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Original Research Article

Antibiotic Susceptibility Pattern of Bacteria Causing Lower Respiratory Tract Infections in HIV/AIDS Patients with Correlation to CD4+T Cell Counts

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ABSTRACT

Introduction: One of the commonest infectious diseases of humans is lower respiratory tract infection (LRTI). Opportunistic pulmonary disease remains an important cause of acute illness and death in patients with advanced HIV disease. A structured approach to respiratory complaints in these can result in a timely and cost-effective evaluation.

Method and Material: Sputum and blood samples were collected from180 HIV sero positives treatment naive patients with lower respiratory tract infection and 100 controls were also taken. Sputum was processed for bacterial pathogens. Antimicrobial susceptibility testing was done using Kirby-Bauer disc diffusion method. CD4 count was estimated using blood sample by Flow cytometry method.

Results: All 180 samples yielded growth. Aerobic bacteria isolated were *Streptococcus pnemoniae* (22.2%) followed by *Moraxella catarrhalis* (19.4%), *Escherichia coli* (16.1%), *Enterobacter aerogenes* (11.7%) and *Klebsiella spp, (3.9%)*. Acid Fast Bacilli were demonstrated in 40.5% samples. Meropenem showed the maximum sensitivity in all Gram negative bacterial isolates in both the groups. Linezolid showed 100% sensitivity to all the Gram positive bacteria in both the groups. CD4 counts in most of them were < 200 cells/cumm.

Conclusion: This sensitivity pattern seen in our study can be helpful for clinicians in presenting empirical antimicrobial treatment in this area.

Key words: Lower Respiratory Tract Infections, HIV Positive, CD4 Count, Antimicrobial Susceptibility Testing.

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INTRODUCTION

Human Immunodeficiency Virus/Acquired Immuno Deficiency Syndrome (HIV/AIDS) is a major public health problem in India and other parts of the world. Globally an estimated 35.3 million people were living with HIV in 2012. There are 2.3 million new HIV infections globally. As per the sentinel surveillance 2012-13 the overall HIV prevalence among general population in India is 0.35% and in Rajasthan is 0.32% slightly less than national prevalence[1].

One of the main cause of morbidity and mortality in HIV infected patients is due to bacterial pneumonias. Pyogenic bacterial infections are common in patients with AIDS and are an important cause of death. As CD4+T cell count decreases rate of bacterial pneumonias increases[2]. *Streptococcus pneumoniae, Haemophilus influenza, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are the most commonly identified pathogens[3].

HIV associated tuberculosis (TB) remains a major global public health challenge. TB is the most common opportunistic infection (OI) among HIV-infected individuals, and co-infected individuals are at high risk of death. Respiratory tract infections are the major cause of significant morbidity and mortality in HIV patients. The clinical spectrum of pulmonary disease is broad and geographic and temporal variations in the etiology of respiratory illnesses in HIV-infected patients are important. This study will further add to our knowledge of the prevailing profile of bacteria and antibiotic susceptibility pattern in sputum of HIV positive patients with Lower Respiratory Tract Infections in this region. It will also define the range of CD4+T Lymphocyte count among various bacterial species suspected as probable pathogen.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Sawai Man Singh Medical College Jaipur from May 2014 to April 2015. One hundred and eighty treatment naive, HIV sero-positive patients and hundred HIV non-reactive patients, of both sexes, having LRTI (Lower respiratory tract infections), attending the

Integrated Counseling and Testing Centre (ICTC) were included. The HIV status of these patients was confirmed by three tests with different antigens or principles as recommended by National AIDS Control Organisation (NACO)[4].

After obtaining informed consent from the patients, the socio demographic details, clinical signs and symptoms, occupation, education and history of risk behaviour were filled on a structured proforma. Ethical clearance for the study was taken from college research review committee. Patients who had prior antiretroviral or antibiotic treatment were excluded.

CD4 +T lymphocyte count estimation was done by Flow cytometry using BD FACS Calibur (Becton Dickinson Immunocytometery System, San Jose, CA, USA) as per manufacturer's instructions [5].

Sputum samples were collected from the patients who were diagnosed HIV positive and who had complains of cough and fever. From each patient, early morning expectorated sputum sample was collected. Patients were asked to collect the expectorated sample in a sterile wide mouth container. The quality of expectorated sputum was evaluated macroscopically and by Bartlett's scoring method.[6] Sputum was stained by Ziehl Neelson method for detection of Acid Fast Bacilli (AFB) according to Revised National Tuberculosis Control Program (RNTCP).Sputum samples were inoculated on blood agar with 10% Sheep blood (BA), Chocolate agar with 10% Sheep blood (CA), Mac Conkey agar (MC) and Thioglycolate broth (TG). The BA, MC & TG were incubated at 37°C for 18 – 24 °C. The CA plates were incubated in 5 – 10% CO_2 in a candle jar at 37°C for 18 – 24 °C. After 24 hours of incubation the colonies grown on BA, CA& MC were identified by standard microbiological procedures.[7]

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Table 1: Age &sex distribution of HIV positive patients with LRTI.

		No. of patients (n=180)	Percentage of patients
Sex	**Male**	119	66.1
	Female	61	33.9
Age Group (years)	**0 to 10**	2	1.1
	11 to 20	7	3.9
	21 to 30	43	23.9
	31 to 40	72	40
	41 to 50	37	20.6
	≥51	19	10.5
Mean Age		**37.21 ± 11.2 years**	

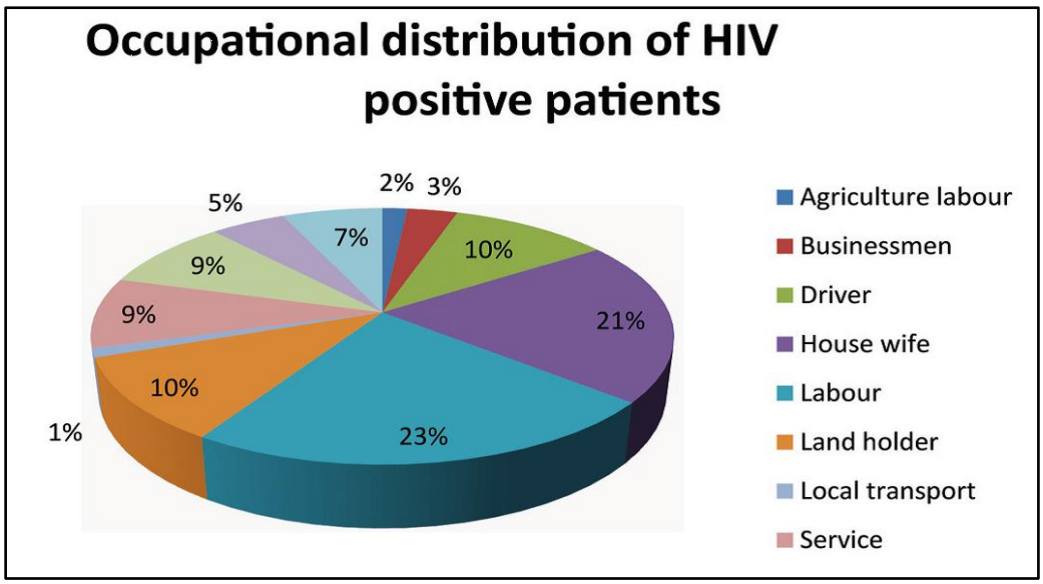

Table 2: CD4+T Lymphocyte cell count (cells/µl) of HIV Positive patients with LRTI.

CD4+T lymphocyte cell Count (cells/c mm)	Number of patients	%
<50	42	23.3
51 to 100	25	13.9
101 to200	46	25.6
201 to 350	31	17.2
351 to 500	20	11.1
> 500	16	8.9
	180	100.00

RESULTS

In the present study the mean age of patients was 37.21± 11.2 years. Majority (63.9%) of the patients were in the age group of 21 to 40 years. Males outnumbered females with male female ratio 1.95:1 [Table 1]. Labourers were the most affected group [Fig 1]. The most common route of HIV transmission was heterosexual contact noticed in 171 (95%) patients, followed by perinatal route in 7 (3.9%) patients and in the rest it was unknown. The mean CD4 +T lymphocyte cell count of patients was 209.05±195.23 cells/µl. The mean CD4 +T lymphocyte count of males was 192.85±189.70 cells/µl and females were 240.66±203.47 cells/µl. The majority of patients had CD4+T lymphocyte counts between 101-200 cells/µl [Table 2]. In the present study 220 strains of bacteria were isolated from all the patients. Amongst them the most common was *Streptococcus pneumoniae which* was isolated from 22.2% of patients followed by *Moraxella catarrhalis* (19.4%*), Eschirichia coli* (16.1%), *Enterobacter aerogenes* (11.7%) and, *Klebsiella spp* (3.9%). *Acinetobacter spp, Streptococcus pyogenes and Klebsiella spp* were isolated in patients with CD4 T lymphocyte counts <350 cells/ cumm, whereas *Coagulase negative Staphylococcus, Coagulase positive Staphylococcus, Enterococcus. spp, Streptococcus pneumoniae, Proteus. mirabilis, Pseudomonas. spp, Eschirichia coli, Enterobacter.spp*, were isolated with CD4 T lymphocyte counts >350 cells/cmm.[Table 3]

Table 4 shows antimicrobial susceptibility pattern of Gram negative bacteria isolated from sputum samples in study and control group. Meropenem showed the maximum sensitivity in all the Gram negative bacterial isolates in both the groups. In *Acinetobacter spp* sensitivity was 50%, *Enterobacter aerogenes (90.5%), Enterobacter cloacae (100%), Eschirichia coli (93.1%), Klebsiella.spp (71.4%), Proteus mirabilis (97.1%*).

Table 5 shows antimicrobial susceptibility pattern of Gram positive bacteria isolated from sputum in study group and control group sample. Linezolid showed 100% sensitivity and ampicillin was the least sensitive to all the gram positive bacteria in both the groups.

Table 6 shows antimicrobial susceptibility pattern of *Pseudomonas spp* isolated from sputum sample in study group and control group. Polymyxin B and colistin showed 100% sensitivity, whereas cefaclor and gentamicin showed least sensitivity that was 13.6%.

In the RNTCP grading the proportion of positive patients was high in 3+ grade in the study group which was 12.8%.

Table 3: Distribution of Bacterial isolates in HIV positive patients and its relation with CD4+T Lymphocyte cell count.

BACTERIA	<50 (N=42)	50-100 (N=25)	101-200 (N=46)	201-350 (N=31)	351-500 (N=20)	500> (N=16)	Total N (100%)
Acinetobacter.spp	1 (25%)	2 (50%)	1 (25%)	0	0	0	4
Coagulase negative Staphylococcus	3 (21.5%)	1 (7.1%)	5 (35.7%)	3 (21.5%)	1 (7.1%)	1 (7.1%)	14
Coagulase positive Staphylococcus	5 (20.9%)	4 (16.6%)	9 (37.5%)	5 (20.9%)	1 (4.1%)	0 (0%)	24
Eschirichia coli	6 (20.7%)	4 (13.8%)	9 (31.0)	6 (20.7%)	2 (6.9%)	2 (6.9%)	29
Enterobacter aerogenes	6 (28.6%)	5 (23.8%)	7 (33.3%)	1 (4.7%)	2 (9.6%)	0 (0%)	21
Enterbacter cloacae	1 (25%)	1 (25%)	1 (25%)	0 (0%)	1 (25%)	0 (0%)	4
Enterococcus.spp	1 (7.1%)	2 (14.3%)	1 (7.1%)	4 (28.6%)	2 (14.3%)	4 (28.6%)	14
Klebsiella.spp	2 (28.6%)	1 (14.3%)	3 (42.8%)	1 (14.3%)	0 (0%)	0 (0%)	7
Streptococcus pneumoniae	9 (22.5%)	8 (20%)	13 (32.5%)	7 (17.5%)	2 (5%)	1 (2.5%)	40
Pseudomonas.spp	11 (50%)	2 (9.1%)	1 (4.6%)	4 (18.1%)	2 (9.1%)	2 (9.1%)	22
Proteus.mirabilis	1 (33.3%)	0 (0%)	0 (0%)	1 (33.3%)	1 (33.3%)	0 (0%)	3
Streptococcus pyogenes	2 (66.7%)	0 (0%)	0 (0%)	1 (33.3%)	0(0%)	0 (0%)	3
Moraxella catarrhalis	3 (8.6%)	3 (8.6%)	9 (25.7%)	5 (14.3%)	8 (22.8%)	7 (20%)	35
TOTAL	51	33	59	38	22	17	220

Note: Figures in parenthesis indicates percentage

Table 4: Antimicrobial susceptibility pattern of Gram Negative Bacteria isolated from sputum sample in Study & Control Group

Antimicrobial	*Acinetobacter spp*		*Enterobacter aerogenes*		*Enterobacter cloacae*		*Eschirichia coli*		*Klebsiella. spp*		*Proteus mirabilis*	
	HIV+ n=4	HIV- n=0	HIV+ n=21	HIV- n=2	HIV+ n=4	HIV- n=3	HIV+ n=29	HIV- n=4	HIV+ n=7	HIV- N=35	HIV+ n=3	HIV- n=3
Amikacin	0	0	38.1	50	75	33.3	86.2	50	57.1	82.8	66.7	66.7
Ampicillin	0	0	0	0	0	0	13.8	0	0	14.2	0	0
Cefepime	0	0	19	50	0	100	0	50	0	20	0	66.7
Cefixime	0	0	0	0	0	0	13.79	75	0	25.7	0	66.7
Ceftriaxone	0	0	0	0	0	0	17.2	25	0	14.2	0	33.3
Ceperazone/ Sulbactam	0	0	42.9	100	50	100	69	100	42.9	85.7	66.6	100
Cefuroxime	0	0	0	50	0	0	13.8	25	71.4	62.8	0	33.3
Cephalexin	0	0	9.5	0	0	0	10.3	25	0	14.2	0	33.3
Ciprofloxacin	0	0	0	50	12.5	66.7	34.5	75	0	71.4	0	66.7
Gentamicin	0	0	23.8	50	25	33.3	41.4	25	28.6	62.8	0	33.7
Netilmicin	0	0	52.4	100	100	100	72.4	100	71.4	100	100	100
Doxycyclin	0	0	23.81	100	75	66.7	55.17	75	28.5	85.7	0	33.3
Meropenem	50	0	90.5	100	100	100	93.1	100	71.4	97.14	33.3	100

Table 5: Antimicrobial susceptibility pattern of Gram Positive Bacteria isolated from sputum sample in Study and Control Group

Antimicrobial	*Coagulase negative staphylococcus*		*Coagulase positive staphylococcus*		*Enterococcus.spp*		*Streptococcus pyogenes*	
	HIV+ N=14	HIV- N=10	HIV+ N=24	HIV- N=8	HIV+ N=14	HIV- N=0	HIV+ N=3	HIV- N=3
Ampicillin	57.1	40	16.7	22.2	57.1	0	100	100
Augmentin	57.1	80	95.8	88.8	100	0	100	100
Ceftriaxone	57.1	50	29.2	38.8	-	0	0	100
Cephalexin	57.1	50	29.2	38.8	-	0	100	100
Clindamycin	71.4	70	33.3	88.8	57.1	0	66.7	100
Erythromycin	28.6	70	33.3	77.7	57.1	0	66.7	100
Gentamicin	57.1	50	33.3	55.5	57.1	0	0	100
Vancomycin	100	100	100	100	92.8	0	100	100
Doxycyclin	64.29	90	95.83	83.3	78.57	0	100	100
Linezolid	100	100	100	100	100	0	100	100

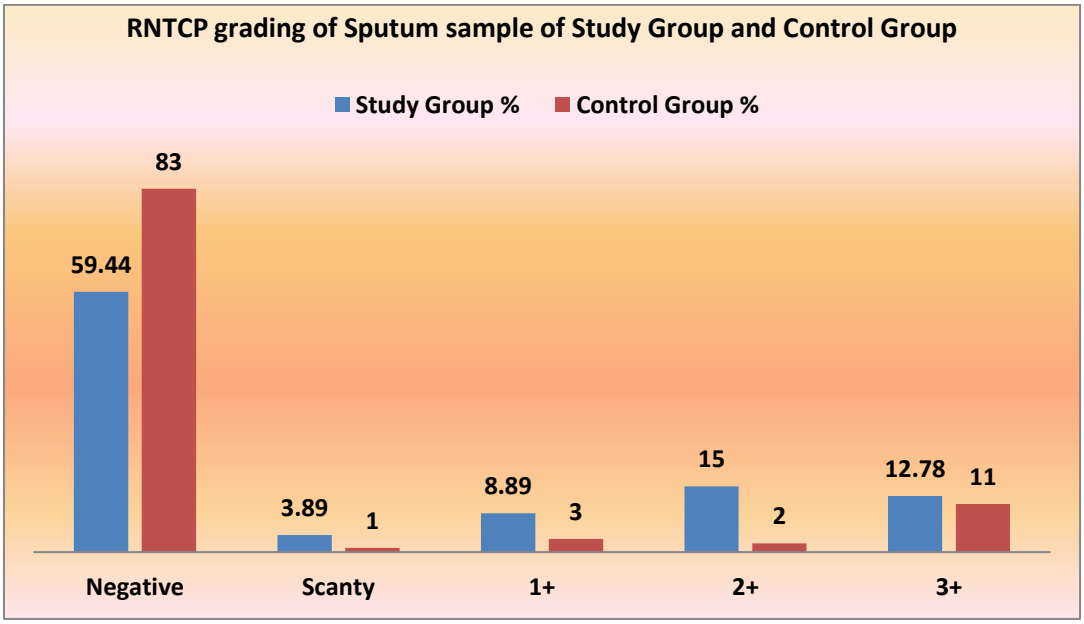

Table 6: Antimicrobial susceptibility pattern of *Pseudomonas .spp* isolated from sputum sample in Study and Control Group

Antimicrobial	Sensitivity%	
	HIV+ N=22	HIV- N=10
Aztreonam	50	70
Cefaclor	13.64	10
Cefoperazone	31.8	20
Ceftazidime	50	60
Cefoperazone/Sulbactam	68.18	90
Gentamicin	13.6	70
Imipenem	86.3	100
Piperacillin/tazobactam	50	100
Meropenem	59.1	100
Polymyxin b	100	100
Colistin	100	100
Levofloxacin	22.7	60

DISCUSSION

Opportunistic pneumonias are the major cause of mortality and morbidity amongst the HIV seropositive patients .Bacterial infections are one of the important causes of LRTI. Identification of the bacterial species is important, as it helps in the initiation of specific antibacterial therapy which improves the outcome of treatment. In the present study most of the HIV positive patients were in the age group of 31-40 years(40.0%) followed by the age of group 21-30(23.9%). The mean age of HIV positive patients was 37.21±11.2 years. The higher mean age of HIV positive patients may be because the index of suspicion is low and testing for HIV infection is carried out only when clinical signs and symptoms start appearing, which happens in the advanced stage of infection. Our findings are consistent with other studies elsewhere in India[2,8,9]. In the present study male patients were more than females. The male female ratio was 1.95:1. Our finding was comparable with Chakraborty et al., and Sangeeta et al.[8,10] The male prevalence seen might have been due to the fact that in the existing social milieu in India, females do not seek medical care because of fearing isolation and loss of family support.

Majority of patients were non agriculture labourers, truck drivers, business/self-employed and housewives. Our findings are consistent with that of Shilpa et al.[2] Males migrate to different places for education and work. Migrants form a link between urban and rural areas, and high-risk and low-risk groups[11]. However, about 75% of women testing positive in India have a husband who is a migrant labourer[11]. Migrants returning back may infect their wives which significantly increases the rate of infection among housewives. Truck drivers themselves are a highly vulnerable group for HIV infection. They stay away from their families for extended period of time and are in close proximity to "high risk" sexual network. Awareness about HIV transmission is low among truck drivers. Approximately 2.6% of the two million truckers in India are living with HIV[12]. In the present study in 95% of patients HIV was transmitted through heterosexual contact. However Chakraborty et al., and Gupta et al., reported transmission by heterosexual route only in 64% and 66.6% of cases respectively.[8,13]

In the present study majority of patients had CD4+T lymphocyte cell counts <200 cells/cmm 113 (62.7%). The number of cases were more in 101-200 cells/cmm 46(25.6%). Our result are similar to Shilpa et al[2] and Nilanjan et al[15]. However, Bharathi et al[16] reported 53% patients between 201 – 500cells/cmm.

In our study the mean CD4+T lymphocyte cell count was 209.05±195.23which is comparable to results of RR Shah et al[17] & Rajeev et al.[18]

In the present study 220 strains of bacteria were isolated from all the patients. Amongst them the most common was *Streptococcus pneumoniae which* was isolated from 22.2% of patients followed by *Moraxella catarrhalis* (19.4%), *Eschirichia coli* (16.1%), *Enterobacter aerogenes* (11.7%) and, *Klebsiella spp* (3.9%). *Acinetobacter spp, Streptococcus pyogenes and Klebsiella spp* were isolated in patients with CD4 T lymphocyte counts <350 cells/cumm, whereas *Coagulase negative Staphylococcus, Coagulase positive Staphylococcus, Enterococcus.spp, Streptococcus pneumoniae, Proteus mirabilis, Pseudomonas. spp, Escherichia coli, Enterobacter.spp*, were also isolated with CD4 T lymphocyte counts >350 cells/cmm, Our study was similar to Shilpa et al[2] and Yarlagadda P et al[14].

In the present study most of the Gram positive bacteria were sensitive to linezolid, vancomycin and doxycycline, while the sensitivity to other microbial agents showed variable resistance. Our results are comparable to Yarlagadda P et al[14] and Ramanlal et al[17]**.** Most of the gram negative bacteria were sensitive to meropenem. Our study was consistent with Shilpa et al[2] and Rajeev et al[18]. Polymyxin B and colistin were most effective for *Pseudomonas. spp*. Our study was similar to Shilpa et al[2] and Yarlagadda P et al[14].

In our study AFB was demonstrated in 40.5% of HIV positive patients and 17% of HIV negative patients. Our results are comparable to Nilanjan et al[15] and Shailaja et al[19]. Whereas, in a study conducted in Manglore by K Shreevidya et al[20] *M. tuberculosis* was isolated in 83.3% of HIV positive patients.

In the present study 27.2% AFB positive had CD4+T lymphocyte cell count <200 cells/cmm. *Mycobacterium tuberculosis* is endemic in India and thus, is one of the commonest opportunistic infections in India. Our result was similar to Usman et al[21] and Shilpa[2] et al.

CONCLUSION

HIV seropositives are susceptible to lower respiratory tract infection especially pneumonia caused by various organisms. During routine CD4 count evaluation whenever there is a decrease in counts, it should alert the clinicians to anticipate opportunistic infections. Routine screening of opportunistic pulmonary infection is essential to know the etiological agent and their susceptibility pattern before treating the patients empirically. As was observed in the present study these isolates show drug resistance. So culture and sensitivity will help to give an effective treatment, which will help to reduce morbidity and mortality.

WHO in Geneva in 2013 has recommended offering antiretroviral therapy (ART) earlier when CD4 cell count falls to 500 cells/mm³ or less – when their immune systems are still strong. This is being followed in Algeria, Argentina and Brazil and has shown that earlier ART will help people with HIV to live longer, healthier lives, and substantially reduce the risk of transmitting HIV to others.

The results of this study can be of use to clinicians regarding common prevailing infectious agents .The sensitivity pattern seen in our study can be helpful for clinicians in presenting empirical antimicrobial treatment in this area.

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