5-Fluorocytosine resistance in clinical isolates of *cryptococcus neoformans*

C.S. Chin, B Sc (Hons)
Y.M. Cheong, MBBS, M Sc
Bacteriology Division
Institute for Medical Research
Kuala Lumpur

Y.H. Wong, B Sc (Hons)
Department of Laboratory Services
General Hospital
Kuala Lumpur

Summary

Thirty six clinical isolates of *Cryptococcus neoformans* were tested for their susceptibility to 5-fluorocytosine and amphotericin B by the determination of minimum inhibitory concentrations and minimum fungicidal concentrations. 22.2% of the isolates were resistant to 5-fluorocytosine and 36.1% indicated 5-fluorocytosine tolerance. All strains were sensitive to amphotericin B.

Key words: 5-fluorocytosine resistance, Cryptococcus neoformans, cryptococcosis.

Introduction

Amphotericin B, a drug active against a variety of fungi, is effective for the treatment of cryptococcosis and several other systemic mycoses. Disadvantages in its use, however, include severe side-effects and the necessity of intravenous or intrathecal administration.[1,2] 5-fluorocytosine, on the other hand, can be administered orally, causes milder side effects and penetrates well into body fluids, but its efficacy is restricted mainly to yeast infections and its usefulness limited by primary fungal resistance and the emergence of secondary resistance during therapy.[1-8]

Both therapeutic success and failure had been reported with the use of 5-fluorocytosine alone in the treatment of cerebral cryptococcosis in Malaysia.[9] The contributory role of fungal resistance in therapeutic failures were however not confirmed by laboratory studies. This study was therefore conducted to ascertain the existence and the extent of 5-fluorocytosine resistance among local clinical isolates of *Cryptococcus neoformans*. The level of susceptibility of these isolates to amphotericin B was also determined.

Materials and methods

The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of 5-fluorocytosine and amphotericin B for 36 clinical isolates of *C. neoformans* were determined by the broth dilution method.[10] These isolates were collected from the year 1982 to 1987. Thirty-five isolates were from patients with cerebral cryptococcosis and one was from a patient with concomitant cerebral and breast cryptococcosis.

The yeasts were tested against one millilitre volumes of drug concentrations that ranged 0.05 to 100 mg/L for 5-fluorocytosine and 0.02 to 50 mg/L for amphotericin B. In 5-fluorocytosine

testing, dilutions were made from aqueous 10,000 mg/L stock solutions, using as diluent, Yeast-Nitrogen Base (Difco) broth supplemented with L-asparagine and dextrose. Yeasts were grown for 48 hours on Yeast Nitrogen Base solidified by Special Noble Agar (Difco), suspended in Yeast Nitrogen Base broth, and adjusted to percent transmission of 90% at 530 nm wavelength. 0.05 millilitres of these suspensions were used as test inocula. For amphotericin B testing suspensions were used as test inocula. For amphotericin B testing, dilutions were made in Antibiotic Medium 3 (Difco) from 10,000 mg/L stock solutions in dimethyl sulfoxide. Inocula used were 0.05 millilitres of aqueous suspensions of 48 hours yeasts grown on Sabouraud Dextrose Agar, similarly adjusted to 90% transmission at 530 nm wavelength.

All strains were tested in duplicates. Drug free diluent controls and dimethyl sulfoxide controls were included. A strain of *Candida pseudotropicalis* (Pasteur Institute) was tested in each test run to allow for between-tests comparability. The MIC was read, after 48 hours incubation at room temperature, as the lowest concentration of drug that inhibited growth in both tubes of duplicate tests. The MFC was determined as the lowest concentration of drug that yielded less than three colonies in both plates after 0.05 mls were subcultured from negative tubes onto Sabouraud Dextrose Agar and incubated for 48 hours.

Results

The relative susceptibilities of the 36 isolates of *C. neoformans* to amphotericin B and 5-fluorocytosine are indicated in Figures 1 and 2. MIC values to 5-fluorocytosine ranged from 0.39 mg/L to more than 100 mg/L, while MFC values ranged from 1.56 mg/L to more than 100 mg/L. Eight isolates (22.2%) had MIC values to 5-fluorocytosine that were greater than 25 mg/L. Six (16.7%) had both MIC and MFC values greater than 100 mg/L.

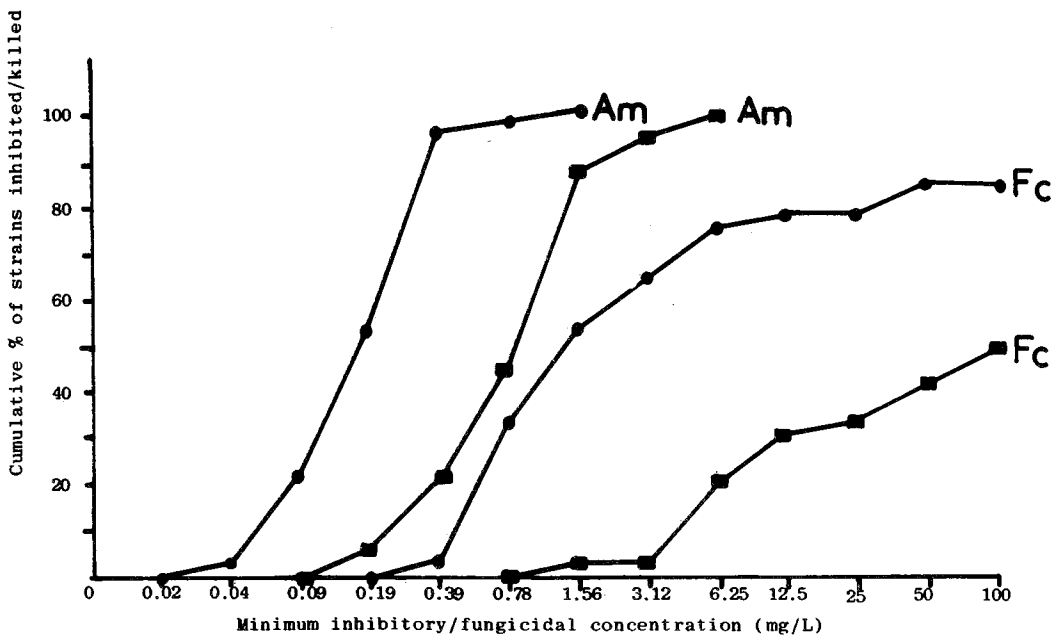

Fig. 1 Susceptibility of *Cryptococcus neoformans* to 5-fluorocytosine (Fc) and amphotericin B (Am). ● – inhibited. ■– killed.

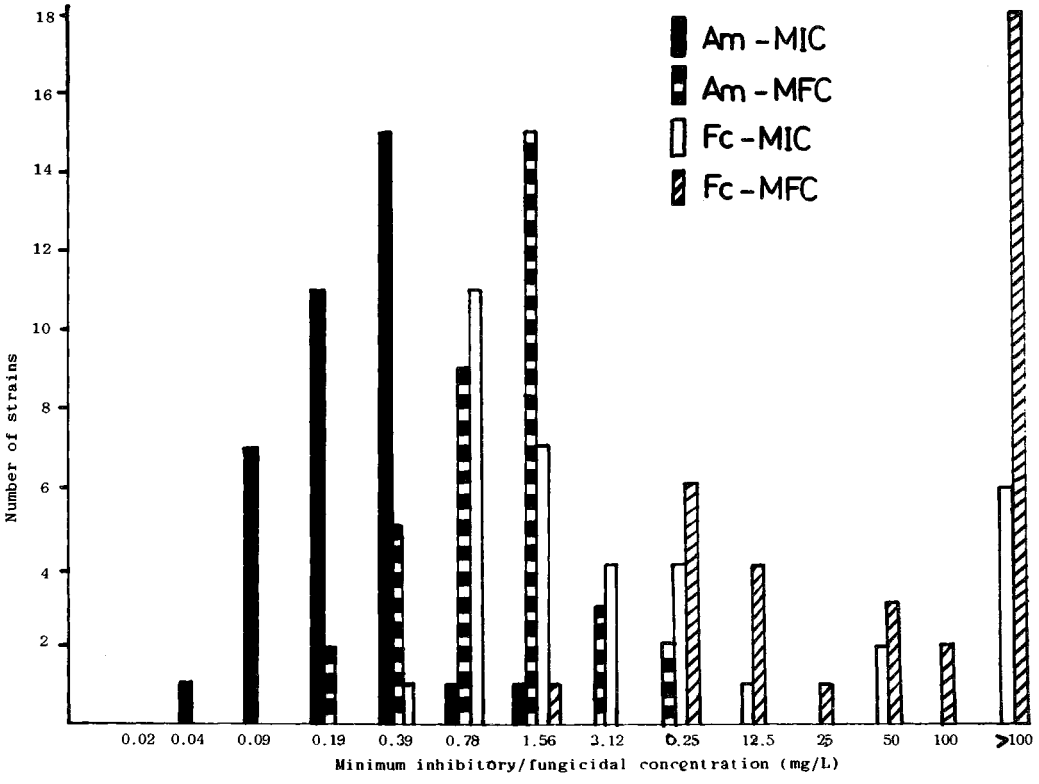

Fig. 2 Comparison of MICs and MFCs (mg/L) of 5-fluorocytosine (Fc) and amphotericin B (Am).

The distribution of resistant strains in the period of study is indicated in Figure 3. There was no detectable increase in the proportion of resistant strains over the years.

MIC and MFC values to amphotericin B ranged from 0.04 to 1.56 mg/L and 0.19 mg/L to 6.25 mg/L respectively. All eight isolates with MICs to 5-fluorocytosine greater than 25 mg/L had MICs to amphotericin B that were less than or equal 1.56 mg/L.

The MFC values of amphotericin B, a fungicidal drug, exceeded the MIC values in all strains mainly by one to two dilutions, maximally by four dilutions. In contrast, MFC and MIC values of 5-fluorocytosine, a static drug that can also be fungicidal at higher concentrations,[2] differed by as much as eight dilutions. Thirteen strains (36.1%) had MFCs to 5-fluorocytosine that were >32 fold higher than MICs and thus met the definition for 5-fluorocytosine tolerance. All 13 strains had MICs to 5-fluorocytosine that were <25 mg/L.

Discussions

Although MIC values within the wide range of 2–31 mg/L had been taken as indicative of susceptibility to 5-fluorocytosine by various investigators,[8] MIC values of 16 mg/L or 25 mg/L are generally accepted as the cut-off points between resistance and susceptibility.[2,11,12] Strains with MIC values of ⩾64 mg/L may be considered highly resistant strains.[11] Thus, 22.2% of the clinical isolates tested were resistant and 16.7% were highly resistant strains. Proctor and

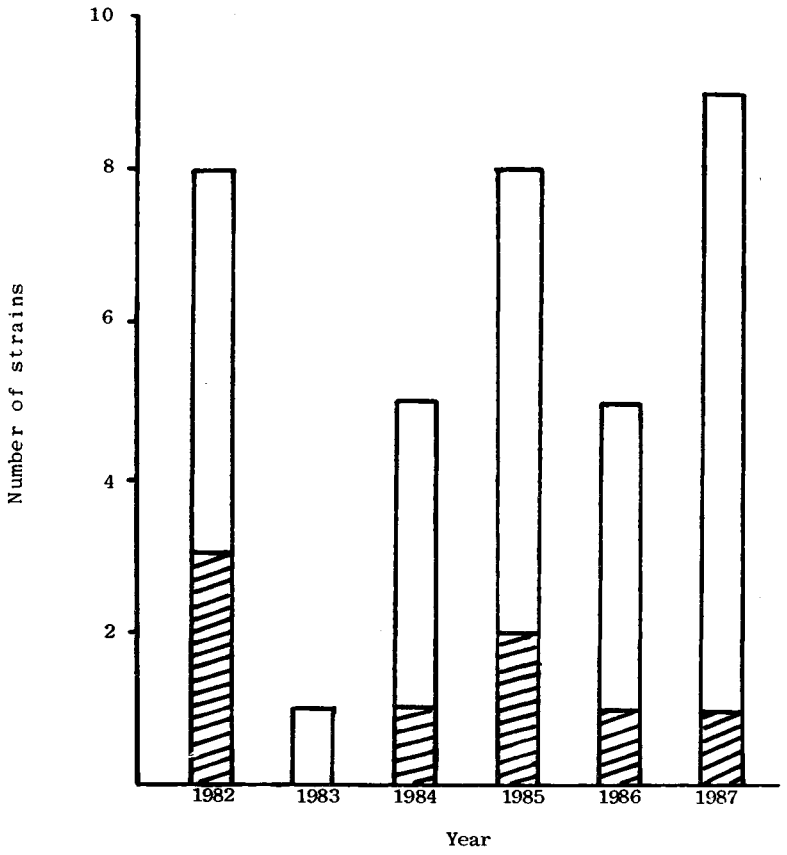

Fig. 3 Distribution of resistant strains, 1982–1987.

Mackenzie (1980) noted that strains that were not originally sensitive to 2–4 mg/L of the drug were more likely to develop resistance during treatment.[8] Hence, a further 13.9%–25% of our strains might have become resistant during treatment.

In comparison, the level of susceptibility of these strains to amphotericin B remained high. MIC values of fungi known to be susceptible to amphotericin B are reported to range from 0.02–100 mg/L.[1] An MIC $\geqslant$2 mg/L is usually accepted as indicative of resistance while probable intermediate susceptibility is denoted by an MIC of 1.56 mg/L[12,13] By these criteria, all isolates tested were susceptible to amphotericin B, with one strain in the range of intermediate susceptibility.

Cryptococcosis, particularly Cryptococcal Meningitis, can be a fatal disease if untreated. On the other hand, partially treated cryptococcosis is just as undesirable because it not only results in continued infection and relapses, but also encourages the emergence of resistant strains of *C. neoformans.* It is therefore imperative that the treatment initiated is effective. In view of the high incidence of 5-fluorocytosine resistance and tolerance in our local isolates of *C. neoformans*, combination treatment with 5-fluorocytosine and amphotericin B which had been recommended for the treatment of cryptococcal meningitis,[14] should be considered the treatment of choice, especially in the absence of sensitivity tests. Should 5-fluorocytosine be used alone, sensitivity testing is mandatory for all isolates obtained before and during the course of therapy.

Although the high level of susceptibility to amphotericin B in this study does not indicate the need for routine sensitivity testings, it must be stressed that resistance to amphotericin B amongst yeasts, although a rare clinical phenomenon, had been reported.[15-18] A significantly high incidence of polyene resistance was recorded among yeast strains isolated from oncology patients.[17] Thus sensitivity tests to amphotericin B should be considered for isolates from immunocompromised patients receiving therapy with cytotoxic drugs, antibiotics and polyenes, patients who are granulocytopenic and patients whose clinical status deteriorate despite adequate therapy with amphotericin B.

Acknowledgements

We thank the Director, Institute for Medical Research for his permission to publish this paper, M. Kanaganayaghy for technical assistance and Norangizan Abdul Hamid for typing the manuscript.

References

1. Utz J.P. Chemotherapy for the Systematic Mycoses. *Br J Hosp Med. 1976; 15: 112–121.*
2. Medoff G, Kobayashi G S. Strategies in the treatment of systemic fungal infections. *N Engl J Med* 1980; 302: 145–155.
3. Hermans P E, Keys T F. Antifungal agents used for deep-seated mycotic infections. *Mayo Clin Proc* 1983; 58: 223–231.
4. Schoebeck J, Ansehn S. 5-fluorocytosine resistance in *Candida* Spp and *Torulopsis glabrata. Sabouraudia* 1972; 11: 10–20.
5. Holt E J, Newman R L. The antimycotic activity of 5-fluorocytosine *J Clin Path* 1973; 26: 167–174.
6. Block E R, Jennings A E, Bennett J E. 5-fluorocytosine resistance in *Cryptococcus neoformans.* Antimicrob Agents Chemother 1973; 3: 649–656.
7. Bennett J E. Flucytosine *Ann Intern Med* 1977; 86: 319–322.
8. Speller D C E (edit.). *Antifungal chemotherapy* Chichester John Wiley & Sons, 1980.
9. Richardson P M, Mohandas A, Arumugasamy N. Cerebral cryptococcosis in Malaysia. *J Neurol Neurosurg Psychiatry* 1976; 39: 330–337.
10. Haley L D, Callaway C S. *Laboratory Methods in Medical Mycology.* (Fourth Edition). U.S. Department of Health, Education and Welfare. 1978.
15. Pappagianis D, Collins M S, Hector R, Remington J. Development of resistance to amphotericin B in *Candida lusitaniae* infecting a human. *Antimicrob Agents Chemother* 1979; 16: 123–126.
16. Drutz D J, Lehrer R J. Development of amphotericin B resistant *Candida tropicalis* in a patient with defective leukocyte function *Am J Med Sci* 1978; 276: 77–92.
17. Dick J D, Merz W G, Saral R. Incidence of polyene-resistant yeasts recovered from clinical specimens. *Antimicrob Agents Chemother* 1980; 18: 158–163.
18. Ahearn D G, McGlohn M S. In vitro susceptibilities of sucrose-negative *Candida tropicalis, Candida lusitaniae* and *Candida norvegensis* to amphotericin B, 5-fluorocytosine, miconazole and ketoconazole. *J Clin Microbiol* 1984; 19: 412–416.
11. Report of a Working Group of the British Society for Mycopathology, Laboratory Methods for flucytosine. *J Antimicrob Chemother* 1984; 14: 1–8.
12. Lennette E H, Balows A, Hausler W J, Truant J P. *Manual of Clinical Microbiology.* (Fourth Edition) American Society for Microbiology.
13. Mercz W G. *Candida Lusitaniae*: Frequency of recovery colonization, infection, and amphotericin B resistance. *J Clin Microbiol* 1984; 20: 1194–1195.
14. Bennett J E, Dismukes W E, Duma R J *et al* A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med* 1979; 301: 126–131.