**Original article:**

**Study of microbiological profile of sputum specimen isolates with special reference to pulmonary nocardiasis in rural tertiary care center**

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**Abstract:**

**Background**: Respiratory Tract Infection (RTI) is by large one of the leading causes of the morbidity and mortality in the world. In lower respiratory tract infections sputum is the common and easily available specimen. The etiologies of respiratory infections assume an important role in the choice of empirical antibiotics and hospitalization measures. Among the various etiological agents *Nocardia*, a branching, filamentous bacteria, is widely distributed in the environment causing pulmonary disease especially in a rural areas.

Microbiological profile of sputum isolates with special reference to Nocardiosis . Present study was done at Dept. of Microbiology, RMC, and Loni over a period of one year aiming to study the bacteriological profile of sputum specimen isolates and its sensitivity pattern with special reference to Nocardiasis.

**Materials and method:** A total of 381 patients sputum specimens were received in the Dept. of Microbiology during the study period. Direct microscopy (Gram staining, Ziehl Neelsen (ZN) and 1% modified Ziehl Neelsen) was done for all sputum specimens. All the sputum specimens were inoculated and subjected to culture study. The sputum isolates were identified and antibiotic sensitivity pattern was studied.

**Results:** Sputum culture positivity in our study was found to be 45.40%. The sputum isolates included both bacteria and yeasts. Gram negative organisms were found to be predominant isolates 143(82.65%) followed by Candida and Gram positive organisms. We found four cases of Nocardiasis.

**Conclusion:** Sputum specimen staining and culture is still a standard method to detect the pathogen causing lower respiratory tract infections at earliest. Specific recommendation for modified ZN staining in suspected TB, Bronchitis and chronic pulmonary disease patients is need of hour. Early detection can lead to the prompt treatment can reduce mortality in these patients.

**Keywords:** Sputum, Nocardia spp. ZN staining, Gram negative organisms, Candida

**Introduction:**

Lower respiratory tract infections (LRTI’s) may be defined as those infections presenting with symptoms including cough, expectoration, dyspnea, wheeze and /or chest pain/discomfort usually for a period ranging from 1-3 weeks.[1] In this infection , there is an inflammation of the respiratory tract instigated from trachea to the alveoli with ensuing proliferation of an infectious agent.[2] It encompasses bronchitis, bronchiectasis, bronchiolitis, emphysema, lung abscess, pleural effusion and pneumonia. Many studies had incorporated all prevailing bacterial isolates from sputum, endo-tracheal aspirate and bronchoalveolar lavage (BAL). [3]

This study we included only organisms which are isolated from sputum. We present microbiological profile of sputum along with its sensitivity pattern with special reference to a case series of four patients of pulmonary nocardiasis. The disease had different presentations and also responded differently to treatment. That may help in improving disease management.

*Nocardia* is an aerobic Gram-positive rod belonging to the *Actinomycetales* order and is mainly distributed in the soil.[4] Pulmonary nocardiosis is a major cause of morbidity and mortality in immune-compromised patients. Lack of suspicion, non-specific clinic radiological presentation (often mimicking tuberculosis and fungal infections), diagnostic intricacies, and lack of systematic reporting are the probable reasons that have hindered the true estimation of its incidence. Although nocardial infections are usually opportunistic infections in the compromised host, at least 15% of the infections in this series occurred in patients without a definable predisposing condition.[5]

 **Materials and methods:**

This was a prospective study done in Dept. of Microbiology ,Rural Medical College ,Loni (PIMS –DU), Maharashtra, a teaching tertiary care centre which is attached to 1275 bedded hospital serving since approximately thirty five years in Western Maharashtra to bulk of nearby rural people. The study was conducted for one year duration. The institutional ethics committee approval was obtained.

Inclusion criteria: All the sputum specimens received during the study period from both gender and all age groups

Exclusion criteria: The repeat specimens and those on antibiotics prior to hospitalization were excluded from the study.

On arrival of sputum specimens gross was noted and then all specimens were subjected to direct microscopy and culture.

Direct microscopy was done by Gram staining, Ziehl-Neelsen staining (ZN) and modified Ziehl- Neelsen staining using 1% H2SO4 as decolorizer. Both Ziehl-Neelsen staining (ZN) and modified Ziehl- Neelsen staining using 1% H2SO4 stained smears were observed for any acid fast element.[6][7]

 In the Gram Staining, we followed Barlett’s grading of sputum. Sputum with a score of 1 and above was deemed as a suitable sample. [8 ] [9] [10] [11] Culture was done on Mac Conkey’s agar and Blood agar. All microscopy positive samples for Nocardia were inoculated on Lowenstein-Jensen (LJ) slopes and Sabouraud’s Dextrose Agar (SDA) incubated at 37℃ for six weeks and examined by alternate day for visible Nocardial growth . [12]

Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and on Blood agar for fastidious organisms. After incubation at 37°C for 18-24 hours, the results were read and interpreted as per CLSI guidelines. CLSI Anti-microbial sensitivity was performed using the Modified Kirby-Bauer’s disk diffusion method using discs of Trimethoprim /Sulfamethoxazole (1.25 + 23.75 μg), Gentamicin (10μg), Tobramycin (10μg), Amikacin (30μg) and Erythromycin (10μg) as per CLSI guidelines[13]

For quality control we used ATCC strains 25922 E. coli & S. aureus 25923 strains.………

Result:

**Observations and tables:**

Table no 1: Age wise distribution of specimens

|  |  |  |  |
| --- | --- | --- | --- |
| Total specimens |  | Total | % |
| 381 | Adult | 274 | 98.6 |
| Paediatrics | 7 | 1.83 |

Table 2 : Barlett’s grading of sputum specimens

|  |  |
| --- | --- |
| No.of sputum speci mens(n=381) | Barlett’s grade |
| 160 | -2 |
| 48 | -1 |
| 138 | +2 |
| 35 | +1 |

Table no 3: ZN staining result

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sputum | Zn Staining | Modified Zn Staining | Total | % |
| n=381 | 10 | 4 | 14 | 3.67 |

Table 4 : Growth positivity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables |

|  |  |
| --- | --- |
| n=381 | N=number |

 | Percentage % |
|

|  |  |
| --- | --- |
|  |  |
| Bacteria & Candida |  |
|  |  |

 | 173 | 45.40 |
|  Normal flora | 208 | 54.59 |

Table 5: Percentage of mono, poly microbial growth

|  |  |  |
| --- | --- | --- |
| Growth | Number(n) | Percentage(%) |
| monomicrobial | 158 | 91.32 |
| polymicrobial | 15 | 8.78 |

Table 6: Antibiotic sensitivity parttern for gram negative organisms

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sensitivity (%) | Klebsiella | Ecoli | Acinetobacter | Enterobacter | Citrobacter | Pseudomonas | Sphingomonas |
| Ampicillin(Amp) | 2 | 00 | - | 00 | 00 | - | - |
| Amoxyclav(AMC) | 64 | 21 | - | - | 100 | 100 | - |
| Trimethoprim/Sulfamethoxazole(Cot) | 45 | 12 | 11 | 50 | 50 | 17 | 100 |
| Gentamicin (G) | 79 | 60 | 56 | 67 | 100 | 83 | 00 |
| Amikacin(AK) | 49.44 | 85 | 43 | 60 | 100 | 88 | - |
| Ciprofloxacin(Cip) | 56 | 19 | 40 | 67 | - | 85 | - |
| Levofloxacin(Le) | - | 82 | 43 | 67 | - | 82 | 00 |
| Tetracycline(Te) | 40 | 17 | 44 | 20 | 00 | 60 | 00 |
| Meropenem(Mrp) | 65 | 85 | 50 | 67 | 100 | 94 | 00 |
| Imipenem(Imp) | 73 | 52 | 43 | 100 | 100 | 93 | 00 |
| Piperacillin Tazobactem(Pit) | 30 | 29 | 40 | 00 | 00 | 80 | 00 |
| Cefixim(Cxm) | 14 | 13 | 86 | - | 00 | 00 | 100 |
| Cefotaxim(Ctx) | 30 | 22 | 00 | 17 | - | 35 | 00 |
| Ceftriaxone(Ctr) | 53 | 21 | 00 | 00 | 00 |  | - |
| Ceftazidime(Caz) | 30 | 19 | 8 | - | 00 | 75 | 00 |
| Cefepime(Cpm | 54 | 16 | 00 | 00 | 00 | 77 | 00 |
| Aztreonam(At) | - | - | - | - | - | 66 | - |
| Polymyxin B(Pb) | 100 | - | 99 | - | 100 | 100 | 100 |
| Colistin(Cl) | 100 | - | 100 | - | 100 | 100 | - |
| Ticarcillin Clavulunate(Tcc) | 100 | - | - | - | - | - | - |
| Tigecycline(Tgc) | - | - | 00 | - | - | - | 00 |

Table 7 : Antibiotic sensitivity parttern for gram positive organisms

|  |  |  |  |
| --- | --- | --- | --- |
| Sensitivity (%) | Staphylococcus | Streptococcus | Enterococcus |
| Linezolid(Lz) | 100 | 100 | 98 |
| Vancomycin | 100 | 75 | 98.81 |
| Teicoplanin(Tei) | 100 | 80 | 97.56 |
| Gentamycin(G) | 50 | 67 | 00 |
| Trimethoprim/Sulfamethoxazole(Cot) | 50 | 00 | 00 |
| Cefoxitin(Cx) | 14 | - | - |
| Erythromycin(E) | 14 | 00 | 15.11 |
| Clindamycin(Cd) | 67 | 60 | 00 |
| Ciprofloxacin(Cip) | 00 | - | 17.70 |
| Cefepime(Cpm) | - | 100 | 00 |
| Benzyl Penicillin(P) | - | - | 37.25 |
| Tetracycline(Te) | 50 | 100 | 19.35 |
| Chloramphenicol(C) | 80 | 89 | 70.51 |
| Doxycycline(Do) | 75 | 25 | - |
| Levofloxacin(Le) | 50 | 100 | - |
| Sparfloxacin(Spx) | 50 | - | - |
| Amikacin(Ak) | - | - | 31.37 |

Table 8 : Nocardia distribution in sputum

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Case No. | Age/ Gender | Predisposing Factors | AKT\* | Isolate | Drug sensitivity |
| 1 | 65/F | Newly detected type 2 DM with microcytic anaemia | No | *Nocardia* Species | S: TOB,G,AK,LZ,IPM,CIP,RIF,COTR: AMC,CPM,CAZ,G,PIT,CFM,Cefazolin ,Cephodoxamine |
| 2 | 60/F | Pulmonary tuberculosis | Yes | *Nocardia* Species | S:LZ,CIP,AK,IPMR: COT,DO,CTX,CTR,AMC,CIP |
| 3 | 62/F | Bilateral pneumonia with fungal bronchitis | No | No growth | ------------------- |
| 4 | 68/F | Ovarian cancer with acute renal failure | No | *Nocardia* Species | S: IPM,LZ,TOB,G,AK ,TER: COT ,CTX,CTR |

\*AKT : Anti Koch’s treatment (Anti TB treatment)

S: Sensitive , R: Resistant

**Discussion:**

 Among 381 received specimens 245(64%) were male and 136 (36%) were Female in present which is in concordance with a study by Narayangowda.[14] Males were affected more than females because they were more involved in smoking & start it in younger age group, therefore more chance of inhalation and increased environmental exposure or temperature [15] In contrast a study by Egbe *etal* stated that prevalence of LRTIs did not differ significantly between men and women.[16]About 45.40 % of cultures were positive for growth of pathogenic bacteria and candida. Deepthi babu *etal* (2017)[17] also reported 42.77 bacterial growth along with 57.23% normal flora. In Ramanna etal [18] study (71.6%) and (39.4%) no pathogen( normal flora )was grown in sputum samples. Growth of normal flora could be due to prior administration of antibiotic or due to inability to include viral, mycological pathogens cultivation methods. In present study normal flora was grown in 208 specimens (54.59%), we have processed all the received samples in microbiology lab because they were paid samples. With this inoculation we can point out that this barlett score is necessary to be followed because all the samples which shows growth of normal flora were of -1 or -2 in grading. [8]

Out of 173 culture positive specimens, 158(91%) monomicrobial and 15(9%) specimens poly microbial growth is seen in present study . In observations by kulkarni *etal*, they reported similar findings.[19] Which is similar to present study. Where as Hassan *etal* reported 77% monomicrobial ,13% polymicrobial growth in their study which was lower than present study.[10]

Out of 173 culture positive specimens 20(11.56%) Gram positive organism, 143(82.65%) gram negative and 25 (14.45%) candida was grown. In a study by Regha *etal* they reported. (84.7%) GNB and (15.3%).[3]Gram negative organisms (77.78%) and gram-positive bacteria (22.22%) was seen in a study by Deepthi babu *etal* [17]

About 45.40 % of cultures were positive for growth of pathogenic bacteria and candida. Similar to present study Candida (14.45%) was also reported by Ramana *etal*[18] and Sarmah *etal* [20]they reported growth of *Candida spp-*5.2% and (17.39%) respectively*.*

In present study Klebsiella pneumonia (29.78%) was preponderant pathogen. This is in concordance with various authors [1] [9]  [17] [21] Followed by Pseudomonas aeruginosa as second most prevalent pathogen, which is similar to study by Regha *etal* [3], Elumalai *etal* [9] , Deeptihi babu *etal* [17] Vishwanathan *etal* .[22] Where as in a study by Patel *etal* reported that *Streptococcus pneumoniae*  were causative agent for respiratory tract infections. [11]  Hospital antibiograms are most important component for detecting and monitoring trends in antimicrobial resistance which is a guide to antimicrobial therapy.[3] [14]

MRSA is seen in all S. aureus strains in present study which coincides with the study of an Egypt Hassan researcher.[10] This high prevalence of MRSA among local hospitalized patients is an alarming situation. HIV infection (33.3%) was the most common predisposing factor in a study by Dharmshale *etal*  (2018),in contrast it was not present in our patients[12]. In .present study only one patient was on steroidal therapy, all the patients were immune-compromised with underlying disease one patient was suffering from Diabetes, anaemia the second one was on anti -tubercular drugs(AKT) for tuberculosis, the third patient was suffering from bilateral pneumonia with fugal bronchitis and the last patient was of oncology suffering from ovarian cancer with acute renal failure. In these *Nocardia* patients even though only 1 patient was sensitive to Trimethoprim/Sulfamethoxazole and rest were resistant to it , Trimethoprim/Sulfamethoxazole combination theraupy was the drug of choice.

Agrawal D *etal* stated in his research that most of the patients of pulmonary nocardiosis was immunocompromised due to chronic corticosteroid therapy (20 patients).[23]In one observational study over a 13 year period, specific predisposing factors were seen in 94% of cases and the most common factor being prolonged steroid therapy (64.5%) followed by immune suppression in transplant patients(29%), COPD (23%), HIV infection (19%) and alcoholism (6.5%). [12]

In a study by Blackmon *etal*  they found in radiographic analysis that discrete nodules were more often associated with immunosuppression compared with the non-immunosuppressed patient group (66% vs. 11%) .[24] Discrete nodules was absent in this study. Present study shows that all Gram negative organisms showed maximum susceptibility towards carbapenems (imipenem,meropenem) aminoglycosides (gentamycin,amikacin), polymyxin B and colistin. which coincides with the study by Regha *etal .* All gram positive cocci were sensitive to linezolid, vancomycin, teicoplanin and chloramphenicol in present study. Which was in concordance with study by Regha *etal.* All 3 Nocardia spp were sensitive to Linezolid and imipenem,amikacin .All isolates were sensitive to Gentamicin, Amikacin and Trimethoprim/Sulfamethoxazole in research done by Dharmshale *etal .*In contrast only one growth of Nocardia was sensitive to Trimethoprim/Sulfamethoxazole rest were resistant.

**Conclusion:**

We believe that continuous surveillance of antibiotic trends is vital to attaining a goal of low levels of HCAI.

**Limitations:**

A limitation of the present study was the small number of patients with nocardiosis and the focus on a single geographical region in maharashtra. However, although these epidemiological and microbiological results may not be applicable to other regions, we could not identify at species level.

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