Note

Candidostatic Effect of Heavy Metals

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The *Candida* strains isolated from human pathogenic lesions were tested with different concentrations of heavy metals to determine the in vitro minimal growth inhibitory concentration of the salts. The test, being a new one, can help to develop ancillary candidostatic measures.

Key words : Heavy metals/*Candida* species/Pathogenic lesions.

In the last decade, an increased incidence of mycotic infections in various groups of patients has been documented (Chakraborti et al., 1992 ; Das et al., 1995 ; Meunier, 1989). Mycotic infections are classified by the site of infection, route of acquisition and types of virulence (Kobayashi, 1991). When classified by the site of infection, the diseases are designated as superficial, cutaneous, subcutaneous or deep. Infections of candidiasis, commonly caused by *Candida albicans*, are classified either as superficial or deep. On this basis, superficial candidiasis may involve epidermal or mucosal surfaces including those of the oral cavity, pharynx, oesophagus, stomach, intestine and urinary bladder, etc. The kidneys, liver, spleen, brain, eyes, heart and other tissues are major organ sites involved in deep or visceral candidiasis. These fungal infections may further be categorized as endemic or opportunistic mycoses. In endemic mycoses, the susceptibility to infection is acquired by living in a geographic area which is the natural habitat of the particular fungus. The opportunistic mycoses pose an equally pressing problem since these infections are associated with a particularly high mortality rate, and they seem to be occurring at a significantly increasing frequency (Pfaller, 1994). Candidiasis is one such opportunistic mycotic disease (Datta et al., 1989; Martin and Kobayashi, 1993; Rippon, 1988; Rüchel, 1991).

This disease is acquired from the natural environment, and females, especially housewives, maid servants, cooks, dairy workers, and fish and vegetable

vendors, often suffer from this disease due to continuous contact with water (Das et al., 1995; Evans, 1985; Jawez, 1987; Stewart, 1978). Climatic conditions such as a tropical climate with high temperatures and high humidity predispose human beings to acquire this type of disease (Majumdar and Chakraborti, 1962). Due to the increasing occurrence of candidial infections, it is of paramount importance to understand the characteristics of the pathogen and the ways and means by which it can be managed and controlled.

The aim of the present investigation is to ascertain the minimal inhibitory concentration (MIC) of certain heavy metals against the in vitro growth of two forms of *C. albicans* isolated from pathogenic lesions of the patients in the steel city of Rourkela, Orissa, India.

The heavy metal salts used in this investigation were silver, cadmium, copper, bismuth, mercury and lead. Since these are toxic to the organisms or show effects in trace quantities they can be prescribed for topical treatment of superficial candidal infections in the form of ointments.

The samples were collected for a period of one year from patients suspected to be suffering from superficial skin infections and thus, who were visiting the Skin and Venereal Disease Department, Ispat General Hospital, Rourkela, Orissa, India, for treatment. The samples were obtained from infected nails and skin lesions. Parts of the scrapings were mounted in 10 % (w/v) KOH and observed directly under the microscope to detect fungal elements (spores and hyphae). The remaining part of the samples which

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showed positive results were inoculated into Sabouraud's dextrose agar (SDA) (40g of dextrose, 10g of peptone, 15g of agar in 1 l of distilled water) containing cycloheximide (0.5g) and chloramphenicol (0.05g) at pH 5.6. The incubation was carried out for one week at 30°C.

The macro-and micromorphology of the fungi grown in SDA media were studied to identify the variants of *C. albicans*. Out of 195 positive cases of superficial fungal infections, 84 samples (44.8%) were identified to be candidiasis (Das et al., 1995). Only two forms of *C. albicans* were isolated. Upon culturing in SDA, 58 samples developed smooth, creamy, white colonies which were observed to be budding yeast forms under the microscope. The remaining 26 samples developed rough, white colonies with irregular margins. Viewed through the microscope, they were observed to be mycelial forms.

Since only two forms of *C. albicans*, i.e., yeast and mycelial, were collected from the superficial infection sites and all of them developed chlamydospores in confirmatory tests, one from each category was used separately to study its sensitivity to different heavy metals. Both cell forms of *C. albicans* were initially grown in SD broth in order to prepare their stock cultures. To determine the candidostatic effect of heavy metals, SD broth was prepared in double concentrations at pH 5.6 and was dispensed in 5-ml aliquots to culture tubes. The culture tubes were then sterilized under 15lb/inch² steam pressure for 15min. The heavy metals used were silver nitrate (AgNO₃), copper sulphate (CuSO₄·5H₂O), cadmium sulphate (CdSO₄·8H₂O), bismuth nitrate [Bi(NO₃)₃·5H₂O], mercuric chloride (HgCl₂) and lead acetate [Pb(CH₃COO)₂·3H₂O]. To the culture tubes different heavy metal salts

were added in 0.05, 0.1, 0.25, 0.35, 0.5, 1.0, 1.5, 2.5, 3.5 and 5 ml amounts aseptically from the sterile heavy metal salt solutions (200 µg/l). Each set of solutions was made in triplicate and the final volume was made to be 10 ml with sterile distilled water. The stock cultures of the fungus were kept on a rotary shaker (110 rpm) for 1 h before inoculation to the sets of culture tubes with varying concentrations of heavy metal salts. Then 0.1 ml of thoroughly mixed stock cultures were inoculated aseptically and incubated at 30°C for 10 d. The growth of two forms of the organism was noted visually.

Organisms grew best in cultures which contained no heavy metals. In these cases, profuse ingrowing and surface growth were detected. The MIC values of different heavy metals varied, and the growth of both forms of the fungus differed according to the different heavy metal ions and the concentrations which were used (Table 1). It was further observed that the salts of bismuth among all the heavy metal salts tested, inhibited the growth of the two forms of *C. albicans* at the lowest concentration. The MIC was 5 µg/ml against the yeast form and 10 µg/ml against the mycelial form (Table 1). The MIC of the remaining heavy metals against the growth of both the forms of *C. albicans* could be arranged in the following order: Ag>Cd>Hg>Cu>Pb (Table 1). The growth of the fungal forms appear very dense and deep at the concentration preceding the MIC value. Besides, it was observed that the yeast form of *C. albicans* was more susceptible to heavy metals than the mycelial forms in all cases of heavy metal salts (Table 1).

Deleterious effects of heavy metals on the growth of microorganisms are well established. Horsefall (1956) had listed the toxic effect of different heavy

TABLE 1. Growth inhibitory activities of heavy metals against two forms of *C. albicans*.

Metal ion as salt	Cell form	Concn (µg/ml)											
		1	2	3	4	5	7	10	20	30	50	70	100
AgNO ₃	Y	++	++	++	+	+	+	+	-	-	-	-	-
	M	+++	++	++	++	++	++	+	+	-	-	-	-
CdSO ₄ ·8H ₂ O	Y	+++	++	++	++	++	+	+	+	-	-	-	-
	M	+++	+++	++	++	++	++	+	+	+	+	+	-
CuSO ₄ ·5H ₂ O	Y	+++	++	++	++	++	+	+	+	+	-	-	-
	M	+++	++	++	++	++	++	+	+	+	+	+	-
Bi(NO ₃) ₃ ·5H ₂ O	Y	+	+	+	+	-	-	-	-	-	-	-	-
	M	++	++	+	+	+	+	-	-	-	-	-	-
HgCl ₂	Y	+++	+++	+++	+++	+++	+++	+++	+	+	-	-	-
	M	+++	+++	+++	+++	+++	+++	+++	+	+	+	-	-
Pb(CH ₃ COO) ₂ ·3H ₂ O	Y	+++	+++	++	++	++	++	+	+	+	+	-	-
	M	+++	++	++	++	++	++	++	+	+	+	+	-

^aY, yeast form; M, mycelium form.

^b+++ , Good growth; ++, moderate growth; +, poor growth; -, no growth.

metal ions against fungi in the following order : Ag>Hg>Cu>Cd>Ni>Pb>Co>Zn>Fe>Ca. This is, however, a generalised scheme, as the order of the effectiveness of different elements may differ against different species. Disinfectant properties of mercuric chloride and silver nitrate are utilized in different standard techniques and in medicines.

Many investigators have reported the effect of heavy metals on the pure culture of bacteria (Jones, 1971; Starr and Jones, 1956; Zwarum, 1973), algae (Barflett et al., 1974; Steeman et al., 1970; Sundow and Guillard, 1976), dermatophytes (Choudhury, 1985) and on many natural microbial populations (Albright et al., 1972; Albright and Wilson, 1974; Nelson and Colwell, 1975). The inhibitory effect of zinc, mercury and silver on the respiration of dermatophytic fungi has been reported (Nickerson and Chadwick, 1946). Lead, copper and cadmium were found to have toxic effects on the growth of the fungus, *Trichophyton rubrum* (Chandra and Banerjee, 1974). Of the group comprising cadmium, lead, and mercury, the last has been extensively characterized as an inhibitor of enzymes due to its binding to a number of functional groups (Oster, 1961). However, reports on candidostatic properties of heavy metals have not been available excepting the reports of Malavasic and Cihlar (1992) who studied the retardation of colony growth of certain *C. albicans* species by heavy metals. An attempt has been made here to determine the effect of heavy metal compounds on the in vitro growth of two forms of mycoses isolated from superficial human pathogenic lesions. The MIC of the heavy metal compounds are shown in Table 1.

Anions in heavy metal salts generally reduce their toxicity by precipitation. Sulphur, in its sublimated inorganic form, is generally, an integral part of antifungal ointment (Oster, 1961). Yet, sulphates of cadmium and copper were third and fifth in the order of their candidostatic effects among the six heavy metal salts tested. Bismuth was observed to have the greatest candidostatic property and, as it is, nontoxic in nature, it can be prescribed for superficial candidiasis. It can be used as a supplement to several effective therapeutic agents available. The microbial forms are pleomorphic in nature and upon isolation and while being maintained in media containing peptones and yeast extracts of certain buffer solutions, they react chemically with heavy metals, to become resistant in nature (Oster, 1961). Since the two forms of *C. albicans* tested here were maintained and cultured throughout in SDA media that always contained 1% (w/v) peptone, the inhibitory potency of certain heavy metals shown in Table 1 can be used as a very good tool in controlling such supposedly resistant

mycoses. The mycelial form of *C. albicans*, being more pathogenic (Datta et al., 1989; Odds, 1988; Rippon, 1982; Rook et al., 1987), resisted higher dosages of heavy metals than the yeast form did.

Presently available antifungal candidostatic agents are taken orally, topically or both, but have a multitude of adverse side effects such as persistent diarrhoea, nausea, vomiting, elevated liver function, and intestinal reactions. Hence, alternate sources of drugs which are free of or have fewer side effects are still being probed. Hence, in vitro experiments with heavy metal salts and their topical use to control the candidiasis lesions seem to herald a new dimension to the antimycotic potency of nontoxic salts. The finding here regarding Bismuth, is really very inspiring.

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