



## Original Research Article

## Antitubercular drug resistance pattern of *Mycobacterium tuberculosis* isolates from CSF samples of suspected meningoencephalitis patients

Satish Kumar<sup>1</sup>, M V Narasimham<sup>2,\*</sup>, Akash Panigrahi<sup>2</sup>, Banojini Parida<sup>2</sup>

<sup>1</sup>Dept. of Microbiology, S.L.N. Medical College, Koraput, Odisha, India

<sup>2</sup>Dept. of Microbiology, M.K.C.G. Medical College, Berhampur, Odisha, India



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## ABSTRACT

**Background:** Meningoencephalitis refers to the inflammation of the meninges and brain, and is considered an infectious neurological emergency. Tuberculous meningoencephalitis is caused by *Mycobacterium tuberculosis* and is the most common form of central nervous system (CNS) tuberculosis.

**Materials and Methods:** CSF samples were collected aseptically and processed with an aim to identify and isolate *Mycobacterium tuberculosis* from clinically suspected cases of meningoencephalitis and observe the antitubercular drug resistance pattern.

**Results:** The study group included 117 patients clinically diagnosed cases of chronic meningoencephalitis which were subjected to Z-N staining, culture on LJ media. From 117 cases, 21 cases were AFB positive, 20 cases were culture positive for *Mycobacterium tuberculosis*. Antitubercular drug susceptibility pattern was obtained using commercial kit provided by Himedia. (SL023)

**Conclusion:** Tuberculous meningoencephalitis associated with a high frequency of neurologic sequelae and mortality if not treated promptly.

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### 1. Introduction

Meningoencephalitis can be caused by bacteria, viruses, fungi, and protozoan or as secondary sequel of other inflammations like AIDS. Tuberculous meningoencephalitis is still one of the common infections of central nervous system (CNS) and poses significant diagnostic and management challenges, more so in the developing world. Tuberculous meningoencephalitis which accounts for approximately only 6% of all cases of extrapulmonary tuberculosis is one of the most serious clinical forms of TB, with a high mortality rate and disabling neurological sequelae.<sup>1,2</sup> Individuals with increased risk for Tuberculous meningoencephalitis include young children with primary TB and patients with immunodeficiency caused by aging, malnutrition, or disorders such as HIV and cancer.

Drug-resistance testing (DRT) for *Mycobacterium tuberculosis* has two objectives: (i) Individual management (treatment and prevention) of tuberculosis cases and (ii) Anti-TB drug-resistance surveillance. Since the first use of anti-TB drugs in the late 1940s,<sup>3</sup> relapse cases of tuberculosis with acquired resistance have been repeatedly observed for all new effective drugs.<sup>4-6</sup> Because the resistant strains were then transmitted to treatment-naïve patients, DRT for *M. tuberculosis* was recommended for all definite cases of tuberculosis.<sup>7</sup>

#### 1.1. Problem statement

Tuberculous meningoencephalitis is still one of the common infections of central nervous system (CNS) and poses significant diagnostic and management challenges, more so in the developing world.

\* Corresponding author.

E-mail address: [mayur9.prajaj@gmail.com](mailto:mayur9.prajaj@gmail.com) (M. V. Narasimham).

First described by Willis in the 17<sup>th</sup> century, Tuberculous meningoencephalitis is the most severe form of tuberculosis. It continues to be a worldwide burden, with majority of new active cases occurring in underdeveloped countries.<sup>8</sup> As per the World Health Organization (WHO) statistics, five countries, viz., India, China, Pakistan, Indonesia and South Africa, account for over 70% of the global burden of disease. Although central nervous system TB accounts for 5–10% cases of extra-pulmonary TB and only 1% of all cases of TB, it is responsible for more deaths than any other form of TB, owing to the inherent seriousness of this illness.<sup>9</sup>

## 2. Materials and Methods

The present study was conducted in the department of Microbiology, M.K.C.G Medical College, Berhampur in collaboration with department of Pediatrics and Medicine.

### 2.1. Type of study

Original Prospective study.

### 2.2. Period of study

The study was carried out over a period of 24 months, from February 2018 to January 2020.

### 2.3. Study group

Clinically diagnosed cases of meningoencephalitis admitted in the department of Pediatrics & Medicine, M.K.C.G Medical College and Hospital were included in the study. This study was approved by IEC of M.K.C.G Medical College and Hospital.

### 2.4. Selection of cases

#### 2.4.1. Inclusion criteria

Clinically diagnosed meningoencephalitis were included in the study. They had following clinical features,

1. Fever > 38.5°C rectal and 38°C axillary temperature.
2. Headache
3. Vomiting
4. Neck stiffness
5. Altered or reduced level of consciousness.
6. Convulsion, seizure and other neurological features.
7. Poor sucking and irritability.

#### 2.4.2. Exclusion criteria

Clinically diagnosed cases of

1. Cerebral malaria.
2. Metabolic encephalopathy.
3. Altered sensorium due to traumatic or narcotic abuse.
4. Other conditions of fever.

Though these clinical entities have features of meningoencephalitis, they were excluded from the study group.

2.4.2.1. Samples. Cerebrospinal fluid (CSF) collected from all patients.

## 2.5. Specimen collection and transport

### 2.5.1. Cerebrospinal fluid

Under strict aseptic precautions, lumbar puncture was done on clinically diagnosed cases of meningoencephalitis, preferably before initiation of antimicrobial therapy. 3 to 5 ml of CSF for adults and 1 to 2 ml for children were collected in sterile screw capped containers. The specimens were transported to the Microbiology laboratory without any delay. If delay was unavoidable, CSF was kept in an incubator (37 °C).

## 2.6. Sample Processing

### 2.6.1. Cerebrospinal fluid

The samples were processed immediately. It was examined with naked eye for the presence of turbidity or any signs of contamination with blood from the puncture wound.

The samples were subjected to centrifugation at the speed of 1500 X g for 15 minutes. The centrifuged deposits, was mixed thoroughly and was then used for routine staining including Z-N stain and culture.

1. Z-N staining of CSF deposit was done to detect the presence of acid fast bacilli in samples. Ziehl-Neelsen smears were prepared using standard methods with two modifications. The layered smear was then stained according to standard procedures. The ZN smear was meticulously examined for up to 30 min under a 1,000X magnification before being recorded as negative. Observation of a single acid-fast bacillus was considered a positive result.
2. Bacterial Culture:

The centrifuged CSF deposit was mixed thoroughly and inoculated in blood agar, chocolate agar, MacConkey agar and LJ media. However routine bacterial cultures were not included in the purview of this study. CSF deposits were inoculated on LJ slant and were observed once in a week for the first month and then twice a week in the next month. The LJ slants were observed for 8 weeks before declaring negative.

## 2.7. Anti-tubercular sensitivity testing

Resistance of Mycobacterium tuberculosis was determined by proportion method using commercial kit. MTB isolates from LJ media were used to prepare inoculums of 0.5 McFarland's standard, these inoculums were further

diluted to 1:10000 and 10 $\mu$ l of these diluted inoculums were inoculated on drug containing LJ media as per manufacturer's instruction kit.

### 3. Results

A total number of 117 patients were included in the study group constituting 70 children and 47 adults. The study group included chronic meningoencephalitis based on whether duration of signs and symptoms was > 4 weeks.

**Table 1:** Comparison of Z-N staining / Culture isolation using LJ media (n=117)

	Positive samples	Negative samples
Z-N staining	21(17.9%)	96
Culture on LJ	20(17.1%)	97

In ZN staining, 21 smears were AFB positive and 96 were AFB negative whereas Mycobacterium tuberculosis were isolated in 20 cases from culture.

**Table 2:** Antitubercular drug susceptibility pattern of Mycobacterium tuberculosis isolates from CSF (n=20).

Drugs	No. of positive isolates
Pan sensitive	13(65%)
Resistance to rifampicin (R) only	0
Resistance to isoniazid (H) only	0
Resistance to pyrazinamide (Z) only	1 (5%)
Resistance to streptomycin (S)	2 (10%)
Resistance to ethambutol (E)	1 (5%)
Resistance to H & R both	2(10%)
Resistance to Z & S both	1(5%)

### 4. Discussion

This prospective study included 117 patients who were clinically diagnosed as chronic meningoencephalitis. Among the 117 patients of chronic meningoencephalitis, 70 patients (59.8%) were children and 47 (40.2%) were adults. Lumbar puncture and examination of CSF by routine staining including Z-N stain, along with culture on LJ media.

On Z-N staining, acid fast bacilli were seen in 21 (17.9%) CSF samples which were further subjected to culture on LJ media wherein 20(17.1%) cultures showed growth of Mycobacterium tuberculosis. Various studies have shown varying rates of isolation of Mycobacterium tuberculosis. In a study by Rahman et al from 150 CSF samples, the ZN positivity and growth of Mycobacterium tuberculosis was 55.3% and 68.7% respectively.<sup>10</sup>

In another study by Pathrikar et al, the AFB positive cases was 1.4 %, however 16% culture positivity of Mycobacterium tuberculosis was almost similar to our study.<sup>11</sup>

Drug susceptibility testing was done on all the 20 isolates. A total of 13 (65%) isolates were pan susceptible

(sensitive to all the 4 drugs H, R, Z & E). Only 2 (10%) isolates were MDR i.e. resistant to rifampicin & isoniazid. Resistant to pyrazinamide and ethambutol was observed in 1 (5%) case each. 2 (10%) isolates were resistant to streptomycin. Combined resistance to pyrazinamide and streptomycin was found in a single isolate. In a study by Thapa et al. the resistance rates of Mycobacterium tuberculosis towards streptomycin was 24.4 % followed by isoniazid 23 %, rifampicin 17.8 % and ethambutol 15.6 %.<sup>12</sup>

A total of 20 samples from the 117 clinically suspected cases had yielded Mycobacterium tuberculosis on L-J media. Culture positive in CSF samples is still considered to be a gold standard as this method provides a further lead in accessing the drug susceptibility of the grown mycobacterial cultures. Improper technique of lumbar puncture, prior antibiotic therapy, delay in sample transport and culture, low bacterial load, may be the possible reasons for low isolation.

### 5. Conclusion

Tuberculous Meningoencephalitis has a considerable mortality & morbidity and serious long term sequelae despite of advances in medical care. There is increasing trend of drug resistance tuberculosis and anti tubercular drug susceptibility testing will aid in identifying resistance cases.

### 6. Source of Funding

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### 7. Conflict of Interest

The authors declare they have no conflict of interest.

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### Author biography

**Satish Kumar**, Senior Resident

**M V Narasimham**, Assistant Professor

**Akash Panigrahi**, 3rd Year Junior Resident

**Banojini Parida**, Professor and HOD

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