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## Activity of garlic extract against the *Cryptococcal* isolates obtained from cerebrospinal Fluid from patients suffering from fungal meningitis

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### Abstract

*Cryptococcosis* is increasing due to an ever rising population of immunocompromised subjects, including AIDS patients. Ayurveda, the ancient medical science of India requires today documentary evidence to establish for its regular use as remedy, which will be useful for the control and cure of the various infectious conditions. Garlic, has been reported as effective treatment for *Cryptococcal* meningitis. Keeping in mind the above facts, the study was conducted to evaluate susceptibility of *Cryptococcus* isolates against Garlic extract in Nair Hospital, Mumbai, including 180 patients, who were clinically suspected for fungal meningitis. Collection and microbiological analysis of cerebrospinal fluid for identification of *Cryptococcal* isolates was carried out as per standard procedures. Garlic extract was prepared and its Minimum Inhibitory and Lethal concentration was ascertained. Total 20 cases of *Cryptococcus* species obtained in the study was evaluated against antifungal agents and garlic extract. Highest susceptibility was shown by the Amphotericin B to 60% of the strains. MIC values against garlic extract fell in the range of 128 to 256 mcg/ml, while MLC values in the range of 256 to 512 mcg/ml. Hence by comparing the present study findings it can be suggested that Garlic can help in augmentation of antifungal drugs effect in the treatment of *Cryptococcosis*.

**Keywords:** *Cryptococcus*, antifungal agents, Garlic, Susceptibility study, Ayurveda

### 1. Introduction

*Cryptococcosis* is a potentially fatal fungal disease, which is a defining opportunistic infection for AIDS. The prevalence of *cryptococcosis* has been increasing over the past 20 years for many reasons, including the increase in incidence of AIDS and the expanded use of immunosuppressive drugs. In humans, it causes three types of infections namely Cutaneous *Cryptococcosis*, Pulmonary *Cryptococcosis* and *Cryptococcal meningitis*. *Cryptococcal meningitis* is believed to result from dissemination of the fungus from either an observed or unappreciated pulmonary infection. Often there is also silent dissemination throughout the brain when meningitis is present. *Cryptococcosis* is often fatal, even if treated. It is estimated that the three-month case-fatality rate is 9% in high-income regions, 55% in low/middle-income regions, and 70% in sub-Saharan Africa. <sup>[1]</sup> *Cryptococcosis* has been termed the 'Awakening giant' among the mycoses since 1970 <sup>[2]</sup> and now being regarded as one of the AIDS- defining infections. The organism is unique among pathogenic fungi because of its production of mucinous capsule in tissue and culture <sup>[3]</sup>.

According Stenens DA *et al.*, the field of antifungal chemotherapy is presently rapidly moving <sup>[4]</sup>. In 1903, it began with the successful use of potassium iodide. Then after 50 years, in 1951 Nystatin was introduced, the first useful polyene. Four years later Amphotericin-B was the historical standard regimen. After 1970's Azole drugs were introduced beginning with Ketoconazole, Itraconazole, and Imidazole. Study carried out by Shindo *et al.* in 1990, has proved that Amphotericin- B was essential to administer to the patient as the initial treatment for *Cryptococcal meningitis*, when antigen titer and India ink is positive <sup>[5]</sup>.

Ayurveda, the ancient medical science of India, is not merely a doctrine of medical treatment, but a way of healthy long life, comprising mostly medicinal plants. More over the increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggest that, in order to find active components, a systemic study of medicinal plant is very important.

In 1980, a study in the Chinese medical journal suggested that garlic, when administered alone either intravenously, intramuscularly or by mouth was an effective treatment for *Cryptococcal meningitis* [6, 7, 8]. In 1977, Moor GS *et al.* had studied the effect of garlic extract on *Candida spp.* and found that Garlic extract appeared more effective at body temperature than at 30° c. He also stated that isolates of *Cryptococcus* species, were inhibited *in vitro* in the presence of an aqueous extract of Garlic. Scientific study towards various plant drugs [9] and its effect on various pathogens *in vitro* as well as *in vivo* are not well documented. [10] Hence, it is need of today to establish such documents for its regular use as remedy, which will be useful for the control and cure of the various infectious conditions. Keeping in mind the above facts, we have, conducted a study to evaluate susceptibility of *Cryptococcus* isolates against Garlic extract.

## 2 Materials and methods

This prospective longitudinal study was carried out over a period of three years, from January 1997 to December 1999 at the Department of Microbiology, after taking the permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai.

### 2.1 Participants

The study included 180 patients, who were clinically suspected for fungal meningitis. These subjects were showing sign and symptoms of fever with chronic headache, body ache, Nausea, vomiting, staggering gait, altered sensorium and neck stiffness. Also patients on long-term immunosuppressive drug therapy and malignancy and organ transplant were included.

### 2.2 Collection and microbiological analysis

Cerebrospinal Fluid (CSF) was obtained from each patient from various clinical units by trained clinicians by the standard procedure [11] and sent to Microbiology department for the further analysis. CSF samples were centrifuged at 3000 rpm for 10 minutes immediately and sediment was studied by microscopy and culture methods.

### 2.3 Identification of *Cryptococcal* isolates from CSF samples

- A. Initially Direct microscopy of CSF samples was done by conventional as well as Modified India Ink Preparation [12] and Gram staining as per standard method.
- B. Further, Sediment of CSF obtained after centrifugation was cultured on Blood agar, Chocolate agar and MacConkeys agar along with Sabourauds Dextrose Agar (SDA) in duplicate and incubated at 37 °C and Room Temperature. Agar plates were checked for any growth everyday till 4<sup>th</sup> week, before reporting it as “no growth” occurred on agar plates.
- C. Blood Culture [13] was also done in brain heart infusion

- D. broth to detect *Cryptococemia* in patients showing *Cryptococci* on direct mount. Inoculated bloods cultures, were incubated for 48-72 hours at 37°C and were subcultured on SDA without cycloheximide to check the colonies of *Cryptococcus isolates*.
- E. Confirmatory tests were carried out by inoculating *Cryptococcal* isolates on Niger seed agar [14] and L-Canavanine Glycine Bromothymol Blue medium. [15] Also carrying out Rapid selective urease test [16] and Rapid Nitrate Reduction test [17].

### 2.4 Determination of *In vitro* antifungal activity

- A. All *Cryptococcal* isolates were tested *in vitro* against Amphotericin B (100 units/disc) and Fluconazole (10 mcgm/disc) by Stokes method [18] using *Candida kefyr* as control strain.
- B. All *Cryptococcal* isolates were also tested against Garlic Extract to ascertain their Minimum inhibitory and lethal concentrations [19].

### 2.5 Preparation of Garlic extract

- A. Garlic was identified and collected from the farm. It was further certified by the botanist and brought into use. One hundred grams of garlic were homogenized in 100 ml of distilled water in a warning blender for 10 minutes. The extract was filtered through the 5 layers of gauze and the filtrate was centrifuged for 20 minutes at 2000 g and filtered through Whatmanpaper number1 and then passed through Seitz filter for sterilization.
- B. Sterility testing of garlic extract was done to confirm that extract was devoid of any contaminant. [20]

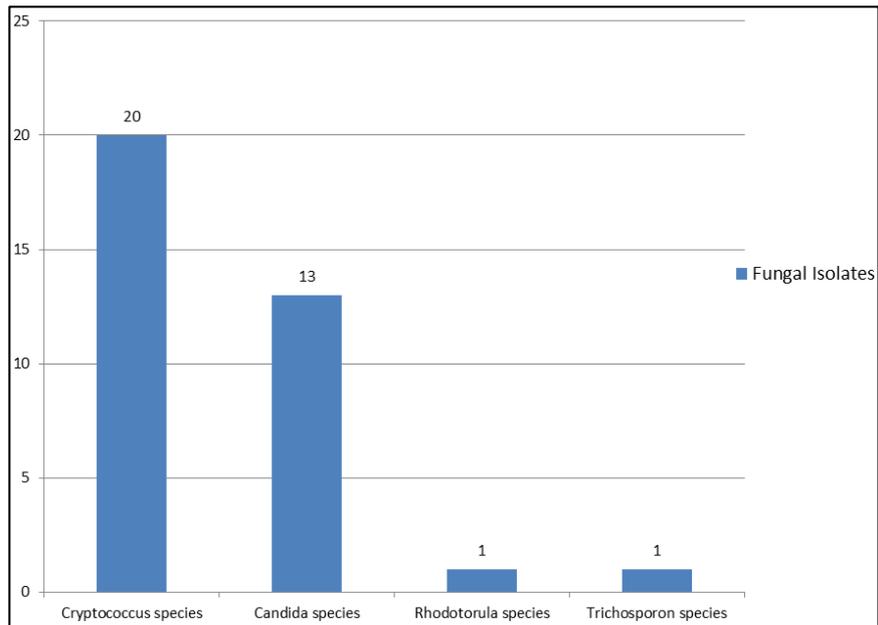
### 2.6 Inoculum preparation

*Cryptococcus isolates* were washed off from Sabouraud agar slants and a final concentration of suspension was matched with the Macfarland Standard 1 for the inoculum density. [21]

### 2.7 Minimum Inhibitory Concentration and Minimum Lethal concentration

MIC and MLC of garlic extract was determined by broth dilution method using Sabouraud's Broth. Serial dilutions of extract were done in Sabouraud's Broth from dilution 2 to 1024. Medium and positive control was also tested in each test. 20 micro liter of *Cryptococcus isolates* suspension of each strain was inoculated in each dilution tube and mixed well. All the tubes were incubated at 20-25 °c for 24-48 hrs or until growth in control tube was observed. The inhibitory concentration was defined as the lowest concentration of antimicrobial agent that showed no growth of original inoculum. MIC was reported as the concentration showing no growth, while MLC of garlic extract was determined by culturing loopful suspensions from tubes showing no growth on the SDA without cycloheximide plates and inoculated at room temperature for 24- 48 hrs. The lethal concentration was defined as the lowest concentration of antimicrobial agent that provided 99.9%--100% mycotoxic activity when compared with the growth of original inoculum.

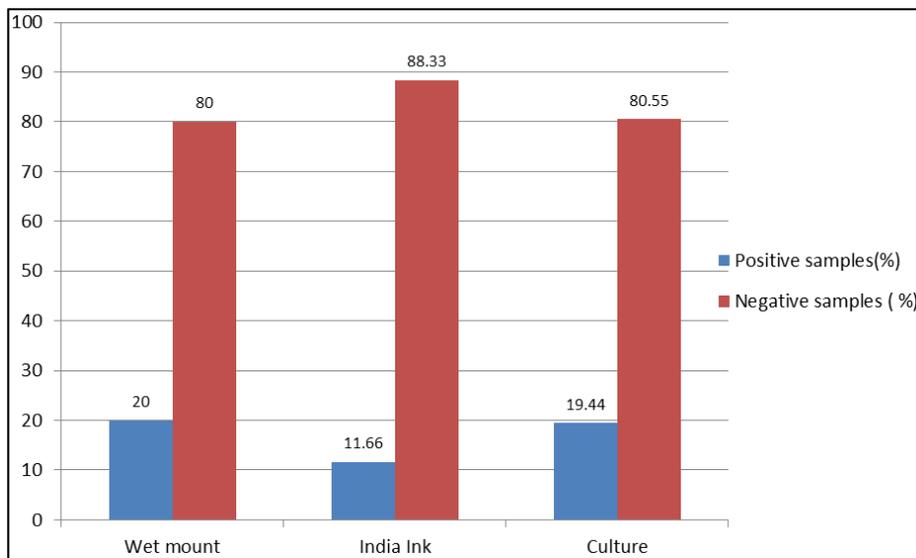
**3. Results**



**Graph 1:** Number of Fungal isolates in CSF samples. n=180

Study revealed *Cryptococcus* species in 20 cases, *Candida* species in 13 cases, *Rhodotorula* and *Trichosporon* species in one case each. Hence total 35 fungal strains were isolated

from total 180 cases. A concomitant growth of *Cryptococcus* and *Candida* species was observed in one case.



**Graph 2:** Microscopic findings n=180

Microscopic findings of 180 cases revealed yeast cells in 36 cases by wet mount. Out of these 36 cases, 21 cases demonstrated capsulated yeasts cells by India ink staining

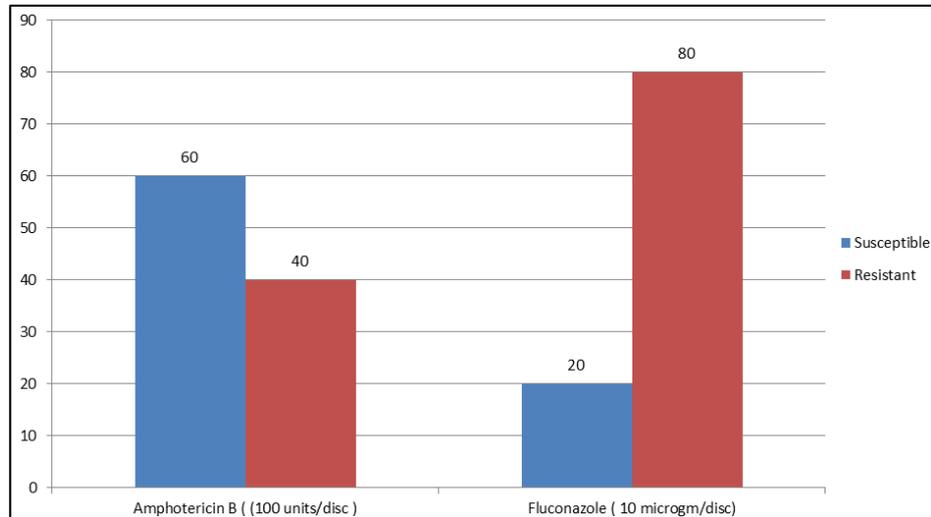
which was confirmed as *Cryptococcus* species. Out of these 21 cases, one case was positive by India ink and negative by culture for *Cryptococcus* species.

**Table 1:** HIV Status in *Cryptococcosis* cases n=180

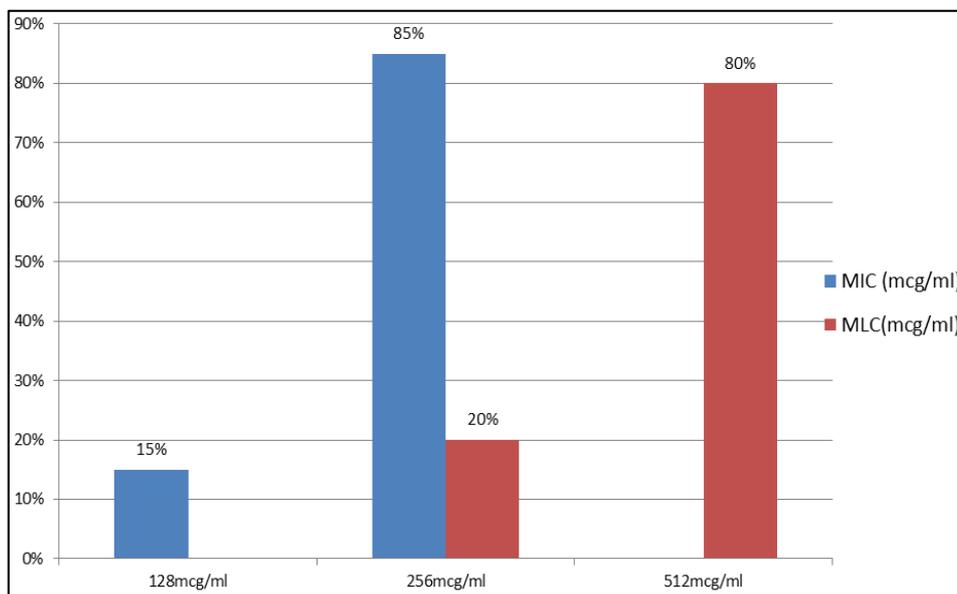
Groups	Suspected Cases	Diagnosed Cases
Group I (HIV Positive)	45 (25%)	19 (42.22%)
Group II (HIV Negative)	135 (75%)	03(02.22%)
Total	180	22

*Cryptococcal* meningitis was diagnosed significantly higher in Group I as compared to Group II patients. *Cryptococcosis* was diagnosed more in adult males than in adult females.

The ratio of Male to Female was 2. 6: 1 (Chisquare =46.7(P, 0.001))



**Graph 3:** Susceptibility pattern of *Cryptococcus neoformans* strains to antifungal agents (n=20)



**Graph 4:** MIC & MLC pattern of *Cryptococcus neoformans* strains to garlic extract. (n=20)

### 3. Discussion

Our observations in Nair Hospital from the year 1992 to 1998 has shown gradual rise in the incidence of *Cryptococcosis* infection in HIV positive patients and those patients who received immunosuppressive drug therapy and having other predisposing factors. Incidence was seen to increase from 6.6% to 13.75% in this time period. The study undertaken from 1997 to 1999 had shown the increase in the prevalence rate of *Cryptococcosis* which goes in accordance with the data published earlier at Nair Hospital. Over all prevalence of *Cryptococcal meningitis* in the study was found to be 12.2% which is in accordance with the WHO incidence rate of 5-33% worldwide [22]

In the pre-AIDS era of 1970-82 Talwar *et al.* from Chandigarh had reported only 9 cases of *Cryptococcal meningitis* [2]. An occurrence of 22 *Cryptococcal meningitis* cases in a 3 years period, that was from 1997 to 1999, maximum in HIV positive patients as observed by us, was an indication of increased incidence of *Cryptococcosis* as a consequence of AIDS. In the era of AIDS pandemic, Khan *et al.*, from Lucknow, have reported 10% positivity of *Cryptococcosis* or *Cryptococcus meningitis* from 1991 to 1994 [23] which shows a rise in the prevalence rate as compared to only 9 cases of *Cryptococcosis* in 12 years

period reported by Talwar *et al.* from Chandigarh. In the present study 86.09% of the *Cryptococcus meningitis* cases were HIV positive which correlates with a retrospective study of Pedrol *et al.* from Spain in 1985-90 which shows that 76.9% of the *Cryptococcus meningitis* cases were HIV positive [24].

According to Aquinas *et al.* from Bangalore in 1996, *Cryptococcus meningitis* was the most common opportunistic fungal infection in patients with AIDS contributing to the increased morbidity and mortality. [25] Hence our present study correlates with this findings that *Cryptococcosis* was maximally diagnosed in HIV positive patients than HIV negative patients. However, Petty *et al.* had noted the prevalence rate as low as 3.3% from HIV positive patients [26]. Hence a high index of clinical suspicion and routine mycological surveillance is essential to identify this infection.

Significant advances in antifungal therapy have occurred in the last decade. Most of these advances have been tried to the introduction of the Itraconazole and Fluconazole. In the present study *in vitro* antifungal susceptibility test has been carried out using Amphotericin B and Fluconazole against all fungal isolates by Stoke's method using *Candida kefyr* as a control strain [27, 28]. It was observed that maximum

number of isolates were sensitive to Amphotericin B as compared to Fluconazole. 60% of *Cryptococcus species* were sensitive to Amphotericin B, hence it can be still considered as a drug of choice. Kauffman CA *et al.* [29] and Treseler CB *et al.* [30] states that Amphotericin B with or without Flucytosine remains the drug of choice for many fungal infection especially those that are life threatening. It is the preferred as initial treatment for many fungal infections [12].

Garlic extract has an antimycocidal activity which can augment the treatment of *Cryptococcosis* when administered along with antifungal drugs. An extract of garlic was studied for its efficacy in treating experimental *Cryptococcosis*. Studies *in vitro* showed a definite anti-*cryptococcal* effect of garlic extract similar to that noted by other workers [31]. In the present study results of MIC and MLC of garlic extract against *Cryptococcus* were well correlated. *Cryptococcus* isolates showed MIC towards garlic extract to the dilution of 1:128 and 1:256 and MLC to the dilution of 1:256 and 1:512 by Tube dilution method. Ratio between MIC and MLC fell between 1 to 2. Micheal *et al.* [32] had reported, garlic inhibited growth of many but not all the species of fungi. Louria *et al.*, in 1989 [8]. Had tried the effect of Garlic on the experimental mice. The modest effect of Garlic in the Mouse model gives some support to the clinical report of Garlic efficacy in the treatment of *Cryptococcal meningitis* but failure to eradicate the infection in mice and the increasing similarity of *Cryptococcal* counts in the brains of control and Garlic treated animals over time, would suggest that Garlic alone is of no value for the treatment of human *Cryptococcosis*.

## 5. Conclusion

Our study highlights the antifungal property of garlic by its inhibitory and lethal effect on *Cryptococcus neoformans*. Hence by comparing the present study findings with *in vitro* findings by Gary *et al.*, it can be suggested that Garlic can help in augmentation of antifungal drugs effect in the treatment of *Cryptococcosis* but Garlic alone is not suitable candidate for the treatment of human *Cryptococcosis*. Extensive trials using different types of extract and determination of MIC and MLC would make interesting *in vitro* study. Considering the potential toxicity of existing antifungal drugs, a possible therapeutic or additive role of garlic in treatment of *Cryptococcal* infections can be envisaged and hypothesized [33].

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## 7. References

- Rippon JW. The Pathogenic fungi and Actinomycetes- Cryptococcosis and other yeast infections. Textbook of Med. Mycology. 1974, 205-227.
- Talwar P, Shama M. Incidence and diagnostic aspects of *Cryptococcosis* in Chandigarh (India) during the period of 1970-80. I.J.P.M. 1986; 29:45-52.
- Baum RR, Jose A, Gonclaves R. Clinical Epidemiological study of 171 cases of *Cryptococcosis*. Clinical infectious diseases. 1994; 18:369-380.
- Stenens DA. Therapy for opportunistic fungal infections, past, present and future. India J Cancer (India). 1995; 1:1-932.
- Shindo K. Treatment of *Cryptococcal meningitis* with five antifungal drugs- the role of Amphotericin -B Drugs. Exp Clin Res. 1990; 16(7):327-32.
- Grant IH, Armstrong D. Fungal infections in AIDS, Cryptococcosis. Infect Dis. Clin. North Am (USA). 1988; 2(2):457-64
- Harrison's Textbook on Principles of Internal Medicine, Ed. 1997; 2:1589-1601.
- Louria DH, Lavenhar M, Kaminski T. and Eng RHK. Garlic (*Allium sativum*) in the treatment of experimental cryptococcosis. Journal of Medical and Veterinary Mycology. 1989; 27:253-256.
- Moore GS, Atkins DR. The Fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. Mycologia, 1977, 69.
- McGinnis MR. Detection of fungi in cerebrospinal fluid. Am J Med (USA), 1983; 75(1B):129-38.
- CSF collection procedure: [https://www.labce.com/spg546367\\_csf\\_specimen\\_collection\\_process.aspx](https://www.labce.com/spg546367_csf_specimen_collection_process.aspx)
- Zerpa R, Huicho L, Guillén A. Modified India ink preparation for *Cryptococcus neoformans* in cerebrospinal fluid specimens. J Clin Microbiol. 1996; 34(9):2290-2291.
- Fuglsang- Damgaard D, Pedersen G, Schönheyder HC. Positive blood cultures and diagnosis of bacterial meningitis in cases with negative culture of cerebrospinal fluid. Scand J Infect Dis. 2008; 28; 40(3):229-3.
- Richard EL, Barton C. The use of Niger seed agar to screen for *Candida* in the clinical microbiology laboratory. Diagnostic Microbiology and Infectious Disease. 46(1):13-17.
- Klein KR, Hall L, Demi SM, Rysavy JM, Wohlfiel SL and Wengenack NL. Identification of *Cryptococcus gattii* by use of L-canavanine glycine bromothymol blue medium and DNA sequencing. J Clin Microbiol. 2009; 47(11):3669-72.
- Zimmer BL, Roberts GD. Rapid selective Urease test for presumptive identification of *Cryptococcus neoformans*. J Clin Microbiol. 1979; 10(3):380-381.
- Hopkins JM, Land GA. Rapid Method for Determining Nitrate Utilization by Yeasts. Journal of Clinical Microbiology. 1977; 5:497-500.
- Gosden PE, Andrews JM, Bowker KE, Holt HA, Mac Gowan AP, Reeves DS, *et al.*. Comparison of the modified Stokes' method of susceptibility testing with results obtained using MIC methods and British Society of Antimicrobial Chemotherapy breakpoints. J Antimicrob Chemother. 1998; 42(2):161-9.
- Casadevall A, Spitzer ED, Webb D, Rinaldi MG. Susceptibilities of Serial *Cryptococcus neoformans* Isolates from Patients with Recurrent *Cryptococcal meningitis* to Amphotericin B and Fluconazole. Antimicrobial agents and chemotherapy. 1993; 37:1383-1386.
- Sterility testing procedure: [www.who.int/.../TestForSterility-RevGenMethod\\_QAS11-413FI\\_NAL\\_March2012.pdf](http://www.who.int/.../TestForSterility-RevGenMethod_QAS11-413FI_NAL_March2012.pdf)
- McFarland J, Jama MD. The nephelometer: an instrument for estimating the number of bacteria in

- suspensions used for calculating the opsonic index and for vaccines. 1907; 49(14):1176-1178.
22. Bannerjee U. Cryptococcosis: Mycosis of future clinical profile and recent strategy of Lab. Diagnosis. Dept. of Microbiology, All India Inst. Of Medical Sciences, New Delhi, Recent Trends in Mycosis, 1998; 27-31.
  23. Khan ZK, Katiyar R, Shukla R, Kulstreshtha R, Mathew A. Cryptococcosis associated with HIV negative Indian Patients and HIV positive Indian blood donors. Drug Research Institute, Lucknow India. 1996; 283(3):360-74.
  24. Pedrol EE, Mallolas J, Gonzalez CJM. Cryptococcosis: Presentation of 26 cases (Spanish) Medicine Clinica. 1992; 98(10):361-5.
  25. Aquinas SR, Tasey SD, Ravindran GD, Nagamani D, Ross C. Cryptococcal meningitis in AIDS-Need for doagmpsos. Lf J Assoc. Physicians, India. 1996; 44(3):178-80.
  26. Petty RK, Kennedy PG. The neurological features of HIV positive patients in Glasgow-A retrospective study of 90 cases. Quarterly Journal of Medicine. 1992; 82(299):223-34.
  27. National Work shop on-Anti-microbial susceptibility testing and control of antibiotic therapy. Dept. of microbiology, Aamed Forces Medical College, Pune. 27:253-256.
  28. Morrow JD. Flucanazole -A new triazole antifungal agent' Am J Med Sc (USA). 1991; 302(2):129-32.
  29. Kauffman CA, Heddenwick S. Opportunistic fungal infection filamentous fungi and cryptococcosis. Geriatrics (USA). 1997; 52(10):40-2.
  30. Treseler CB, Sugar AM. Fungal Meningitis' Infect. Dis Clin North, Am (USA). 1994; (4):789-808.
  31. Tansoy MR, Judith A. Appleton Dept. of Microbiology, Indian Univ. Inhabitation of Fungal Growth by Garlic Extract. Mycologia, 1975; 67:183.
  32. Michal R, Judith A. Appleton Dept. of Microbiology, Indian Univ. Inhabitation of Fungal Growth by Garlic Extract. Mycologia, 1975; 67:183.
  33. Sandhu DK, Wavrach MK, Singh S. Sensitivity of Yeasts isolated from cases of vaginitis to Aqueous Extracts of Garlic. Mycosen, Dept. of Biology, Guru Nanak Dev. Uni. Amritsar, India. 23(12):691-698.