

Original article:

Study of microbiological profile of sputum specimen isolates with special reference to pulmonary nocardiosis 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 Nocardiosis.

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 Nocardiosis.

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 nocardiosis. 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 MacConkey'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°C 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	Percentage %
Bacteria & Candida	173	45.40
Normal flora	208	54.59

N=number

Table 5: Percentage of mono, poly microbial growth

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

Table 6: Antibiotic sensitivity pattern 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 pattern 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	<i>Nocardia</i> Species	S: TOB,G,AK,LZ,IPM,CIP,RIF,COT R: AMC,CPM,CAZ,G,PIT,CFM,Cefazolin ,Cephodoxamine
2	60/F	Pulmonary tuberculosis	Yes	<i>Nocardia</i> Species	S:LZ,CIP,AK,IPM R: 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	<i>Nocardia</i> Species	S: IPM,LZ,TOB,G,AK ,TE R: 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] , Deepthi 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 fungal 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 therapy was the drug of choice.

Agrawal D *et al* 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 *et al* 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 *et al*. All gram positive cocci were sensitive to linezolid, vancomycin, teicoplanin and chloramphenicol in present study. Which was in concordance with study by Regha *et al*. 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 *et al*. 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.

References:

- 1-M. Woodhead, F. Blasi, S. Ewig, G. Huchon, M. Ieven, T. Schaberg, A. Torres, G. van der Heijden, A. Ortqvist and T.J.M. Verhij. Guidelines for the management of adult lower respiratory tract infections Eur Respir J 2005; 26: 1138-1180.
- 2-Pant S, Bhusal KR, Manandhar S. Microbiology of lower respiratory tract infection in workers of garment industry of Kathmandu. *J Col Med Sci* 2014;10(3):14-22.
- 3-R. Regha, B. Sulekha. Bacteriological profile and antibiotic susceptibility patterns of lower respiratory tract infections in a tertiary care hospital, Central Kerala. *Int J Med Microbiol Trop Dis* 2018;4(4):186-90.
- 4-. Beaman BL, Beaman L. *Nocardia* species: Host-parasite relationships. *Clin Microbiol Rev.* 1994;7:213-64.
- 5-. Beaman BL, Burnside J, Edwards B, Causey W. Nocardial infections in the United States, 1972-1974. *J Infect Dis.* 1976;134:286-9. [[PubMed](#)]
- 6-Goodfellow M. Actinomycetes: Actinomyces, Actinomadura, Nocardia, Streptomyces and related genera, In J.G. Collee, B.P. Marmion, A.G. Fraser, A. Simmons (Ed.), Mackie & McCartney, Practical Medical Microbiology, (London: Churchill Livingstone, 1996;343-359

- 7-Smita bagle ,Bagali S, Mantur P. Pleural Nocardiosis in an Immunocompetent Patient: A Case Report. *J Clin Diagn Res.* 2016;10(1):DD01-DD2. doi:10.7860/JCDR/2016/15039.7144
- 8 -Winn W, Allen S, Janda W, et al. Introduction to microbiology-part I. Koneman's color atlas and textbook of diagnostic microbiology, Lippincott Williams & Wilkins 2006;6th edn
- 9- Elumalai A, Raj MA, Abarna V, Bagyalakshmi R, Reddy S. Study of Gram Negative Bacterial Isolates From Lower Respiratory Tract Infections (LRTI) and Their Antibiogram Pattern in A Tertiary Care Hospital in South India. *JMSC* 2015;4(11):14066-70. DOI:https://dx.doi.org/10.18535/jmscr/v4i11.88
- 10- Alaa T. Hassana, Sherif A.A. Mohameda, Mona S.E. Mohamedb, Mohamed A. El-Mokhtarb Acute exacerbations of chronic obstructive pulmonary disease:etiological bacterial pathogens and antibiotic resistance in Upper Egypt Egypt J Bronchol 10, 283–290 (2016)
- 11- Patel AK, Luhadia AS, Luhadia SK (2015) Sputum Bacteriology and Antibiotic Sensitivity Pattern of Patients Having Acute Exacerbation of COPD india – A Preliminary Study. *J Pulm Respir Med* 5: 238. doi: 10.4172/2161-105X.1000238
- 12-Sujata Dharmshale, Anju Kagal, Deepa Devhare,Renu Bharadwaj, Shailaja Desai ,Pulmonary Nocardiasis Among Suspected Cases of Pulmonary Tuberculosis in Pune, India”. Indian Journal of Basic and Applied Medical Research; Diagnostic speciality Issue, June 2018: Vol.-7, Issue- 3, P. 38-45 38www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858
- 13-CLSI Performance Standards for antimicrobial susceptibility testing; Clinical and laboratory standards institute. Twenty-Third Informational supplement. 2013; 33 (1):M100-S23.
- 14- Narayangowda, Narayanagowda DS, Golia S, Jaiswal J, Manasa SS. A bacteriological study of acute exacerbation of chronic obstructive pulmonary disease over a period of one year. *Int J Res Med Sci.* 2015; 3:3141–6.
- 15- Sharan H. Aerobic Bacteriological Study of Acute Exacerbations of Chronic Obstructive Pulmonary Disease. *J Clin Diagn Res.* 2015;9(8):DC1 DC12.doi:10.7860/JCDR/2015/14515.6367
- 16- Egbe CA, Ndiokwre1 C, Omoregie R. Microbiology of Lower Respiratory Tract Infections in Benin City, Nigeria. *Malaysian J Med Sci* 2011;18(2):27-31.
- 17- Deepthi Babu1*, Lia Abraham1, Binu Raj C, Hina P Majeed, Sajna Banu CR and Sareena A2 Sputum Bacteriology in Patients having Acute Exacerbation of Chronic Obstructive Pulmonary Disease in a Tertiary Care Hospital, International Journal of Medical Research & Health Sciences, 2017, 6(9): 1-51 ISSN No: 2319-5886
- 18- K V Ramana, Anand Kalaskar, Mohan Rao, and Sanjeev D Rao, “Aetiology and Antimicrobial Susceptibility Patterns of Lower Respiratory Tract Infections (LRTI’s) in a Rural Tertiary Care Teaching Hospital at Karimnagar, South India.” American Journal of Infectious Diseases and Microbiology 1, no. 5 (2013): 101-105
- 19- Gauri Kulkarni, Deependra Chaudhary, Anup Bhojar, Sushma Dugad and Abhijit Telkhade Bacteriological Profile in Sputum and their Antibiogram among the Patients of Acute Exacerbation of COPD MVP Journal of Medical Sciences, July-December 2017, Vol 4(2), 113–117,
- 20- Sarmah N, Sarmah A, Das DK. A Study on the Microbiological Profile of Respiratory Tract Infection (RTI) in Patients Attending Gauhati Medical College & Hospital. *Ann. Int. Med. Den. Res.* 2016; 2(5):MB11-MB15.
- 21-Koripella RM, Perala BMK, Cheemala SS et al. Bacterial profile in sputum samples of pneumonia cases in a tertiary care hospital. *Int J Res Rev.* 2016; 3(8):27-31
- 22- Vishwanathan eta Viswanath S, Chawla K, Gopinathan A. Multi drug resistant Gram negative bacilli in lower respiratory tract infections. *Iran J Microbiol* 2013;5(4):323–27.
- 23- Aggarwal D, Garg K, Chander J, Saini V, Janmeja AK. Pulmonary nocardiosis revisited: A case series. *Lung India* 2015;32:165-8
- 24- Blackmon, Blackmon KN, Ravenel JG, Gomez JM, Ciolino J, Wray DW. Pulmonary nocardiosis: computed tomography features at diagnosis. *J Thorac Imaging.* 2011 Aug;26(3):224-9. doi: 10.1097/RTI.0b013e3181f45dd5. PMID: 21785288.

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