IN VITRO DRUG TOLERANCE OF ORAL *CANDIDA* ISOLATES FROM HEALTHY INDIVIDUALS

J. R. Hatherill* and P. A. Volz**

*Department of Toxicology, The University of Michigan
Ann Arbor, Michigan 48104

**Mycology Laboratory, Eastern Michigan University
Ypsilanti, Michigan 48197

Abstract: The isolation frequency of *Candida albicans*, *C. krusei*, *C. guillermondii*, and *C. stellatoidea* were noted on the dorsum of the tongue and the maxillary gingival sulcus of 202 healthy human subjects. *C. albicans* was the predominant isolate and the tongue was slightly more common for *Candida* isolation. Drug tolerance indexes demonstrated variable resistance in *C. albicans* isolates in comparison with the other *Candida* species.

Introduction: Oral thrush is a common form of candidiasis, well documented as case reports or as general mycotic descriptions (2,4, 7,9,10,11,12). Causal agents of thrush can be present in the oral microflora of healthy test subjects (1). It is widely established that *Candida albicans* is the common causative agent of thrush, however, many investigations involving oral flora did not distinguish between *Candida* species. The present investigation identifies pathogenic species of *Candida* present on the dorsum of the tongue and the maxillary gingival sulcus in healthy human subjects. The mouth areas sampled are frequently invaded in cases of thrush even when no other involvement in the patient occurs (10).

The presence of *Candida* species is important to monitor due to possible disease expression in high-risk patients (3) , particularly in cancer patients before irradiation (5), in patients on long-term immunosuppressive or antibiotic therapy and before the use of general anesthesia (6). Species and isolate variation of *Candida* have been shown in dimorphism with numerous biochemical differences (1,14), and variation is also found in dissimilar drug tolerances.

Materials and Methods: The dorsum of the tongue and the maxillary gingival sulcus of 202 healthy university students were sampled using sterile polyester swabs. Collected material was immediately streaked on Sabouraud dextrose agar plates and incubated 72 h at 37°C. Stocks of the *Candida* isolates were maintained in pure culture at 25°C on Sabouraud maltose agar (SMA) slants. Species identification was accomplished with corn meal agar Dalmau plates for the detection of pseudohyphal characteristics (14). The germ tube test was utilized for positive germ tube production of *C. albicans* and *C. stellatoidea* (8). Further separation of the two species was obtained by carbohydrate assimilation tests and colony characteristics (14,19).

Experimental and known antifungal test drugs were selected to assay fungal growth inhibition (Table 1). The strains of orally isolated *Candida* including *C. albicans*, *C. krusei*, *C. stellatoidea*, and *C. guillermondii* were subjected to drugs dispensed in concentrations of 1000 mcg/ml in assay disks on SMA plates previously spread

with suspensions of the test organism. Zones of growth inhibition were recorded in mm with Candida species and isolates.

Results: Of the 202 university students examined, Candida isolates were present in 53 or 26.2 % of the individuals. A total of 28 or 13.9 % of the subjects contained Candida species on the dorsum of the tongue but not in the maxillary sulcus, while 16 students or 8.0 % had Candida on both the dorsum of the tongue and maxillary gingival sulcus. Candida was recorded exclusively from the maxillary gingival sulci in nine subjects or 4.4 % of the total number of students examined. The 53 isolates of Candida included two isolates of C. krusei, and one isolate each of C. guillermondii and C. stellatoidea. The remaining Candida oral isolates were C. albicans represented by a 95 % confidence interval (T-distribution) as presented in Charts 1 and 2.

All Candida isolates were subjected to further study in the drug investigations. The greatest drug intolerance was noted with 2-cyano-4-nitrothiophene (Abbott 36042). This drug is derived from the addition of a highly toxic cyano group with thiophene. Thiophene is a constituent of biotin, a normal substrate metabolized by yeast organisms.

The experimental drug Abbott 6131 demonstrated the second largest inhibition. Mycophenolic acid exhibited growth inhibition which diminished progressively from the central focus of disk application outward. Isolates C. stellatoidea and C. guillermondii had complete resistance to mycophenolic acid. C. guillermondii, C. krusei, and C. stellatoidea showed significant resistance toward 5-fluorocytosine with respect to isolates of C. albicans. Isolate C. stellatoidea expressed complete resistance to amphotericin B, in contrast to an average inhibition of 2.4 mm with C. albicans. Erythromycin B had negligible effects on C. guillermondii, while C. albicans isolates expressed an average of 4.7 mm growth inhibition. The isolate C. stellatoidea was resistant to exposure of TAEM, while the remaining Candida isolates had a significant response.

Nystatin, chlorambucil, Abbott 25579, DMCTC, and niddamycin did not demonstrate significant differences between isolates of the species.

Discussion: There is considerable variation in the isolation frequency of Candida in healthy and debilitated patients (3,5,20). Isolation frequencies of Candida were recorded as 37 % from gum samplings and 47.7 % in saliva cultures (11). Candida in mouth washings of children and adults were 33.5 % and 50 % respectively (15). Sputum examined in hospital personnel and medical students recovered a 55 % incidence of Candida with 50 % identified as C. albicans and the remaining 5 % as other Candida species (2). Previous investigations did not examine the additional medically important Candida species. C. stellatoidea, C. guillermondii, and C. krusei isolates in the current study displayed significantly different drug tolerance results when compared to the C. albicans group. The current series produced a 26.2 % overall recovery of Candida.

Mycophenolic acid did not inhibit C. stellatoidea and C. guillermondii as similarly found in an earlier study with the drug on C.

guillermondii, C. krusei, and C. pseudotropicalis (16). However, mycophenolic acid did exhibit a response to C. stellatoidea at concentrations of 3.9 mcg/ml. Drugs commonly utilized for candidiasis therapy are nystatin, 5-fluorocytosine, and amphotericin B. No conclusive species variation was noted with nystatin. Growth inhibition with 5-fluorocytosine was either intense or negligible. Variability is reflected in the 95 % confidence interval as shown in Charts 1 and 2.

Tolerance of C. albicans to 5-fluorocytosine was previously examined, and some strains were resistant at concentrations of 1,000 mcg/ml (18). Previous reports have noted successful treatment of systemic candidiasis with 5-fluorocytosine (17). However, numerous strains of Candida have demonstrated abilities to acquire resistance to 5-fluorocytosine (13). The current study indicated that isolates C. stellatoidea and C. krusei were completely resistant to the antifungal agent 5-fluorocytosine, while C. albicans and C. guillermondii demonstrated growth inhibition. The isolate C. guillermondii displayed complete resistance to the systemic antifungal agent amphotericin B. In contrast, C. albicans exhibited significant growth inhibition towards amphotericin B.

Table 1.

Drug Modes of Action

Test Drug	Mode of Action
Niddamycin	Interferes with cell wall
Amphotericin B	or membrane integrity
Erythromycin B	
Nystatin	
Mycophenolic Acid	
Triacetyloleandomycin (TAEM)	
5-Fluorocytosine	Nucleic acid synthesis inhibitor
Abbott 36042	toxic cyano poison
Dimethylchlorotricycline (DMCTC)	Experimental
Abbott 25579	
Abbott 6131	
Chlorambucil	Chemotherapeutic alkylating agent

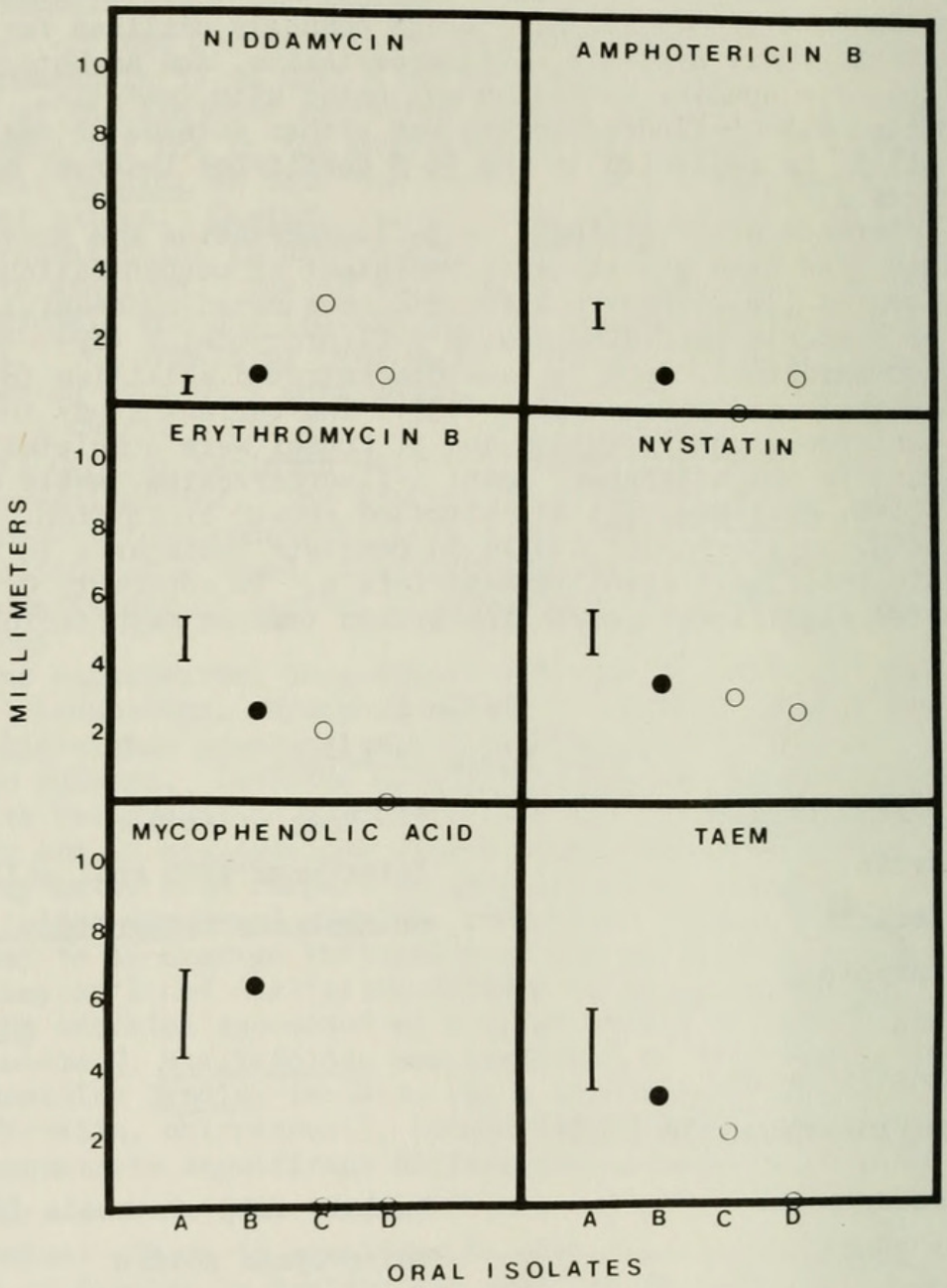

Chart 1.

Growth inhibition of Candida oral isolates with drugs at 1,000 mcg/ml affecting cell wall or membrane structures.

Figure legend:
A C. albicans, t-distribution of 49 isolates |
B C. krusei, 2 isolates ●
C C. guillermondii, 1 isolate ○
D C. stellatoidea, 1 isolate ○

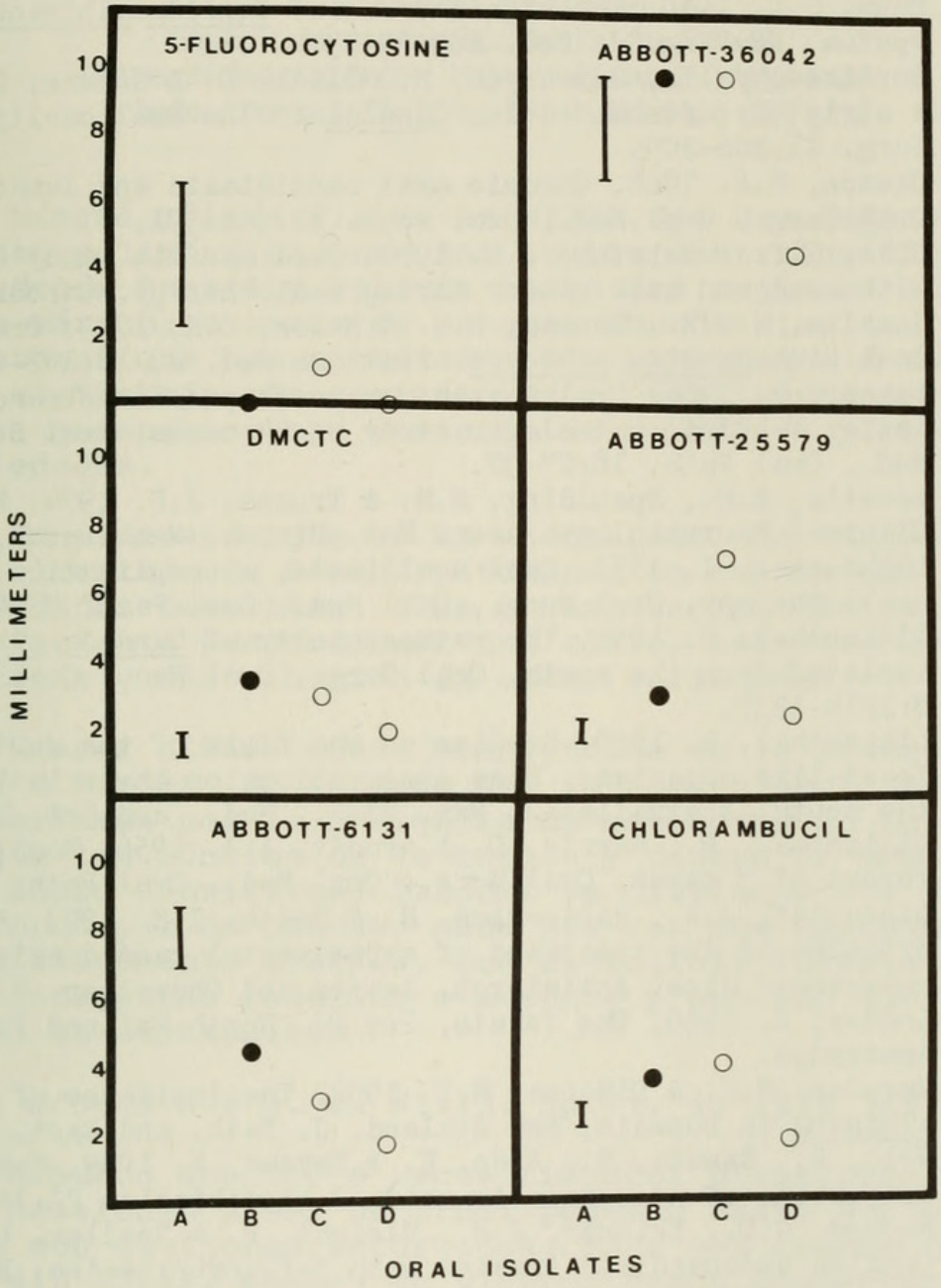

Chart 2.

Growth inhibition of Candida oral isolates with an alkylating agent, experimental, and nucleic acid inhibiting drugs at 1,000 mcg/ml.

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