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Incidence of bacterial and fungal co-infections in some HIV infected Indian population

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Abstract

HIV/AIDS continues to spread globally and remains a worldwide pandemic. In the present study, a total number of 100 biosamples were collected from the HIV positive patients attending the Social Welfare Organizations and HIV Counseling and Testing Centers of Teaching hospital of Trichy in India were enrolled and screened. Bacterial pneumonia and bacteremia occur at a higher frequency among HIV infected patients. Opportunistic infections (OI) are most common in immunocompromised patients which are the leading cause of death in HIV-infected patients. To create awareness among HIV patients for taking control or preventive measures against the opportunistic bacterial and fungal infections, an analysis was done in this present study. Appropriate use of antibiotics against these OI may be one of the strategies to extend the life span of the AIDS patients.

Keywords: HIV, AIDS, immunocompromised patients, opportunistic infections.

Introduction

Acquired Immuno Deficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) is the most important public health problem in 20th century (AIDS Epidemic Update, 2004). Every day approximately 1500 people become infected with HIV and of them young people below 25 years account for over 50% of infections. AIDS is in an advanced stage of the epidemic in some states of the country (Omenaca *et al*., 1999). The infection is alarming due to the unique pathogenesis of the virus that decreases the CD4 cells, signaling the emergence of the opportunistic infections, in the host. HIV/AIDS continues to spread globally and remains a worldwide pandemic affecting about 40million people. HIV does not kill anybody directly. People with advanced HIV infections are vulnerable to infections and malignancies that are called “opportunistic infection”, because they take advantage of the opportunity offered by a weakened immune system. Opportunistic infections are caused by various pathogenic microorganisms such as bacteria, fungi, virus and parasites (Hirschtick *et al*., 1995). An abundance of research and literature has been dedicated to the opportunistic bacteria, fungi, viruses and parasites. Diseases caused by bacteria and fungi are responsible for a significant proportion of the morbidity and mortality seen in HIV population (Graden *et al*., 1992). These organisms attack when there is an ‘opportunity’ to infect. Many opportunistic infections associated with AIDS cause serious illness.

Materials and methods

About 100 HIV patients attending the Social Welfare Organizations and HIV Counseling and Testing Centers of two teaching hospitals of Thanjavur and Trichy in India were enrolled in this study. All HIV positive patients in age group between 10 and 80 were included.

Collection of samples

Blood, urine, sputum, stool, pus and wound, nail and head scrapings were collected from the symptomatic HIV positive patients during the period from Aug 2008 to Oct 2009.

CD_4 count procedure

Saccs method was used for CD_4 count procedure. 50µl of whole blood was taken in a potassium EDTA tube and added with monoclonal antibody with buffer given in the kit. The components were mixed thoroughly and kept the tubes in dark place. Then the fixative 50µl was added and mixed well. Then the sample was run in Saccs count machine for 3 minutes.

Identification of pathogens

Preliminary identification was done by Gram staining, acid fast staining and motility test for bacteria and Lactophenol cotton blue staining for fungi.

Isolation

The collected samples were taken to laboratory and processed for the isolation of bacteria. Before the isolation process, sputum and urine samples were processed by modified Petroffs method.

Culture technique

Culture technique acts as a cornerstone for the identification of positive cultures. The media employed for the isolation of different type of bacteria were: Nutrient agar, Mannitol salt agar, Eosin Methylene blue agar, Blood agar, Mac Conkey agar and Chocolate agar; Saborauds dextrose agar, Rose bengal agar, Potato dextrose agar and Czapek dox agar were used for fungi. To identify and characterize the isolated pathogens, the methods used included test for: Oxidase, Catalase, Sugar fermentation for Glucose, Lactose, Maltose, Mannitol, Sucrose, Triple sugar Iron test, ‘IMViC’, Urease, Niacin and Nitrate reduction.

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Antibiotic sensitivity tests for bacteria

Kirby-Bauer disc diffusion method is commonly employed for antibiotic sensitivity test. This test based on the size of the zone of inhibition related to minimum inhibitory concentration (MIC). The antibiotic discs used were Amikacin, Ampicillin, Cefalothin, Cefepime, Ceftazidime, Clindamycin, Erythromycin, Gatifloxacin, Gentamicin, Lincomycin, Netilmicin, Norfloxacin, Ofloxacin, Penicillin, Piperacillin, Tetracycline and Vancomycin.

Antibiotic sensitivity tests for fungi

Antimicrobial activity test was carried out by using hole-plate diffusion method. Holes were made on the medium by using 6mm cork borer then the antibiotic treated discs namely Amphotericin B, Fluconazole, Fluconazole, Griseofulvin, Itraconazole, Ketoconazole, Miconazole, Nystatin, Trimethoprim and Voriconazole used to detect the antibiotic sensitivity of the isolated fungal pathogens.

Results and discussion

A total number of 100 biosamples collected from the HIV positive patients attending the Social Welfare Organizations and HIV Counseling and Testing Centers of teaching hospital of Trichy were enrolled and screened. Age and Sex distribution of HIV positive patients showed in Table 1; 62 patients were male and 38 were female. Among them, 54 patients showed positive results for bacteria and 22 patients showed positive results for fungi. We concluded that the significant samples had 7 bacterial spp. and 6 types of fungal spp. They were isolated and identified. The predominant isolates of bacteria were *M. tuberculosis* (21), *E. coli* (17), *S. typhi* (8), *P. aeruginosa* (3), *E. aerogenes* (2), *S. aureus* (1), *C. botulinum* (1) and *S. dysentriae* (1). Isolated bacterial species were represented in Table 2. The various etiologies of bacterial pathogens causing OI were showed in Table 3 and predominant isolates of fungi were *C. albicans* (11), *Aspergillus niger* (7), *H. capsulatum* (1), *P. marneiffei* (1), *B. dermatidis* (1) and *C. neoformans* (1). Antibiotic sensitivity of isolated bacteria and fungi represented in Fig. 4 and 5 respectively. Isolated fungal species were shown in Table 6 after that the various etiologies of fungal pathogens causing OI were showed in Table 7.

Table 1. Age and sex distribution of HIV positive patients

Age group	% HIV incident			
	Male	%	Female	%
0-20	19	30	20	42
20-40	33	53	24	50
40-60	9	15	4	8
60-80	1	2	--	--
Total	62		38	

Table 2. Isolated bacteria from HIV positive patients

Bacterial species	Bacteria isolated (%)	CD_4 count/µl
Mycobacterium tuberculosis	21	120-200
Escherichia coli	17	350-470
Salmonella typhi	8	300-380
Pseudomonas aeruginosa	3	500-670
Enterobacter aerogenes	2	560-830
Staphylococcus aureus	1	480-530
Clostridium botulinum	1	570-820
Shigella dysentriae	1	740-960
Total	54	

The Antibiotics pyrazinamide (26.4mm) and rifampin (23.8mm) showed highest inhibition to *M. tuberculosis* and Tuberculin skin testing was compromised in HIV infection, where immunosuppression commonly leads to anergy. Globally 9 % of all new TB cases in adults were attributable to HIV/AIDS, as were 12% of the 1.8 million deaths from TB in the year 2002 (Corbett *et al.*, 2003). A HIV positive person infected with *M. tuberculosis* has a 50-60% life time risk of developing TB diseases as compares to an HIV negative person who has only a 10% risk.

The isolated *E. coli* spp. were sensitive to 8 different antibiotics, among these antibiotics Amikacin showed highest inhibition (21.5mm), followed by Cefixime (20.8mm), Oflaxacin (19.5mm) Gentamycin (19.3mm) Gatifloxacin (18.6mm) and Lincomycin (18.2mm). Breton *et al.* (2006) reported that Ofloxacin (97.4%) and Gatifloxacin (97.4%) is the most effective antibiotic in the treatment of opportunistic infections caused by enteroinvasive pathogenic *E. coli* which resembles the present study.

The isolated *S. typhi* treated against the antibiotic Chloramphenicol showed the highest zone of inhibition (23.6mm) followed by Amphicillin (20.6mm). Rolston *et al.* (1994) reported that, *S. typhi* was 71.6% sensitive to Ampicillin, 80.5% sensitive to Vancomycin which supports the present study.

Antibiotics are Trimethoprim (22.3mm), Tetracycline (21.0mm) and Amikacin (17.0mm) showed the highest zone of inhibition of isolated *P. aeruginosa* and remaining antibiotics were showed moderate zone of inhibition and Oflaxacin showed very least zone of inhibition against *P. aeruginosa*. The species of *Pseudomonas* are emerging as important opportunistic pathogens in the HIV infected host as described in the earlier studies. It has emerged as one of the most common causes of gram negative bacteremia and Pneumonia in HIV infected hospitalized patients (Whimbey *et al.*, 1986).

Antibiotics are Trimethoprim (22.3mm), Amikacin (20.2mm) and Tetracycline (17.8

Table 3. Etiology of opportunistic bacterial infections in HIV positive patients

Etiology	Number of Suspected cases	Positive cases	Organisms isolated
Tuberculosis	32	21	*Mycobacterium tuberculosis*
Typhoid	13	8	*Salmonella typhi*
Wound infection	16	3	*Pseudomonas aeruginosa*
		2	*Enterobacter aerogenes*
Food Poisoning	29	17	*Escherichia coli*
		1	*Clostridium botulinum*
Skin Infections	5	1	*Staphylococcus aureus*
Dysentry	5	1	*Shigella dysentriae*
Total	88	54	

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mm) showed the highest zone of inhibition to *E. aerogenes* and other antibiotics showed moderate susceptibility to the isolated bacteria and the isolated *E. aerogenes* resistant to Cefalothin and Oflaxacin.

Table 4. Morphology, staining and biochemical characteristics of isolated organisms

Name of the organism	Gram staining	Acid fast staining	Morphology	Motility	Biochemical tests							
					Indole	MR	VP	Citrate	Urease	Nitrate reduction test	H_2S Test	Carbohdrate fermentation test
Mycobacterium tuberculosis	+	+	Bacilli	_	_	_	_	_	_	+	_	_
Escherichia coli	-	-	Bacilli	+	+	+	_	_	_	+	_	+(A,G)
Salmonella typhi	-	-	Bacilli	+	-	+	-	+	-	+	-	+(A,G)
Pseudomonas aeruginosa	_	_	Bacilli	+	_	_	_	+	_	+	+	_
Staphylococcus aureus	+	_	Cocci	_	_	+	_	_	+	+	_	+(A)
Clostridium botulinum	+	_	Bacilli	+	+	_	_	_	_	+	_	_
Shigella dysentriae	_	_	Bacilli	_	+	+	_	_	_	+	_	+(A,G)
Enterobacter aerogenes	_	_	Bacilli	+	_	_	+	+	_	+	_	+(A,G)

Table 5. Antibiotic sensitivity pattern of isolated bacteria

Antibiotics	Zone of inhibition (mm)							
	Mycobacterium tuberculosis	*Escherichia coli*	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Enterobacter aerogenes*	*Staphylococcus aureus*	*Clostridium botulinum*	*Shigella dysentriae*
Amikacin	2.6	21.5	3.4	17.0	20.2	18.8	0	16.7
Amphicillin	0	10.9	20.6	10.8	10.7	13.0	5.9	26.8
Cefalothin	3.2	19.1	8.2	15.4	0	16.8	12.6	14.8
Cefixime	4.1	20.8	3.4	16.0	10.2	0	10.8	12.3
Gentamycin	0	19.3	7.6	12.6	11.4	10.2	0.3	15.6
Gatifloxacin	0	18.6	10.2	15.0	11.4	10.8	9.7	11.5
Lincomycin	0	18.2	0.8	10.8	13.3	12.2	14.2	12.9
Chloramphenicol	0	15.5	23.6	18.6	12.6	11.4	12.5	17.2
Oflaxacin	0	19.5	6.9	0.2	0	10.2	8.7	12.1
Norfloxacin	0	12.3	4.2	13.0	10.1	17.2	11.3	11.3
Penicillin	2.3	16.4	5.9	16.2	11.4	18.0	13.2	12.9
Pyrazinamide	26.4	10.2	9.8	8.3	7.8	10.6	7.6	9.2
Rifampin	23.8	9.8	5.9	7.2	8.6	12.3	6.8	7.2
Tetracycline	3.4	14.8	24.6	21.7	17.8	21.11	17.2	17.3
Trimethoprim	0	0	10.2	22.3	22.3	11.22	11.2	15.2
SD	4.51	5.71	7.40	5.74	6.13	5.06	4.78	4.54
SE	1.17	1.47	1.91	1.48	1.58	1.31	1.28	1.17

Antibiotic Tetracyline showed highest zone of inhibition (21.11mm) to the isolated *S. aureus* among the antibiotics of Penicillin (18.0mm) and Norfloxacin (17.2mm). Similarly, Whimbey *et al.* (2005) reported that *S. aureus* predominantly sensitive to Tetracycline.

C. botulinum was highly resistant to Amikacin and Gentamycin and showed moderate sensitivity to all other antibiotics and Lincomycin showed the highest zone of inhibition (14.2mm) followed by Penicillin (13.2mm). Earlier investigations reported that *C. botulinum* were resistant to Amikacin, Gentamicin, Ceftazidime & Levofloxacin (Berman *et al.,* 1996).

S. dysentriae is highly susceptible to Amphicillin and showed the highest zone of inhibition (26.8mm) among the antibiotics of Chloramphenicol (17.3mm), Tetracycline (17.2mm), Amikacin (16.7mm). Viviani *et al.* (2006) reported that the antibiotic spectrum of Amikacin, Gentamicin, Chloramphenicol and tetracycline to *K. pneumoniae* which is similar to the present study.

The higher value Standard Deviation (SD) and Standard Error for antibiotic sensitivity of bacteria found for *S. typhi* followed by *E. aerogenes, P. aeruginosa, E. coli, S. aureus, C. botulinum, S. dysentriae* and *M. tuberculosis*.

C. albicans produced moist opaque creamy colony on blood agar. Clusters of round blastoconidia were present at some septae. Thick walled chlamydospores were seen. *A. niger* borne with septate hyaline hypahe with long conidiophores of smooth, and hyaline, becoming darker at the apex. The conidia were brown to black, very rough and measured 4-5 µm in diameter. The cells of *H. capsulatum* were 5-6 microns in diameter and slightly oval in shape when cultured in SDA. It appeared as a

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white, cottony mycelium after 2 to 3 weeks of culture at 25^{0}C. *P. marneiffei* are dimporphic fungi; the mold form has septate hyphae and smooth conidia while the yeast form is round to oval on SDA. *C. neoformans* were observed as round or oval shaped yeast cells with scars on their surfaces where daughter cells had budded off during reproduction. Capsule was seen. The mycelia and the fruiting bodies of *B. dermatidis* were seen under microscope. The culture of *B. dermatitidis* appeared as a white, cottony mold (mycelium) on SD and took 2 to 3 weeks to grow at 25^0 C.

Table 6. Isolated fungi from HIV positive patients

Fungal species	No: of isolates	%	CD_4 count/µl
Candida albicans	11	50	120-200
Aspergillus niger	7	30	250-310
Histoplasma capsulatum	1	5	190-240
Penicillium marneffei	1	5	220-250
Blastomyces dermatidis	1	5	290-305
Cryptococcus neoformans	1	5	230-330

Table 7. Etiology of opportunistic fungal infections in HIV positive patients

Major etiology	Number of suspected cases	Positive cases	Organisms isolated
Thrush, Vaginosis Diabetes mellitus	28	11	*Candida albicans*
Diabetes mellitus Neutropenia, Tuberculosis	9	7	*Aspergillus niger*
Tuberculosis	11	1	*Penicillium marneffei*
Respiratory infections	7	1	*Histoplasma capsulatum*
hepatosplenomegaly	2		
Meningoencephalitis	1	1	*Cryptococcus neoformans*
Granulomatous skin lesions	1	1	*Blastomyces dermatidis*

The results of sensitivity and resistance of isolated fungal pathogens showed in Table 8. The antibiotic Nystatin (23.6mm) and Fluconazole (20.1mm) showed the highest zone of inhibition and all other antibiotics were showed moderate susceptibility to *C. albicans.* Similarly, Whelan *et al.* (1990) also observed such response with *C. albicans* towards Fluconazole, Nystatin, and Amphotericin B.

The antibiotics are Amphotericin B (24.7mm) and Nystatin (23.5mm) showed the highest zone of inhibition against to *A. niger* and all other antibiotics were showed moderate susceptibility to *Aspergillus niger.* Woitas *et al.,* (1998) reported that the antibiotic spectrum of Amphotericin B, Itraconazole and Fluconazole to *A. niger* which is similar to the present study. The antibiotics are Fluconazole (24.6mm) and Itraconazole (23.4mm)

Table 8. Antibiotic sensitivity pattern of isolated fungi

Antibiotics	Zone of inhibition (mm)					
	Candida albicans	*Aspergillus*	*Histoplasma capsulatum*	*Penicillium marneffei*	*Blastomyces dermatidis*	*Cryptococcus neoformans*
Amphotericin B	18.2	24.7	22.6	24.6	26.4	22.3
Fluconazole	20.1	14.2	24.2	22.6	24.6	20.6
Flucytosine	15.3	8.9	13.4	16.8	16.4	21.6
Griseofulvin	10.2	8.2	15.2	12.8	12.6	5.7
Itraconazole	9.8	23.5	23.4	20.4	14.8	14.9
Ketoconazole	13.2	16.5	20.6	22.8	10.2	14.2
Miconazole	14.6	12.9	18.9	23.7	12.4	9.9
Nystatin	23.6	12.2	16.5	13.2	12.2	7.8
Trimethoprim	15.6	13.5	9.8	14.6	15.6	10.5
Voriconazole	9.5	9.7	8.6	20.3	10.3	10.9
SD	5.24	5.70	5.56	4.48	5.65	5.93
SE	1.16	1.80	1.76	1.42	1.79	1.88

showed the highest zone of inhibition against to *Histoplasma capsulatum*. Yang (2003) reported that the *H. capsulatum* spp., were sensitive to Amphotericin B ,Itraconazole and Fluconazole, the reports were similar to the present study.

The antibiotics are Amphotericin B(24.6mm), Miconazole(23.7mm), Ketoconazole(22.8mm) and Fluconazole (22.6mm) showed the highest zone of inhibition against *Penicillium marneffei.* Supparatpinyo *et al* (1999) reported that the *P. marneffei* spp., were sensitive to Amphotericin B , Itraconazole and Fluconazole, the reports were similar to the present study. The antibiotics are Amphotericin B (26.4) and Fluconazole (24.6mm) showed the highest zone of inhibition against to *Blastomyces dermatidis* and all other antibiotics were showed moderate susceptibility to *B. dermatidis.* Pappas *et al.* (1992) reported that the *B. dermatidis* spp., were sensitive to Amphotericin B, Itraconazole and Fluconazole, the reports were similar to the present study.

The Antibiotics Amphotericin B (22.3mm) showed the highest zone of inhibition against to *Cryptococcus neoformans* and all other antibiotics were showed moderate susceptibility to *C. neoformans.* Viviani *et al.* (2003) reported that the *C. neoformans* spp., were sensitive to Amphotericin B, Itraconazole and Fluconazole, the reports corroborate the present study.

The higher value Standard Deviation (SD) and Standard Error (SE) for antibiotic sensitivity of fungi found for *C. neoformans* followed by *A. niger, B. dermatidis, H. capsulatum, C. albicans* and *P. marneffei.*

Conclusions

Out of 100 samples obtained from HIV infected patients, 35 samples (70%) were found to be infected by bacteria and 22 samples (44%) by fungi. The infected samples were further subjected to the isolation of

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causative agents were found to be bacteria viz., *M. tuberculosis*, *E. coli*, *S. typhi*, *P. aeruginosa*, *E. aerogenes*, *S. aureus* and *K. pneumonia* and fungi isolates *viz*., *C. albicans*, *A. niger*, *H. capsulatum*, *P. marneiffei*, *B. dermatidis* and *C. neoformans*. Those human pathogens are known to infect and induce many diseases to HIV positive patients. These diseases lead to early death of them. We suggested that the appropriate use of recommended antibiotics to extend the life period of HIV positive patients.

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