European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 10, 2020

Study of nontuberculous mycobacterial diseases diagnosed by rapid immunochromatotography method

Jeppu udayalaxmi, Ganesh Arjun

ABSTRACT: Background: Most laboratories do not have facilities for identification of mycobacteria to species level. BD MGIT TBc immunochromatographic strip differentiates mycobacteria grown in liquid media as MTC or NTM. In the present study we clinically correlate NTM identified by above method. Methods: Culture was done by BD BACTEC ^{TM}MGIT 960. All positive cultures were subjected to a BD MGIT TBc immunochromatographic strip method for differentiating MTC and NTM. Results: Of the 29 cases of suspected NTM there was a single case of UTI, a case of right frontal lobe abscess, a case of synovitis, a case of post hernioplasty infected mesh and 25 patients were having respiratory symptoms. All patients responded well to antibiotic therapy which included ATT, fluoroquinolones, cephalosporins, azithromycin, clarithromycin singly or in combination. Conclusions: Diagnosis of NTM done with BD MGIT TBc immunochromatographic card test was found to useful in the absence of a definitive species identification.

Key words: Atypical mycobacteria, Antibacterial agents, Biopsy, Frontal lobe abscess, Immunochromatography,

1. INTRODUCTION:

History shows, among the different mycobacteria *M. tuberculosis* and *M. bovis* caused the majority of human diseases [1]. However in recent years, other mycobacteria have emerged as potential pathogens [1,2]. These mycobacteria have been referred to as environmental mycobacteria, mycobacteria other than tubercle bacilli (MOTT), “atypical mycobacteria” and nontuberculous mycobacteria (NTM) [1,2].

Treatment guidelines for both the diseases caused by M. tuberculosis complex (MTC) and NTM are different [3,4]. Speciation of mycobacteria has been performed based on their cellular fatty acid and/or mycolic acid profile, molecular techniques [1,2]. But most of the laboratories cannot afford to buy expensive equipment like gas liquid chromatography or equipments for molecular techniques. BD MGIT™ TBc Identification Test is used to detect MPT 64 Ag in *M.tuberculosis* complex [5]. The test is negative in case of NTM. In the present study we correlate these NTM positive cultures with the clinical history and treatment given to the patient.

2. METHODOLOGY:

Study population included samples which were received for isolation of *Mycobacterium* spp. during a period of one year in a tertiary care hospital. All samples

received for diagnosis of tuberculosis during a period of one year which were positive by culture and negative by BD MGIT TBc identification test, were included. Patients with severe co morbidities like cancer and those patients who were discharged from the hospital without any antibiotic therapy were excluded. Institutional ethics clearance has been obtained for this study.

Culture was done by BD BACTEC ^{TM}MGIT 960, fluids like CSF were centrifuged and the deposit was directly inoculated into the MGIT tube containing the Middle brooke 7H9 liquid media. All other samples were subjected to NALC concentration method and then inoculated into the MGIT tube. All the positive cultures were confirmed by Gabbet's staining for acid fast bacilli [6]. All positive cultures were subjected to a immunochromatography strip method for differentiating MTC and NTM [5].

Immunochromatography strip method: BD MGIT TBc Identification test detects MPT 64 Ag present in MTB complex. A little fluid is aspirated from MGIT tube which has beeped positive and inoculated into the strip. Bands appear within few minutes, if both the control and test bands are present, report goes as MTB complex grown. If only the control band is present, then the report goes as *Mycobacterium* spp grown, kindly correlate clinically [7,8].

3. RESULTS:

In a period of one year, 1017 samples beeped positive in BD BACTEC ^{TM}MGIT 960 system. Among these, 68 were card test negative. Out of these 22 cases were excluded because the patients were discharged from the hospital without prescribing any antibiotics. Further 17 cases were discarded as they were cancer patients.

Now we were left with 29 cases of suspected NTM. We found that all these cases responded well to therapy. Of these 29 cases there was a single case of UTI, a case of right frontal lobe abscess, a case of synovitis where a tissue biopsy was sent, a case of post hernioplasty, the mesh was infected but deep tissue biopsy was sent. The rest of the 25 patients were having respiratory symptoms. Of these 11 were old case of tuberculosis with completed antitubercular therapy (ATT) and 4 were HIV positive. Of the 25 cases with respiratory symptoms, 9 (36%) were women and 16 (64%) were male. Of these 5 (20%) were of the age group 20-40 years, 11 (44%) belonged to age group 40-60 years and 9 (36%) belonged to age group >60 years.

The single case of UTI was HIV positive and presented with typical symptoms of UTI and was put on trimethoprim sulfamethoxazole, clindamycin and third generation cephalosporin. The single case of right frontal lobe abscess presented with headache since a year and blurred vision since a month. Frontal craniotomy was done and excision of the lesion was done. The patient was put on rifampin, ethambutol along with cefixime. Patient was discharged with decreased vision in the right eye. The single case of synovitis presented with joint pain and swelling since 10 days, was treated with conventional rifampin, ethambutol along with amoxicillin/clavulanic acid and clarithromycin. The single case of infected hernioplasty mesh had undergone post right inguinal open mesh hernioplasty five months back and had developed intermittent fever and pain in surgical site since 4 months. Deep tissue biopsy was

taken and it grew *Staphylococcus aureus* and NTM. The patient was put on clarithromycin, azithromycin and doxycycline.

Of the 25 with respiratory symptoms, all had cough with expectoration, 9 had breathlessness, 8 had fever, 6 had chronic obstructive pulmonary disease (COPD), 5 had bronchitis/bronchiectasis, 7 had pneumonia, 3 had hemoptysis, 2 had pneumothorax and one had empyema [Fig 1]. Seven patients were put on only cephalosporin, two patients were put on only azithromycin, one patient on doxycycline, three on fluoroquinolone, the rest on a combination of drugs [Fig 2]. Specimens received from these patients included 13 bronchoalveolar lavage (BAL) and 12 sputum samples [Fig 3].

4. DISCUSSION

NTM are ubiquitous in the environment with the heaviest concentrations found in soil and water sources. So growing NTM in the laboratory must be correlated clinically. NTM infections have frequently been overlooked in developing countries like India because of high incidence of tuberculosis infection, unfamiliarity of clinicians with NTM infection due to nonspecific clinical manifestations and inadequacy of laboratory services [1-4]. Currently, there are more than 150 species of genus Mycobacterium and it is likely that more will be discovered [1,2]. The rapid increase in identified species in recent years is due to improved culturing techniques and more precise differentiations of species [1,2]. The rate of NTM isolation in India has been reported as 0.5% to 8.6% and common species isolated are *M. intracellulare* and *M. fortuitum* [1].

In a study the mycobacterial cultures were identified by biochemical reactions, BIOLINE SD Ag MPT64 TB test and final identification and differentiation between MTBC and different species of NTMwere further confirmed by GenoTypeMycobacterium CM/AS assay. Out of 227 cultures tested, 165 (72.6%) strains were confirmed as *M. tuberculosis* complex, and 62 (27.4%) were confirmed as NTM. The most common NTM species identified were *M. fortuitum* 17 (27.5%) and *M. intracellulare* 13 (20.9%) [9]. British thoracic society guidelines suggest drugs like amikacin, ethambutol, clofazimine, rifabutin, rifampicin, new generation quinolones and macrolides are effective against mycobacterial infections when used in appropriate combinations are effective against infections caused by atypical mycobacterium. For MAC infections clarithromycin and azithromycin have been considered as promising drugs [10]. In review on ocular NTM infection showed that history of interventions, trauma, foreign bodies, implants, contact lenses, and steroids are risk factors for NTM ocular infections. Steroid use may prolong the duration of the infection and cause poorer visual outcomes. Early diagnosis and initiation of treatment with multiple antibiotics are necessary to achieve the best visual outcome [11].

In a study conducted in Egypt, out of 1402 patients with AFB smear positive only 47 (3.3%) NTM cases diagnosed according to ATS/IDSA criteria. The mean age of the NTM patients was 61.8 ± 23.2 years and more common in white race patients, NTM disease was more commonly associated with old TB infection (42.6%) and with bed ridden patients on tracheostomy (31.9%). The most common organisms isolated were the MAC complex (55.3%) followed by *M. kansasii* (34.04%). Cases. Identification was done by DNA probes

[12]. An update on pulmonary NTM diseases concluded that a diagnosis of NTM pulmonary disease does not necessarily imply that treatment is required; a patient-centered approach is essential. When treatment is required, multidrug therapy based on appropriate susceptibility testing [13].

A study conducted in Simla, Himachal Pradesh, out of the 1042 processed specimens, 16% were positive for M. tuberculosis complex and 1.2% for clinically significant NTM. *M. intracellulare* was the commonest species isolated. NTM were treated mainly on outdoor basis (92%), involving more extrapulmonary system (62%) and higher age-group of 41-60 years (69%). No significant factor was seen to be associated clinically, radiologically, and biochemically with the NTM infections [14].

A retrospective analysis of a seven year data of a hospital in Japan, One hundred and fourteen patients were diagnosed as NTM lung disease (MAC [n=38] and RGM [n=64]) as per American Thoracic Society criteria. Of these, eight patients were tracheotomized and were subsequently infected with RGM. Bronchiectasis on CT was observed more frequently in MAC group (78.9%) than in RGM group (43.8%) (p=0.0004). Nodular lesions were also frequently seen in MAC group than in RGM group (63.2% vs 29.7%; p=0.0002) [15].

A total of 2026 NTM isolates from 852 patients were identified. M. abscessus-chelonae group (1010, 49.9%) was the most commonly isolated and implicated in pulmonary NTM disease. Pulmonary cases (352, 76%) had the highest prevalence among patients diagnosed with NTM diseases (465/852, 54.6%) with no gender difference. Male patients were older (68.5 years, $P = 0.014$) with a higher incidence of chronic obstructive pulmonary disease (COPD) (23.6%, $P < 0.001$) and recurrent cough with phlegm production (51.6%, $P = 0.035$). In contrast, more female patients had bronchiectasis (50%, $P < 0.001$) and haemoptysis (37.6%, $P = 0.042$). Age and COPD were associated with multiple NTM species isolation per patient [16].

A multi-centric study conducted in India showed that common pulmonary isolates from 160 paired respiratory samples found to be 44 (26.8%) Mycobacterium chelonae, 21 (12.8%) were Mycobacterium fortuitum, 15 (9%) were Mycobacterium gordonae. 9% (15) were Mycobacterium tuberculosis complex, 6.1% (10) were Mycobacterium kansasii, 4_9% (8) were Mycobacterium simiae, 2.4% (4) were Mycobacterium thermophile, 1.2% (2) were Mycobacterium gastri, 0.6% (1) were Mycobacterium scrofulaceum, 0_6% (1) were Mycobacterium avium and 4.9% (8) isolates had chromatogram which was un-interpretable. Identification of the isolates was done by gas liquid chromatography [2].

Clinical management strategies of patients with *M. tuberculosis* complex (MTBC) and nontuberculous mycobacteria (NTM) are different. Conventional biochemical methods for speciation are tedious and require extensive safety precautions, therefore, faster methods for identification and discrimination is required for suitable management [1,2]. Molecular methods of identification require expensive, sophisticated equipments and trained personnel hence unsuitable for resource-poor countries [1,2]. In our laboratory a simple immunochromatographic test (ICT) kit using mouse monoclonal anti-MPT 64 is made use of, to differentiate MTC and NTM grown in MGIT culture tubes is used [5] As per ATS/IDSA

guidelines [17], MTC has been ruled out in all the patients included in the present study, out of the 25 with respiratory symptoms, 13 were BAL samples which were positive for NTM, but the 12 sputum samples received were just single sample being positive for NTM. The outcome of present study was consistent with the studies done in the past. There were more number of NTM pulmonary disease and belonged to age group between 20-40 years. All our patients responded to various treatment strategies but the treatment differed from patient to patient. Even though we didn't have an accurate species identification of the Mycobacteria grown still patient management was done successfully.

5. CONCLUSIONS:

1) The non-tuberculosis Mycobacteria (NTM) were mostly isolated from respiratory samples like sputum, BAL.

2) Most of the patients with respiratory symptoms were male patients and belonged to the age group 40-60 years.

3) There was one case each of urinary tract infection, contaminated hernioplasty mesh, frontal lobe abscess, synovitis and 25 cases of respiratory diseases. Among these there were 6 COPD, 5 bronchitis/bronchiectasis, 7 pneumonia cases, 2 pneumothorax cases and one empyema.

4) The patients with respiratory symptoms were treated with macrolides, fluoroquinolones, third generation cephalosporin, amoxicillin or piperacillin combined with a beta lactamase inhibitor, most often in combination with the conventional anti tubercular drugs (ATT). All patients responded positively to treatment.

5) Even though we didn't have an accurate species identification of the Mycobacteria grown still patient management was done successfully.

6. REFERENCES:

[1] Sharma S, Dhar R. Nontuberculous mycobacterial diseases: Current diagnosis and treatment. Astrocyte 2017;4:67-74.

[2] Sebastian G, Nagaraja SB, Vishwanatha T, Voderhobli M, Vijayalakshmi N, Kumar P. Non-Tuberculosis mycobacterium speciation using HPLC under Revised National TB Control Programme (RNTCP) in India. J Appl Microbiol. 2017;124:267-273.

[3] Margaret M. J, John A O, Nontuberculous mycobacterial pulmonary infections. J Thorac Dis. 2014;6:210-20.

[4] Yon J R, Won-Jung K, Charles L D. Diagnosis and treatment of nontuberculous mycobacterial lung disease: clinicians' perspectives. Tuberc Respir Dis 2016;79:74-84

[5] Vishnu Prasad S, Chiranjay M. Rapid Immunochromatographic Test for the identification and discrimination of Mycobacterium tuberculosis Complex isolates from non-tuberculous mycobacteria. J Clin Diag Res. 2014;8(4): DC13-DC15

[6] Brooks G F, Carroll K C, Butel J S, Morse S A. Mycobacteria. In *Jawetz, Melnick and Adelberg's Medical Microbiology*, 24th ed. Brooks G F, Carroll K C, Butel J S, Morse S A (eds). United States of America: Mc Graw Hill Companies, Inc.2007; pp 320-31.

[7] Anika P, Anka V , Tijana S ,Tatjana K. Use of immunochromatographic assay for rapid identification of Mycobacterium tuberculosis complex from liquid culture. Bosn J Basic Med Sci. 2012; 12(1): 33–36.

[8] Maurya AK, Nag VL, Kant S, Kushwaha RA, Kumar M, Mishra V, Rahman W, Dhole TN. Evaluation of an immunochromatographic test for discrimination between Mycobacterium tuberculosis complex & non tuberculous mycobacteria in clinical isolates from extra-pulmonary tuberculosis. Indian J Med Res. 2012 Jun;135(6):901-6.

[9] A.K. Maurya,1 V. L. Nag,1 S. Kant,2 R. A. S. Kushwaha,2 M. Kumar,3 A. K. Singh,3 and T. N. Dhole. Prevalence of Nontuberculous Mycobacteria among Extrapulmonary Tuberculosis Cases in Tertiary Care Centers in Northern India. Volume 2015, Article ID 465403, 5 pages.

[10] Haworth CS, Banks J, Capstick T, et al. British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD) Thorax, 2017;**72**:ii1–ii64.

[11] Wajiha J. K, Huda S, Maamoun A Fa, Rola N H, Nontuberculous Mycobacterial Ocular Infections: A Systematic Review of the Literature, Bio Med Res Int. Vol 2015; Article ID 164989.

[12] Hany S, Abeer T E. Risk factors for atypical mycobacterial disease in patients with smear positive pulmonary TB. Egyptian J Chest Dis and Tubercul. 2014; 63:657–61.

[13] Jason E.S, Won-Jung K, Wing W Y. Update on pulmonary disease due to non-tuberculous mycobacteria. Int J Infect Dis.2016;45:123-34.

[14] Pooja S, Digvijay S, Kusum S, Santwana V, Sanjay M, Anil K. Are we neglecting nontuberculous mycobacteria just as laboratory contaminants? Time to Reevaluate Things. J Path. Vol. 2018, Article ID 8907629, 5 pages

[15] Hiroaki N, Takeshi K, Yuichirou N, Shin Y, Jirou F, Tomoo K. Epidemiology and clinical features of nontuberculous mycobacterial lung disease in a subtropical region in Japan: Analysis with a 7-year data in two major hospitals. European Resp J. 2016; 48:

[16] Zoe X Z, Benjamin P Z C, Li-Hwei S, Yen E T. Clinical and microbiological characteristics of non-tuberculous mycobacteria diseases in Singapore with a focus on pulmonary disease, 2012-2016. BMC Infect Dis. 2019;19:436.

[17] Griffith DE, Aksamit T, Brown-Elliott BA, Antonino C, Charles D, Fred G et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007; 165: 367– 416.

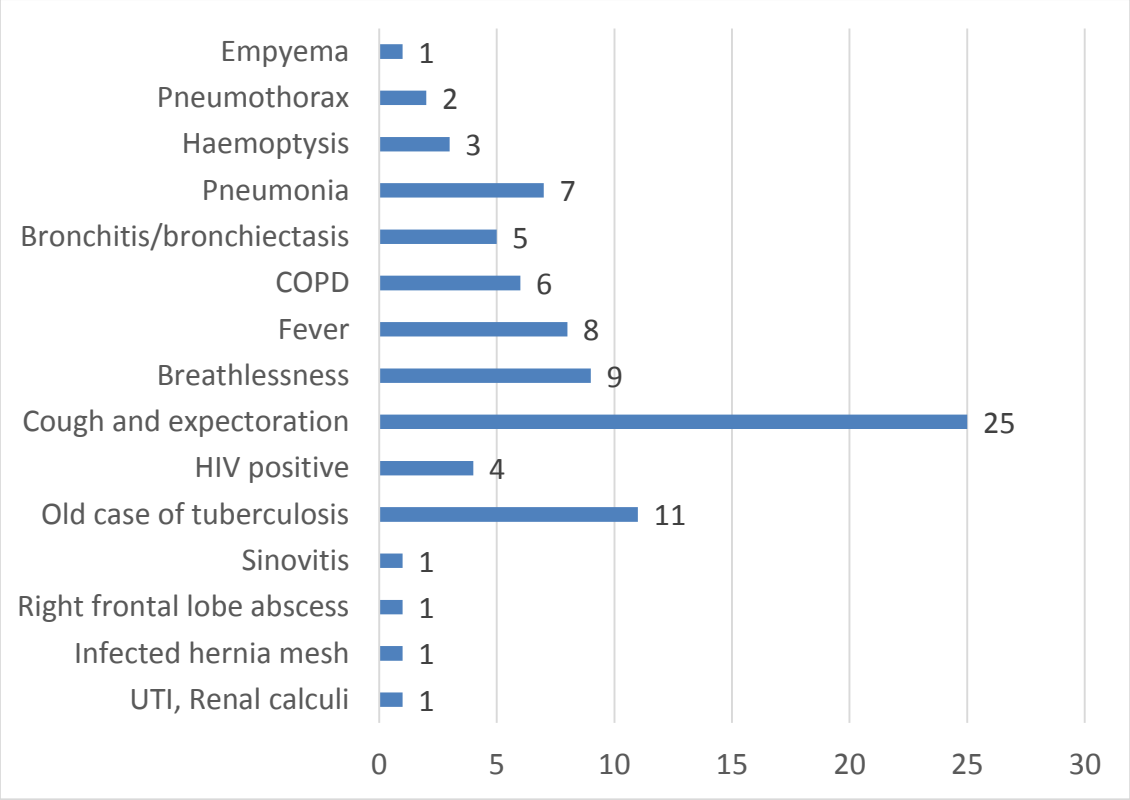

Figure 1: Clinical features of the patients suspected of NTM infection

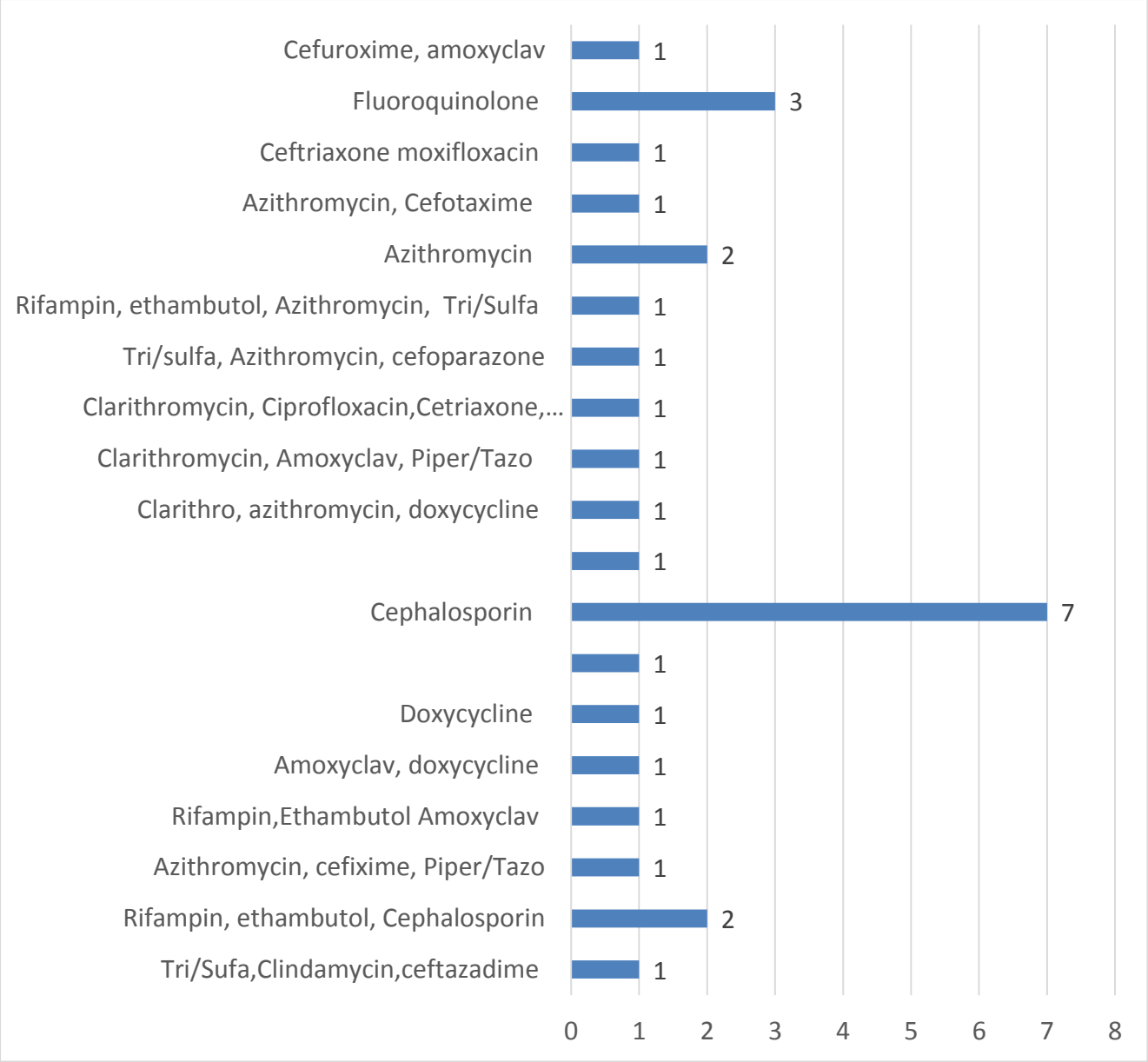

Figure 2: Antibiotics used in the treatment of patients suspected of NTM infection

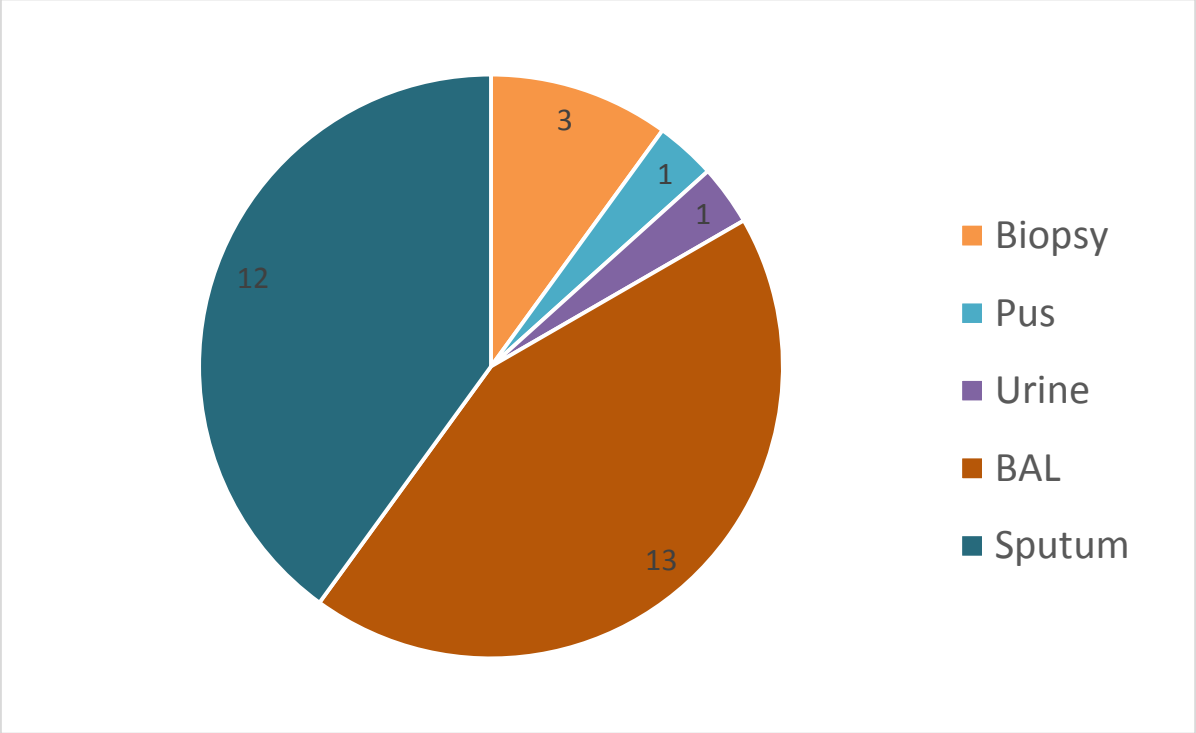

Figure 3: Specimens collected from the patients suspected of NTM infection