



Bacteriological study of Sputum in Adult Respiratory Tract Infection: A study from Eastern India

Rudrajit Paul¹, Jayanti Ray¹, Jayati Mondal², Shubhabrata Das³

1 Department of Medicine, Medical College Kolkata. 700073, West Bengal, India.

2 RMO-Cum-Clinical Tutor, Chittaranjan SevaSadan, Kolkata, West Bengal, India.

3 Clinical fellow, Division of Neurology, Department of Medicine, University of Alberta, Canada

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*Corresponding author:

Email : docr89@gmail.com

Tel.: +91-9433824341

ABSTRACT

Respiratory tract infection (RTI) is one of the commonest community acquired infections. The incidence and severity varies between children and adults but it is a significant cause of both outdoor visits and hospital admissions in all age groups. Indian studies have consistently shown that RTI constitutes a major disease burden in both rural and urban areas [1]. The incidence and morbidity of RTI is more in children, especially in the under-5 age group. But for adults too, this is a major cause of medical consultation. Data from UK general practice clinics have shown that up to 25% of the adult population visit their GP clinic each year for an episode of RTI

INTRODUCTION

Respiratory tract infection (RTI) is one of the commonest community acquired infections. The incidence and severity varies between children and adults but it is a significant cause of both outdoor visits and hospital admissions in all age groups. Indian studies have consistently shown that RTI constitutes a major disease burden in both rural and urban areas [1]. The incidence and morbidity of RTI is more in children, especially in the under-5 age group. But for adults too, this is a major cause of medical consultation. Data from UK general practice clinics have shown that up to 25% of the adult population visit their GP clinic each year for an episode of RTI[2].

As studies from different parts of the world show, RTI is mainly caused by viral infections[3]. The type of virus varies with the age group and season[3]. The human respiratory tract is a reservoir of many pathogenic bacteria but the mere presence of bacteria does not always cause disease[4]. Other local and systemic factors often contribute to bacterial infection of the respiratory tract. Recent studies are showing that a significant proportion of RTIs is caused by bacteria, either alone or in combination with a virus [5]. With the emergence of antibiotic resistant bacteria, RTI with these strains is also becoming common.

A surveillance program in North America, on common respiratory pathogens, has shown a steady rise in antibiotic resistance among these bacteria [6]. In this program, the Canadian data have shown rising resistance (between 4 and 10 times) to common antibiotics in *S. Pneumoniae* between 1988 and 2001 [6]. Another part of this program, the TRUST study in USA, has shown significant levels of resistance to penicillin in *S. Pneumoniae* [6]. Resistance has also been demonstrated for hemophilus and streptococcus, the other two important respiratory pathogens for adults [6]. Studies done elsewhere have shown that presence of co-morbidities like diabetes or HIV infection increases the risk of respiratory infection by a drug-resistant bacterium [5].

Microbiological data about drug resistance of respiratory pathogens are relatively scarce from India. However, available current data have shown that the level of drug resistance in these pathogens are significantly high [7]. This is a major concern for public health, both from an economic and also disease burden point of view. The USA study mentioned earlier have shown that the projected cost of RTI in USA is around 30 billion dollars annually [6]. Similar estimates are not available in India but considering the incidence of the disease, the financial burden is likely to be quite massive. Thus studies to document the microbiology of respiratory pathogens and their drug resistance pattern in various parts of the country are urgently needed.

In this study, we have recorded the antibiotic resistance pattern of respiratory pathogenic bacteria in a sample Eastern Indian population. Our aim was to generate data to help in antibiotic stewardship and formation of rational treatment protocols for local population.

MATERIALS AND METHODS

This was a hospital based observational cross sectional study. A study on the antibiotic resistance pattern of different bacteria isolated from different clinical specimens is underway. As a part of that, we studied the sputum of patients admitted with respiratory tract infection (RTI) in the medicine indoors of a tertiary care centre of Eastern India. The ethical committee of the institution was fully informed of the study and proper approval was obtained.

The patients were included in the study after proper screening and informed consent. RTI was diagnosed by clinical examination and/or imaging (Fig. 1). Patients on steroids or other immunosuppressive drugs, patients with HIV infection, those with history of respiratory tract instrumentation in previous three months or those who had received any anti-microbial agents in last three months were excluded. HIV infection was excluded as these patients are more likely to be on cotrimoxazole prophylaxis or other antibiotics like rifampicin for tuberculosis. Also, patients with dental or oral cavity infection were excluded to prevent contamination of the samples. Sputum was collected after voluntary expectoration or after induction with normal saline nebulisation. Two sputum samples were collected from each patient in sterile containers at room temperature before anti-microbial agent administration and immediately sent to the microbiology department. The sputum sample was collected without regard to time of the day or the time since last meal.

The sputum samples were first analysed for adequacy of specimen. Those samples with >25 polymorphonuclear cells/high power field (HPF) and <10 epithelial cells/HPF were included. All samples were routinely processed for acid fast

staining (as part of standard hospital protocol for sputum analysis in our institution). Then, the sputum sample was homogenized with sterile glass beads. The homogenized sample was used for gram stain and also to inoculate two culture media simultaneously: - blood agar and McConkey Agar. After 18-24 hours of incubation at 37°C , any growth in these media were gram stained and analysed. If any sample grew more than 2 organisms, it was supposed to be contaminated and discarded. Then, antibiotic sensitivity was tested in Muller Hinton Agar by disc diffusion method. Our microbiology laboratory had a limited supply of discs due to financial constraints. Thus, only some selected antibiotics could be tested. The antibiotic sensitivity pattern was interpreted according to the CLSI guidelines. Although gram stain was used as an initial diagnostic test, the final bacteriological data was based only on the culture result. If a fungus like candida was grown, it was not subjected to drug sensitivity tests due to lack of logistics.

The results of the study are expressed as mean $\pm$ S.D. for continuous variables and absolute numbers and percentages for discrete variables. Significance tests are chi square tests for proportions and correlation coefficient for continuous variables. The data was first arranged in Microsoft excel worksheet. Analysis was done by online free software like GraphPad and MedCalc. P value <0.05 was considered significant.

Figure 2 gives a schematic outline of the study.

RESULTS

We had a total of 60 patients in our study with male female ratio of 41:19. Table 1 lists the different demographic and other variables in our patients. It is seen that 37.6% of the patients had pre-existing respiratory disease, previous tuberculosis ($n=6$) being the commonest. 20% of the patients in our study were diabetic. 4 patients had neuromuscular disorders like bulbar palsy or motor neuron disease, which can predispose to respiratory infections.

Anaemia ($\text{Hb} < 10 \text{ g/dL}$) was present in 41.7% ($n=25$) of cases.

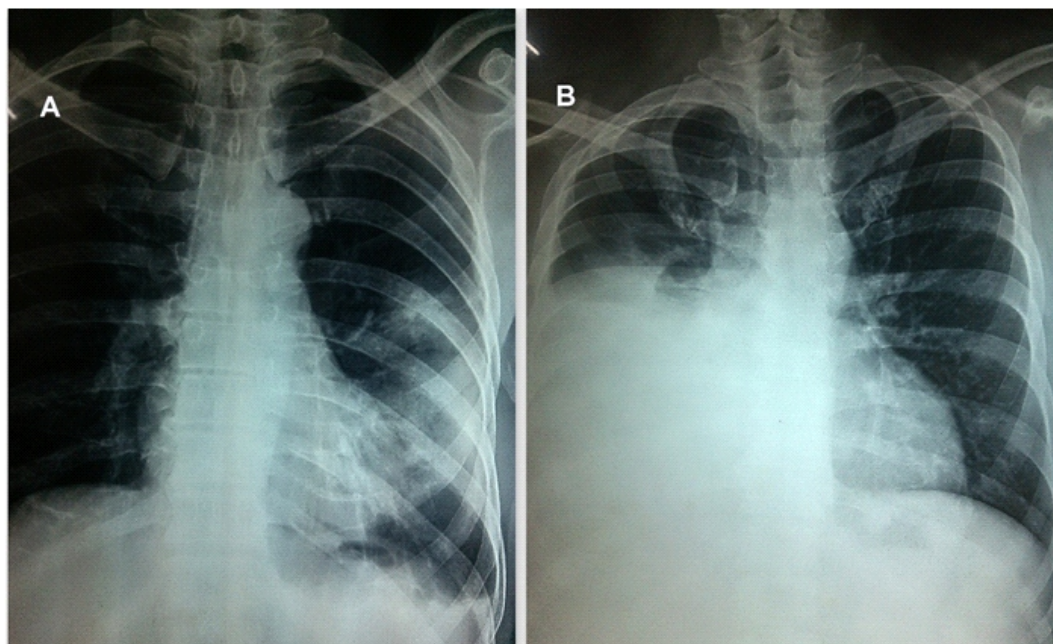


Fig. 1 : Some imaging findings of patients with RTI: A- left sided pneumonia; B- right sided pneumonia with pleural effusion

Table 1 : Table showing the different variables in the patients

PARAMETER		VALUE	PERCENTAGE
Gender	Male	41	68.3
	Female	19	31.7
Residence	Rural	26	43.3
	Urban	34	56.7
Any known respiratory disease (**but without any steroid use)	COPD	5	8.3
	Asthma	5	8.3
	Malignancy	3	5
	Bronchiectasis	2	3.3
	Previous tuberculosis	6	10
	ILD	1	1.7
	None	38	63.3
Diabetes	Yes	12	20
	No	48	80
Alcoholism	Yes	17	28.3
	No	43	71.7
Any neuromuscular disorder	Yes	4	6.7
	No	56	93.3
Any recent (within 6 months) major illness	Viral fever	4	6.7
	Surgery	1	1.7
	Malaria	1	1.7
	Heart failure	2	3.3
	Peptic ulcer/GERD	4	6.7
	CVA	1	1.7
	others	3	5
All the blood test reports are for the first test after presentation			
Blood Hemoglobin	<7	14	23.3
	7-10	11	18.4
	>10	35	58.3
Total Leukocyte count	<12000	7	11.7
	12000-16000	23	38.3
	>16000	30	50
Serum sodium	<135	15	25
	135-145	43	71.7
	>145	2	3.3
Serum ferritin	<200	4	6.7
	200-1000	29	48.3
	>1000	27	45
Oxygen saturation at presentation	<90%	8	13.3
	90-95%	33	55
	>95%	19	31.7

Significant hypoxia ($\text{SpO}_2 < 90\%$) was present in 8 cases. Among the other significant laboratory findings, hyponatremia was found in 25% of the cases and high serum ferritin was present in 27 patients.

Chest X ray was the imaging modality of choice. 13 (21.7%) of the patients underwent CT scan of thorax for different indications. In chest X ray, normal study was the commonest finding (28; 46.7%) followed by lobar consolidation (n=17). Hilar lymphadenopathy was found in 3 cases and cavitation was found in 2. Incidentally, 3 (5%) of the patients were found positive for acid fast bacilli (AFB). All of the patients with positive AFB were also positive for gram staining of sputum.

Table 2 shows the different isolated organisms in our study.

Gram stain of the collected sputum sample was positive in 26 (43.3%) cases. But 48 (80%) of the patients had one or more organisms in their sputum culture. Most of the patients had one organism; only 2(3.3%) had 2 organisms. It is seen that gram positive and gram negative organisms were isolated with equal frequency (23 vs. 21). In 6 cases, the sample grew candida, pathogenic role of which is difficult to ascertain. Among the gram positive ones, streptococcus was the commonest (n=10; 43.5%); among the gram negative group, E. Coli and Pseudomonas were the commonest (6 each; 28.6%).

Presence of any co morbidity like diabetes was associated with increased chance of isolating an organism. 100% of diabetic patients grew an organism in sputum culture against 80% of total

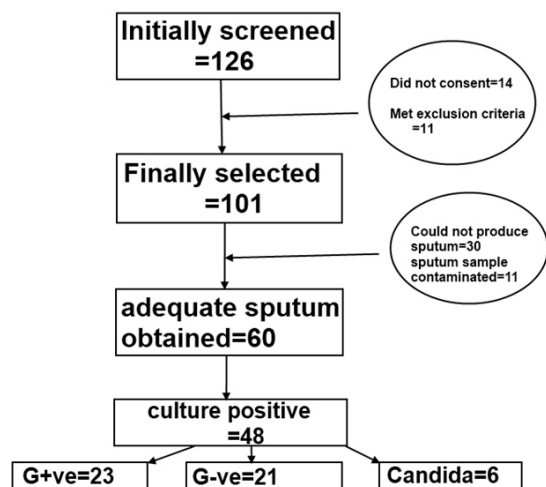
Table 2 : Table showing the different isolated organisms

ORGANISM		TOTAL NUMBER	With any respiratory disease	With diabetes	With alcoholism
Gram positive	Staphylococcus aureus	8	1	1	5
	Streptococcus pyogenes	10	2	0	2
	Streptococcus pneumoniae	5	2	1	2
Gram negative	E. Coli	6	1	4	1
	Pseudomonas	6	5	0	1
	Acinetobacter	4	3	1	0
	Klebsiella	5	1	1	3
Fungi	candida	6	1	4	1

Table 3 : Table showing the antibiotic resistance patterns

Organism	Resistance patterns (in percentage)					
	Amoxicillin	Azithromycin	Ciprofloxacin	Cefepime	Linezolid	AG
Gram positive bacteria						
Staphylococcus aureus	100	100	25	100	37.5	50
Streptococcus pyogenes	100	50	60	80	0	30
Streptococcus pneumoniae	100	50	0	100	0	0
	cefuroxime	AG	Imipenem	Piperacillin	ceftazidime	Aztreonam
Gram negative bacteria						
E. Coli	100	100	0	67	33	0
Pseudomonas	100	50	0	50	50	16
Acinetobacter	100	0	0	0	25	0
Klebsiella	80	0	0	40	0	20

AG: Aminoglycosides;

**Fig. 2 :** The schematic pathway of the study (the total number of culture positive cases and the total number of isolated organisms do not match as 2 patients grew more than one organism)

study population ($p=0.196$ by Fisher's Test, 2 tailed). Also, 88% of the alcoholic group grew an organism. However, among the diabetic population, 2 (16.7%) grew a gram positive bacteria as opposed to 6 (50%) growing a gram negative organism ($p=0.19$ by Fisher's Test). 53% ($n=9$) of alcoholic patients grew a gram positive organism.

Table 3 shows the antibiotic resistance patterns in the isolated organisms. It is seen that the gram Positive organisms are resistant to penicillin and cephalosporin group of drugs. 50% of pneumococcus species were resistant to Azithromycin. Linezolid was the drug with least resistance pattern; but even then, 3 out of 8 staphylococci isolates were resistant to it. Among the gram negative strains, imipenem was the only drug which showed no resistance. Commonly used drugs like cefuroxime showed high degree of resistance (80-100%). E coli were the organisms with highest incidence of drug resistance. 20% of the klebsiella isolates were resistant to aztreonam.

DISCUSSION

Our observational study has revealed streptococcus pyogenes

as the commonest gram positive organism and E.coli and Pseudomonas as the commonest gram negative organisms in respiratory specimens. The gram positive organisms showed very high levels of resistance to penicillins and cephalosporins. In the gram negative subset, the pattern varied according to the organism.

In a similar study from South India, 45% of the respiratory specimens grew Staphylococcus and 3% grew E.coli [7]. In comparison, in our study, staphylococcus was found in 17% cases and E.coli in 12%. But our study was based only on indoor patients while the abovementioned study was done on outpatient attendees. The differing clinical context may cause this variation. In another similar study from Bulgaria, bacteriological study of sputum samples showed staphylococcus in only 4% and E.coli in 13% [8]. This study, which was done on COPD patients, showed a gram negative organism in more than 65% of the sputum samples [8]. But in the South Indian study, which was done in general outdoors, only 17% of the isolates were gram negative [7]. Gram negative infection of the lung is usually found in patients with associated comorbidity and portends a grave prognosis. In our study, 44% of the isolates were gram negative. But in the diabetic subset, 50% (6 out of 12) had a gram negative organism in their sputum. In the subset with background respiratory illness, 45% (10 out of 22) had gram negative organism.

In a study from Nigeria, Ojo-Bola et al have shown that the infecting organism varied significantly with the level of immunosuppression. While in HIV positive persons, the commonest respiratory pathogen was E.coli, for HIV negative ones, the commonest was Klebsiella [9]. Also, the type of sample may affect the organism isolated eventually. In the South Indian study mentioned above, the respiratory sample was throat swab [7]. They got mainly staphylococcus and beta-hemolytic streptococcus. In another study from Bangalore, where the respiratory specimen used was lung aspirate in an ICU, staphylococcus was found only in 14% cases while Pseudomonas was found in 21% [10]. In an Italian study, which used both indoor and outdoor patients, Pseudomonas was isolated from 24% of sputum specimens while streptococcus and staphylococcus were found in 18% and 17% respectively [11]. Thus, it can be concluded that the organism isolated from respiratory infection varies widely according to the clinical condition, pre-existing comorbidities and other factors.

Antibiotic resistance patterns also vary considerably. In the South Indian study, it was seen that all the organisms were uniformly resistant to penicillin while resistance to 3rd generation cephalosporins was considerably lower (around 20%) [7]. In our data, resistance to 3rd generation cephalosporins was much higher (80-100%; Table 3). Aminoglycoside resistance of different organisms in our study varied from 30 to 100%, which is similar to the South Indian observation. Kumari et al have also shown very high mean resistance to penicillins and cephalosporins in respiratory pathogens [10]. In contrast, the Italian study by Varotto et al did not find any significant resistance to beta-lactam antibiotics [11]. In a recent study from Egypt, it was seen that the respiratory pathogens like pneumococcus and klebsiella had more than 90% sensitivity to fluoroquinolones (FQ) and 80-88% of them were sensitive to macrolides [12]. By contrast, in our study, FQ resistance was documented in 60% of streptococci and macrolide resistance varied from 50-100%.

Increasingly, polymicrobial infections of the respiratory system are recognized [12]. Only culture of the respiratory

clinical sample, like sputum or Bronchoalveolar lavage fluid, may not detect all the infecting pathogens. Further serology and other molecular tests may be needed [12]. But in clinical settings like ours, where resources are restricted, such detailed tests are not always feasible and treatment has to be started empirically. But in such cases, antibiotic resistance of each of the organisms will restrict the choice of therapy considerably.

Our study is limited by the small number of study samples and the restricted availability of drug sensitivity equipment. Also, serological diagnosis of respiratory infections (like pneumococcus) was not done due to financial limitations. But still, we have documented the resistance pattern to the common antibiotics in respiratory pathogens.

CONCLUSION

Respiratory infection by drug-resistant bacteria is a cause for concern all over the world. The microbiology of pathogens varies considerably depending on the clinical situation and comorbid illness. Local patterns of drug resistance should be studied and treatment protocols should be devised accordingly. Concentration of many drugs are suboptimal in the lung. Thus, the doses of antibiotics should be adjusted to ensure proper microbicidal concentrations for these respiratory pathogens. Finally, our study is just one facet of the picture. Further studies including other respiratory specimens like tracheal aspirate should be done from this part of the country to obtain a better panoramic picture of the microbiology of respiratory pathogens and their resistance patterns.

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