoenosis of the respiratory system is characterised by rapid changes of the dynamics and biodiversity of the fungi. The findings of the present authors' studies, conducted on patients treated at the Independent Public Complex of Pulmonology and Oncology in Olsztyn, corroborate the claim. Seven fungal species were recorded between 1986 and 1990 (Dynowska 1990), while a few years later as many as 17 species were recovered (Dynowska 1993). In 2001, the number of fungi isolated in different sections of the respiratory system reached 29 (Biedunkiewicz 2001). Apart from the species that are common potential etiological factors of candidiases of the respiratory system, there were also rare species, previously not recovered from this ontocoenosis. *Saccharomycopsis capsularis* (Dynowska and Biedunkiewicz 1999) or *Trichosporon beigelii* (Dynowska 1996) may be good examples. The continuity of studies on the subject and a great biological diversification of the clinical material, collected mostly from individuals suffering from chronic diseases of the respiratory system and from oncological patients, contribute to the detection of many interesting and important species.

In the last few years, the studies have been extended to include healthy individuals. Special attention is paid to the age and the place of residence of the subjects. The group analysed in this project comprised randomly chosen students from whom biological material was collected from primary infection routes. *Candida dubliniensis*, a species not recorded in Poland previously, was found among the 7 fungi identified in the reconnaissance studies. It seems that a closer description of its properties and its systematic position will interest mycologists specialising in both theoretical studies and practical work.

MATERIAL AND METHODS

The studies were conducted between 2002 (Nov.) and 2003 (March). Research material (swabs from the oral cavity, the nose and the throat) was collected from 100 randomly chosen students, aged 22-24 (men and women). The material collected was treated in keeping with generally accepted recommendations for diagnostic mycological laboratories (Dynowska 1995; Kurnatowska 1995).

Initial cultures were inoculated on Sabouraud medium (plates). To obtain pure, bacteria-free strains, fungal colonies were passaged on fresh Sabouraud medium (slant cultures) a number of times.

Macroscopic analysis (size, colour, consistency, shape and smell of the colonies) was conducted on Sabouraud medium. Microscopic features (shape, size and location of blastospores and chlamydospores, diameter and shape of pseudohyphae) were analysed on Nickerson medium (Kurnatowska 1995).

CHROMagar *Candida* substrate (bioMérieux) was used for rapid identification of the most common yeast-like fungi.

Studies by Barnett, Payne and Yarrow (1990), Lodder, Kreger–van Rij (1967), Kurnatowska (1995), De Hoog et al. (2000) as well as Kurtzman and Fell (2000) were used to determine the fungi.

RESULTS AND DISCUSSION

Mycological analysis of the material studied yielded 7 fungal species: *Candida albicans, Candida dubliniensis, Candida glabrata, Candida guilliermondii, Candida tropicalis* as well as *Saccharomyces cerevisiae* and *Saccharomycopsis capsularis.* Particular attention was paid to *Candida dubliniensis*, a novel species in the respiratory system, not recorded in Poland. It was isolated from the oral cavity and the throat in three 22-year-old individuals (one man and two women) who complained of frequent throat and sinus infections.

Candida dubliniensis is described as an opportunistic pathogen strictly related to *Candida albicans*, however, different from it epidemiologically. It was first isolated in AIDS patients in Dublin (Ireland). Sullivan et al. (1995) had it separated as a new species, believing it had been partly identified as *Candida albicans.* They showed its different phenotypic properties, the production of chlamydospores (up to three chlamydospores) characteristic only of this phenotype, resistance to fluconazole, lack of intracellular β-glucosidase and very poor growth at 42°C. It grows on cornmeal agar at 25°C and produces blastospores, pseudohyphae and chlamydospores after 72h. The arrangement of chlamydospores is characteristic of this species (appearing in triplets), and discriminates it phenotypically from *Candida albicans*. Other authors incubated cultures in peptone-dextrose broth at 42°C. *Candida dubliniensis* did not grow at 45°C, while *Candida albicans* grew in both temperature ranges (Pinjon et al. 1998). The colonies incubated on CHROMagar *Candida* at 37°C were dark green (Jabra-Rizk et al. 1999). Similarly to *Candida albicans*, germ tube production that occurs in blood serum after two hours is a characteristic feature of the species.

The new presumptive taxon is controversial although molecular evidence suggests its distinctiveness from *Candida albicans*. Apart from routine laboratory methods applied for confirmation of the presence of *Candida dubliniensis*, molecular and serological methods, such as DNA fingerprinting, rapid PCR tests, rRNA sequence analysis, multilocus enzyme electrophoresis and hybridisation, were also used (Meyer, Maszewska and Sorrell 2001; Pinjon et al. 1998; Piet et al. 1999).

After the isolation of *Candida dubliniensis* by Sullivan et al. (1995), Brandt et al. (2000) recorded four cases of infection with the species in patients from North America suffering from chronic leukaemia, chronic pulmonary diseases, thrombocytosis, after long-term chemiotherapy and prolonged treatment with antibiotics.

Candida dubliniensis occurs as a cosmopolitan species. It has so far been isolated from the oral cavity, urine, vagina, lungs, faeces and saliva from HIV (+) patients and, to a smaller degree, from HIV–negative patients (Coleman et al. 1997; Sullivan et al. 1997; Pinjon et al. 1998; Polacheck et al. 2000).

De Hoog (1996) believes that *Candida dubliniensis* is a species classified as BSL-2 in the biosafety classification of fungi potentially pathogenic for people and animals, which means that it may cause not only superficial infections but also deep, opportunistic infections in highly immunocompromised patients.

As the present authors' studies show, *Candida dubliniensis* confirms the features of this species. The strains recovered from the students are characterised by specific growth, including unusual chlamydospore formation.

Candida dubliniensis was isolated from the oral cavity and the throat. On Sabouraud agar, its showed poor or medium growth, and the colonies were creamy-coloured, soft and smooth. On Nickerson medium, it produced pseudomycelium with characteristic thick and inflated pseudohyphae (Fig. 1A, 1B) on which grape-like blastoconidia, and big, darkly pigmented terminal chlamydospores, appearing characteristically between 1 and 3, were formed (Fig. 2A, 2B). Chlamydospore formation under the inflated terminal cell is also characteristic of this species (Fig. 3). The results were obtained in microcultures after 48, 72 and 144 h, respectively (Figs. 4, 5, 6).

No available study identifies an unambiguous systematic position of *Candida dubliniensis*. It is only known that it was separated from *Candida albicans*, and it may be only surmised now that, similarly to *Candida albicans*, it does not produce the perfect stage. Thus, according to *Dictionary of the Fungi* (Hawskworth et al. 1995), it is an anamorphic fungus of the order *Saccharomycetales*. It is not known whether it belongs to the family *Saccharomycetaceae* or *Cryptococcaceae*. Studies on the species have been conducted only for a short period of time now, and great caution should be exercised as an increasing number of species in which the production of asci is identified have occurred within the genus *Candida*.

The isolation of *Candida dubliniensis* from the respiratory system strictly corresponds to the studies by Dynowska (1993) on the blurring of physiological and ecological boundaries between trophic groups of potentially pathogenic fungi and corroborates her hypotheses on the continuous occurrence of new species in organ ontocoenoses. The phenomenon is connected not only with improved diagnostic methods but first of all with increasing expansiveness of fungi (Dynowska, Biedunkiewicz 2001) and their search for natural ecological niches, the human body in this case.

REFERENCES

Barnett J. A., Payne R. W., Yarrow D. 1990. Yeast: Characteristic and identification. Cambridge Univ. Press.

Biedunkiewicz A. 2001. Dynamics of human respiratory system mycoflora. Acta Mycol. 36 (2): 211–239.

Brandt M. E., Harrison L. H., Pass M., Sofair A. N., Huie S., Li R. – K., Morrison C. J., Warnock D. W., Hajjeh R. A. 2000. *Candida dubliniensis* fungemia: the First Four Cases in North America. Emer. Inf. Dis. 6 (1): 46–49.

Coleman D. C., Sullivan D. J., Bennett D. E., Moran G. P., Barry H. J., Shanley D. B. 1997. Candidiasis: the emergence of a novel species, *Candida dubliniensis*. AIDS 11: 557–567.

de Hoog G. S. 1996. Risk Assessment of Fungi Reported from Humans and Animals. Mycoses, 39: 407–417.

de Hoog G. S., Guarro J., Gené J., Figueras H. J. 2000. Atlas of clinical fungi. 2nd ed. Centraalbureau voor Schimmelcultures/ Universitat Rovira i Virgili, Reus, Spain: 194–195.

Dynowska M. 1990. Występowanie grzybów z rodzaju *Candida* w układzie oddechowym mieszkańców województwa olsztyńskiego. Acta Mycol. 26 (1): 99–107.

Dynowska M. 1993. Changes in mycoflora of man's respiratory system – observed during last some years – yeasts. Ibid. 28 (2): 151–155.

Dynowska M. 1995. Drożdże i grzyby drożdżopodobne jako czynniki patogenne oraz bioindykatory ekosystemów wodnych. Studia i Materiały WSP 77. Olsztyn.

Dynowska M. 1996. *Trichosporon* species isolated from human respiratory system. Acta Mycol. 31 (2): 137–141.

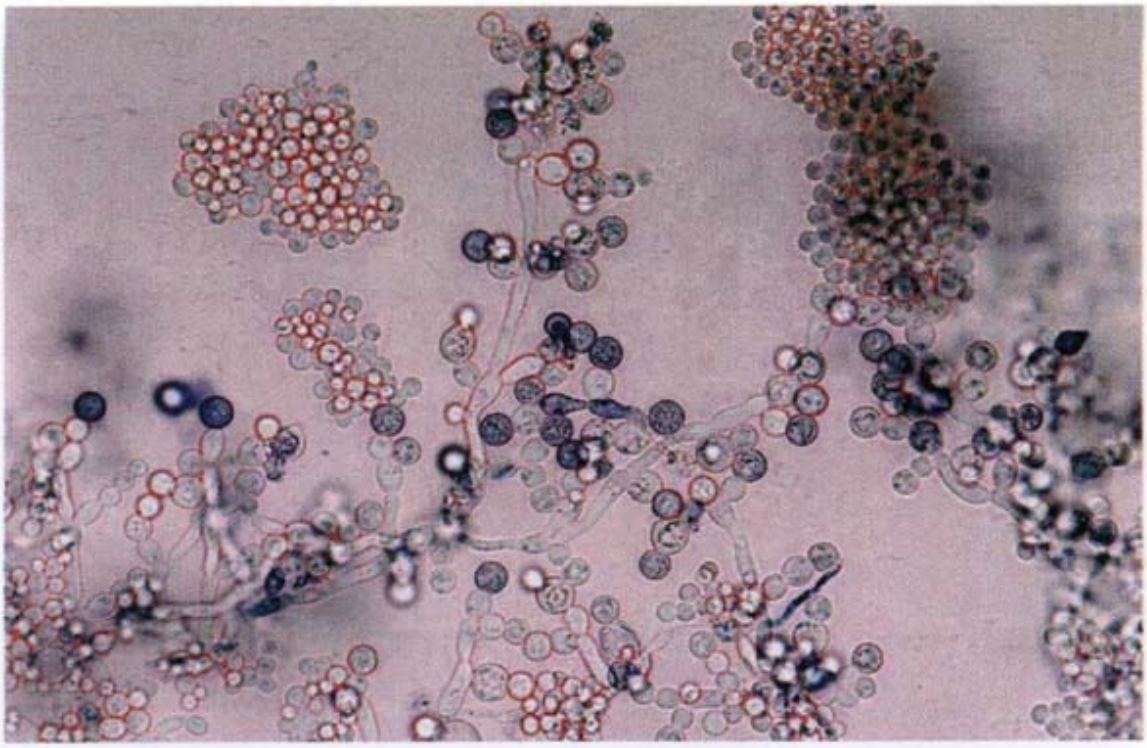

Fig. 1A. *Candida dubliniensis* – microculture on the Nickerson agar.

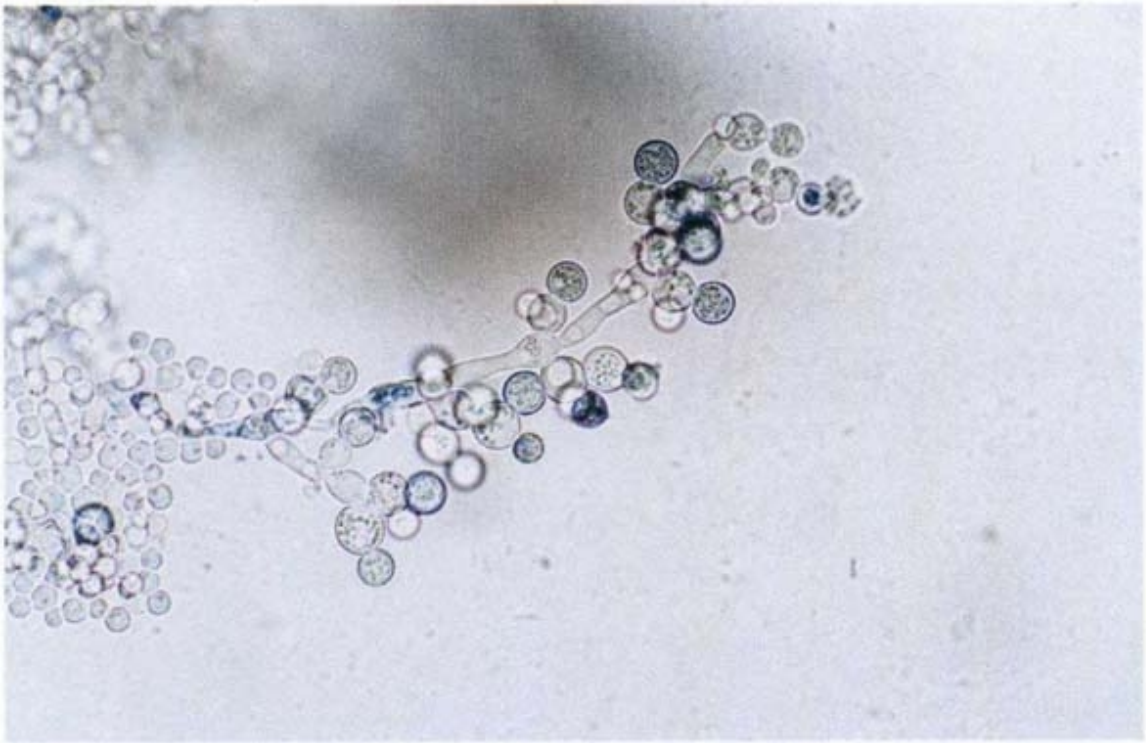

Fig. 1B. *Candida dubliniensis* – microculture on the Nickerson agar, thick pseudomycelium with chlamydospores.

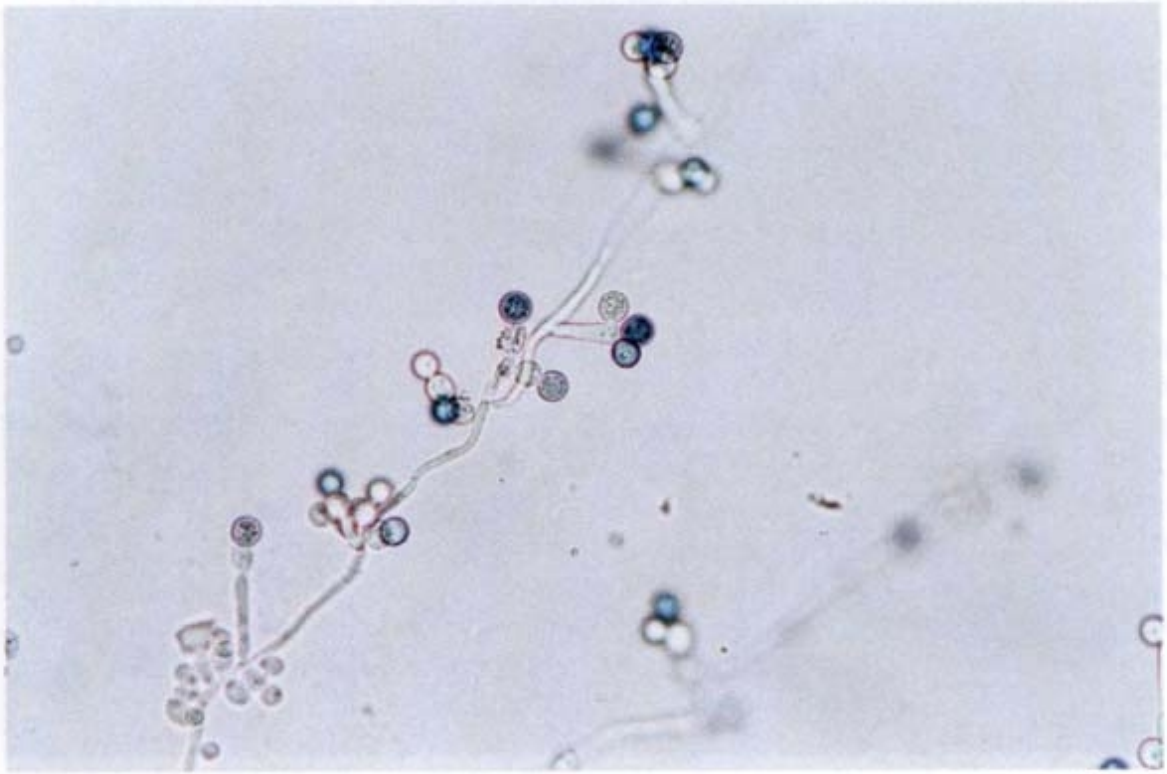

Fig. 2A. *Candida dubliniensis* – microculture on the Nickerson agar, (chlamydospores in chains of 1-3) × 400.

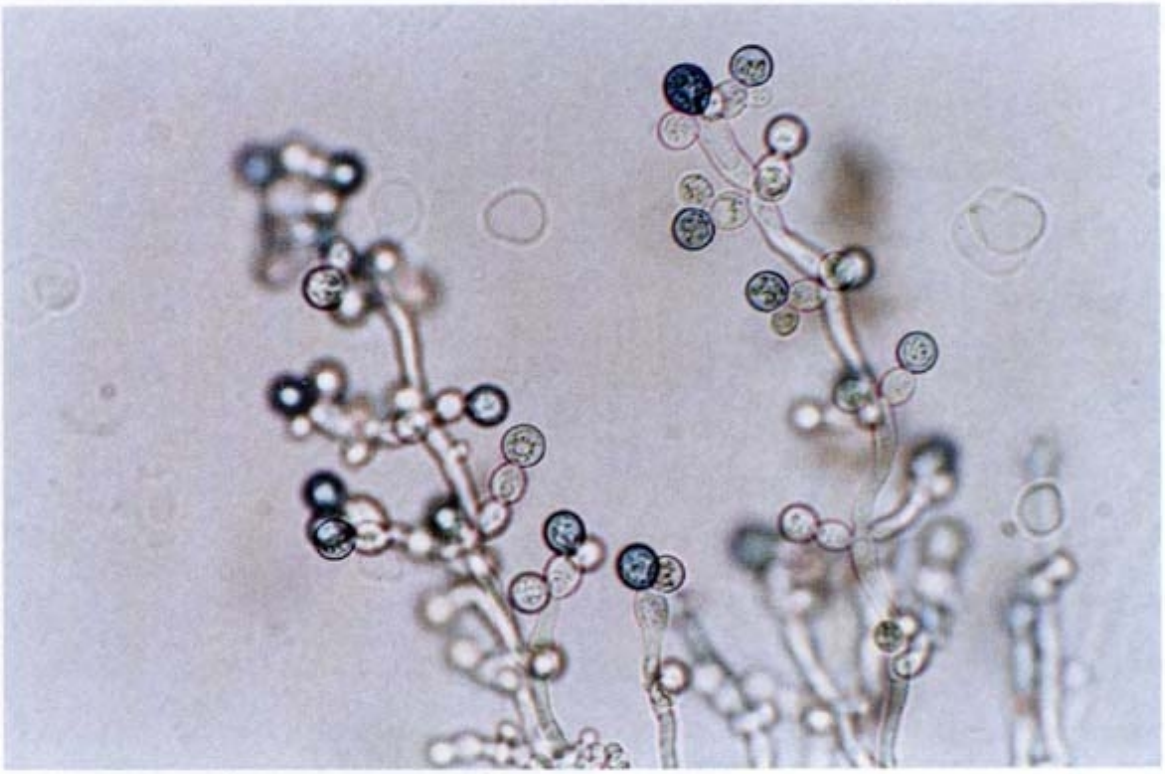

Fig. 2B. *Candida dubliniensis* – microculture on the Nickerson agar, (chlamydospores in chains of 1-3) × 600.

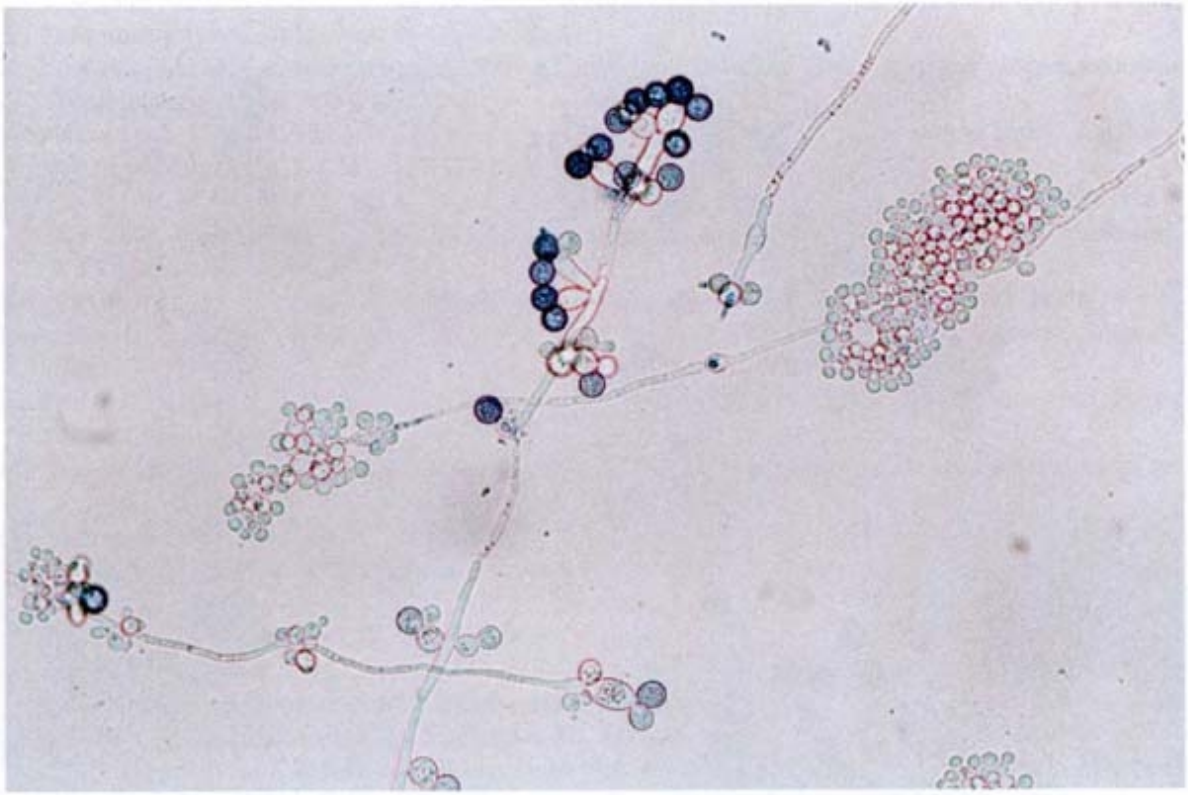

Fig. 3. *Candida dubliniensis* – microculture on the Nickerson agar.

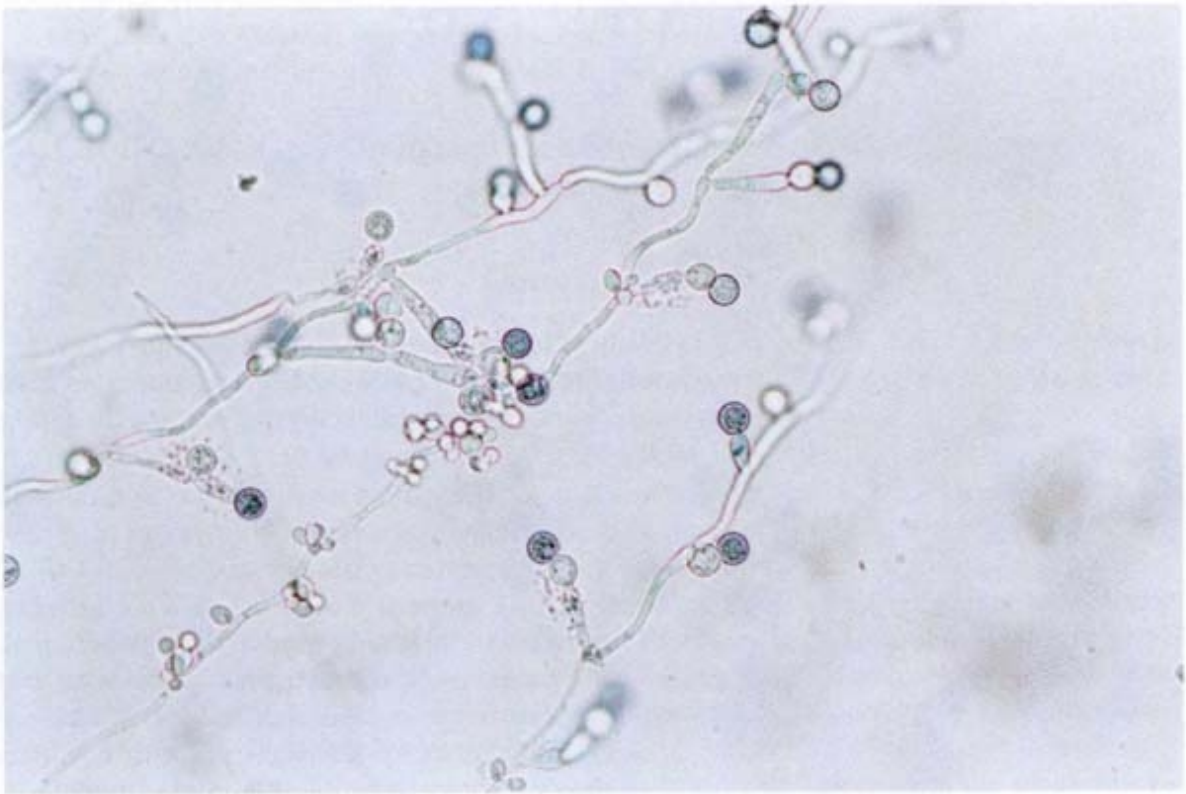

Fig. 4. *Candida dubliniensis* – microculture on the Nickerson agar, after 48h incubation.

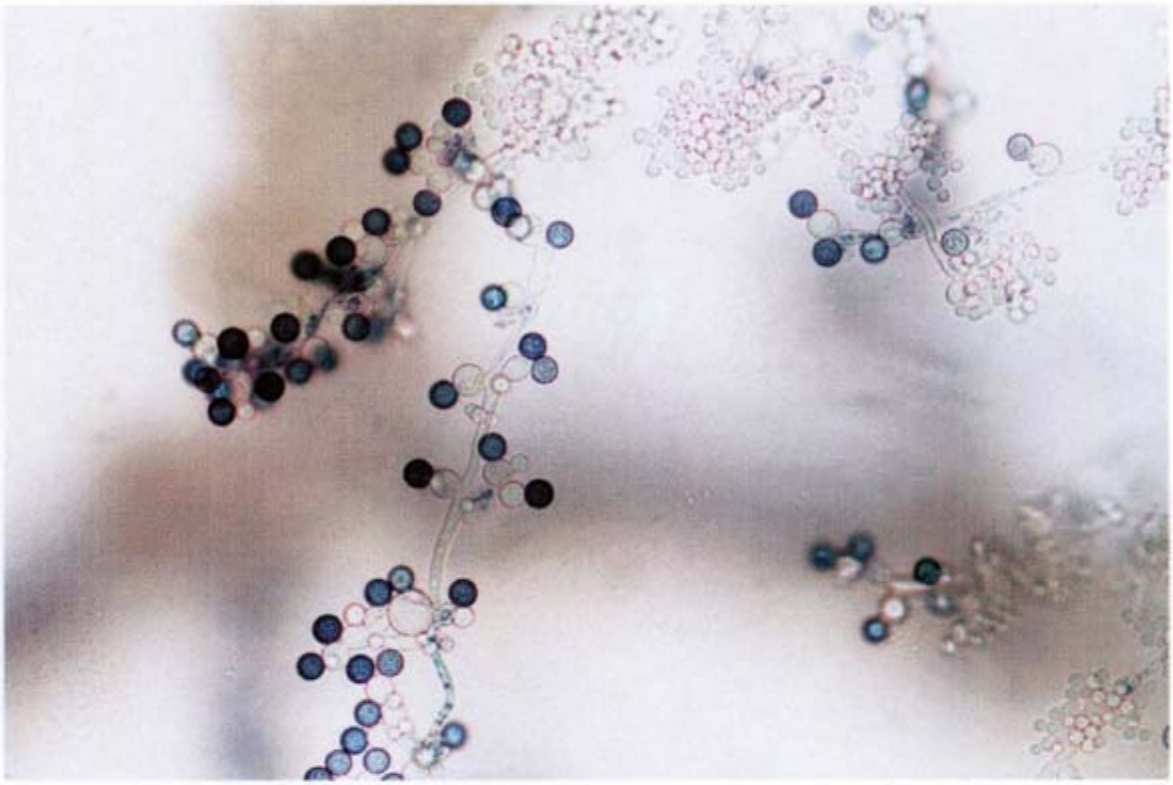

Fig. 5. *Candida dubliniensis* – microculture on the Nickerson agar, after 72h incubation.

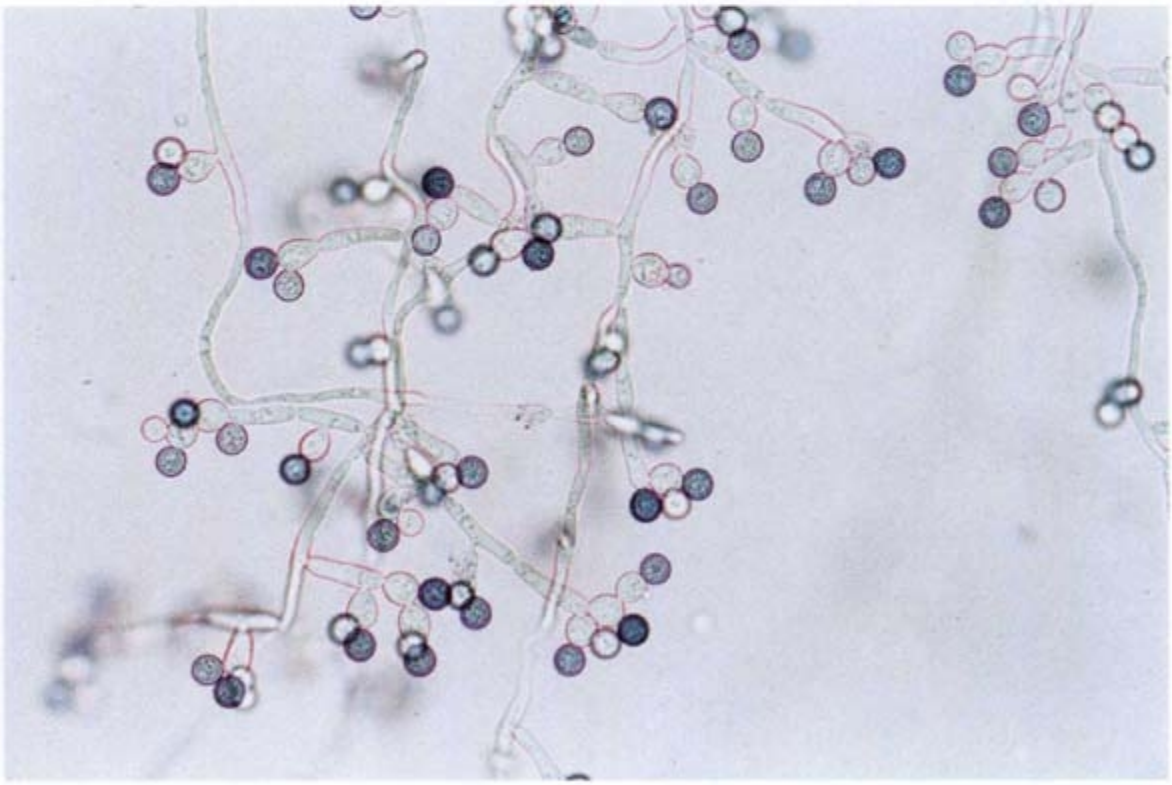

Fig. 6. *Candida dubliniensis* – microculture on the Nickerson agar, after 144 h incubation.

Dynowska M., Biedunkiewicz A. 1999. Presence of *Saccharomycopsis capsularis* in the human respiratory system. Ibid., Vol. 34 (2): 281–287.

Dynowska M., Biedunkiewicz A. 2001. Grzyby chorobotwórcze o wzrastającej ekspansywności. Wiad. Parazyt. 47 (4): 609–613.

Hawskworth D. L., Kirk P. H., Sutton B. C., Pegler D. M. 1995. Ainsworth & Bisby's Dictionary of the Fungi, Ed. 8. CMI, CAB International, England.

Jabra–Rizk M. A., Baqui A. A. M. A., Kelley J. I., Falker Jr. W. A., Merz W. G., Meiller T. F. 1999. Identification of *Candida albicans* in a prospective study of patients in the United States. J. Clin. Microbiol. 37 (2): 321–326.

Kurnatowska A. 1995. Wybrane zagadnienia z mikologii medycznej. Wyd. II. Promedi. Łódź.

Kurtzman C. P., Fell J. W. 2000. The yeasts, A Taxonomic Study. Fourth edition. Elsevier. Amsterdam.

Lodder J., Kreger-van Rij N. J. W. 1967. The yeasts a taxonomical study. North – Hall. Publ. Comp., Amsterdam.

Meyer W., Maszewska K., Sorrell T. C. 2001. PCR fingerprinting: a convenient molecular tool to distinguish between *Candida dubliniensis* and *Candida albicans*. Med. Mycol. 39 (2): 185–193.

Pinjon E., Sullivan D., Salkin I., Shanley D., Coleman D. 1998. Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. J. Clin. Microbiol. 36 (7): 2093–2095.

Pincus D. H., Coleman D. C., Pruitt W. R., Padhye A. A., Salkin I. F., Geimer M., Bassel A., Sullivan D. J., Clarke M., Hearn V. 1999. Rapid identification of *Candida dubliniensis* with commercial yeast identification systems. Ibid. 37: 3533–3539.

Polacheck I., Strahilevitz J., Sullivan D., Donnelly S., Salkin I. F., Coleman D. C. 2000. Recovery of *Candida dubliniensis* from Non – Human Immunodeficiency Virus – Infected Patients in Israel. Ibid. 38 (1): 170–174.

Sullivan D. J., Westerneng T. J., Haynes K. A., Bennett D. E., Coleman D. C. 1995. *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV – infected individuals. Microbiology 141: 1507–1521.

Sullivan D., Haynes K., Bille J., Boerlin P., Rodero L., Lloyd S., Henman M., Coleman D. 1997. Widespread geographic distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus – infected individuals. J. Clin. Microbiol. 35: 960–964.

Candida dubliniensis Sullivan et al., nowy gatunek w układzie oddechowym człowieka

Streszczenie

Wieloletnie obserwacje dotyczące szeroko pojętej charakterystyki mikologicznej różnych ontocenoz narządowych człowieka wskazują, że ontocenozą w której zachodzą bardzo szybkie zmiany w bioróżnorodności i dynamice grzybów jest układ oddechowy.

Wychwycenie wielu ciekawych i ważnych gatunków jest możliwe dzięki ciągłości badań oraz dużemu zróżnicowaniu biologicznemu materiałów klinicznych pochodzących głównie od osób z przewlekłymi schorzeniami układu oddechowego oraz pacjentów onkologicznych.

W ostatnich latach badania rozszerzono na osoby zdrowe ze szczególnym zwróceniem uwagi na ich wiek i miejsce bytowania. Grupą aktualnie analizowaną są wybrani losowo studenci, od których pobrano materiał biologiczny z głównych wrót zakażenia. Wśród 7 grzybów uzyskanych w badaniach rekonesansowych znalazł się gatunek dotychczas nie notowany w Polsce – *Candida dubliniensis* - oportunistyczny patogen ściśle powiązany z *Candida albicans* ale różny pod względem epidemiologicznym.

Pobrany materiał traktowano zgodnie z ogólnie przyjętymi zaleceniami dla diagnostycznych laboratoriów mikologicznych.

Candida dubliniensis wyizolowano z jamy ustnej i gardła. Na agarze Sabourauda tworzył kolonie o wzroście słabym lub średnim, barwie kremowej, powierzchni gładkiej i miękkiej. Na

podłożu różnicującym Nickersona tworzył pseudomycelium o charakterystycznych grubych i rozdętych pseudostrzępkach, na których tworzyły się blastokonidia układające się groniasto oraz duże, ciemno wybarwione chlamydospory ułożone terminalnie o bardzo charakterystycznym układzie w liczbie od 1 do 3. Specyficzne również dla tego gatunku jest także tworzenie się chlamydospor pod komórką szczytową, która wówczas jest silnie rozdęta.

Wyizolowanie *Candida dubliniensis* z układu oddechowego ściśle koresponduje z wynikami badań Dynowskiej (1993) dotyczącymi zacierania się granic fizjologicznych i ekologicznych pomiędzy grupami troficznymi grzybów potencjalnie chorobotwórczych oraz potwierdza jej przypuszczenia o ciągłym pojawianiu się w ontocenozach narządowych nowych gatunków.