Original Article

Prevalence of opportunistic infections in HIV-positive patients in Bahrain: a four-year review (2009-2013)

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Abstract

Introduction: This study aimed to examine the prevalence of opportunistic infections in HIV-infected patients in Bahrain and its relation to absolute CD4 count, CD4%, and CD4/CD8 ratio.

Methodology: This retrospective cohort study used laboratory records (January 2009 - May 2013) from a major hospital in Bahrain. Opportunistic infections (OIs); absolute CD4 counts, CD4%, and CD4/CD8 ratio were recorded.

Results: CD4% and absolute CD4 count in HIV patients with associated infections (157 ± 295) was significantly lower than in those without associated infections (471 ± 285) ($p < 0.001$). There was no significant difference in CD4/CD8 ratio between the two groups. Infection with *Staphylococcus aureus* was the commonest infection, present in 9.8% of total HIV-infected patients and 28.7% of members of the AIDS patient group with OIs, followed by yeast infections (9.2% and 27.2%, respectively). *Mycobacterium tuberculosis* was present in 3.6% of total HIV-infected patients and 10.6% of the group with OIs, while mycobacteria other than tuberculosis (MOTT) was present in 2.5% and 7.5%, respectively. *Pneumocystis jirovecii* pneumonia (PCP) was observed in 5.1% and 15.1%, respectively. Herpes simplex II (HSV-II) was observed in 3% and 9%, respectively, while *Cytomegalovirus* antigenemia was only present in 2% and 6%, respectively. *Streptococcus pneumoniae*, *Streptococcus milleri*, *Stenotrophomonas maltophilia*, and *Citrobacter species* were bacterial infections observed least frequently.

Conclusions: Studying the pattern of OIs in HIV-infected patients in Bahrain is of paramount importance due to the scarcity of data in the Arab world. This will help to improve physicians' awareness to improve care of HIV-infected patients.

Key words: Opportunistic Infections; HIV; AIDS, CD4 count; CD4/CD8 ratio.

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Introduction

Bahrain is an island in the Arabian Gulf with a total population of 1,248,348, including 235,108 non-nationals. Bahrain is highly dependent upon its foreign workforce. Half of Bahrain's 1.25 million-strong labor pool is comprised of foreign-born persons, which increases the risk of travel-related diseases. The first case of HIV discovered in Bahrain was in 1986, when a boy contracted the disease after receiving a blood transfusion [1]. According to Bahrain's Ministry of Health, statistics showed that from a total 415 HIV-infected patients registered in Bahrain, 69 cases of people infected with HIV/AIDS were added in 2012; 51% of them were foreigners. The commonest mode of infection was injecting drug use (57.3%), followed by unsafe sex practice (33.9%). Congenital infection was reported in 2.2% of cases where HIV-positive mothers transmitted infection to their children. Intravenous drug abuse was a major risk factor (38.8%) among Bahraini nationals, while transmission through sexual contact was more common (45.7%) among foreigners. Out of the 415 registered HIV-infected patients, 203 (48.9%) have died, with OIs being the major cause of death [2].

These OIs take advantage of the weak immune systems of HIV-infected patients; some of these infections could be used as a stage indicator of HIV disease, and can predict the disease progress [3]. The frequency of OIs differs from one region to another [4,5].

Infections were the principal cause of morbidity and mortality in HIV-infected patients worldwide in both developed and developing countries [6]. However, other studies have demonstrated that severe bacterial infections, particularly pneumonia, still occurred at high rates even in the absence of severe CD4 cell depletion [7-9]. The CD4-cell count has been a pivotal tool in HIV care. CD4 count can indicate the stage of HIV disease, guide treatment, and predict the disease progression and prognosis. It can help to guide

decisions to use OI prophylaxis and antiretroviral therapy [10].

There are few studies that have analyzed the incidence of opportunistic and other infections in HIV-infected patients in Bahrain. This study aimed to examine the prevalence of opportunistic and other infections in HIV-infected patients in Bahrain and to relate these infections to the absolute CD4 count, CD4%, and CD4/CD8 ratio.

Methodology

This research was conducted as a retrospective, cohort study to explore the types of associated infections in Bahraini patients with AIDS. It was performed using laboratory records from the main tertiary care hospital, Salmaniya Medical Complex (SMC) in Bahrain, from January 2009 to May 2013. Data pertaining to all microbial isolates, to bacterial, parasitic and viral infection, and to CD4%, absolute CD4 count, and CD4/CD8 ratio were retrospectively collected from the laboratory information system. CD4/CD8 ratio was studied, though CD4 % and absolute CD4 count were reported, as CD4/CD8 ratio may reflect adequacy of antiretroviral treatment. Data were tabulated into Microsoft Excel and analyzed separately for the predominant microbiologic isolates using TexaSoft version 6 (WINKS SDA Software, Cedar Hill USA). Data are presented as mean (±SD) values. Comparisons between the studied groups were done using student's *t*-test, with $p < 0.05$ considered statistically significant. The study was approved by the research and ethics committee of SMC. This was a purely record-based study with no ethical issues.

Cases were discovered either accidentally during a routine health check or premarital screening, or when suspected in certain cases when clinical data suggested HIV infection. Cases were defined as HIV positive when confirmed by a confirmatory test such as a western blot if the screening by enzyme immunoassay (EIA) tests was positive. The duration of the disease was calculated from the time of first diagnosis to the time of acquiring OIs. All the included HIV-infected patients (194 patients; 6 were children below seven years of age) were admitted to the hospital and were on adequate antiretroviral medication. They were further subdivided into two groups based on the presence or absence of associated OIs. The first group did not have any associated OIs during the study period (128 patients), while the second group acquired different types of OIs (66 patients). It was assumed that all patients followed during the study were prescribed effective therapy as they were all seen by the infectious disease attending physicians; however, whether the patients were taking their therapy continuously or correctly could not be ascertained. Any available demographic and clinical data, such as age, age at diagnosis, and sex, were collected.

All patients had a flow cytometry performed at various times, as deemed appropriate by their physician, using automated gating four-color flow cytometry (Beckman Coulter Cytomics FC 500 Flow Cytometer; Coulter, Miami, USA) according to the operator's guide and the manufacturer's procedure. Identification of all causative microorganisms was performed by the standard microbiologic methods. Bacterial cultures were performed when indicated on routine culture media, including blood agar, MacConkey's agar, chocolate agar, and xylose lysine deoxycholate (XLD) agar. Identification of cultured bacteria was done using Gram staining and conventional methods. Diagnosis of mycobacteria was done using different methods, including molecular techniques by DNA strip technology (Hain Lifescience GmbH, Nehren. Germany), thermal cycler (Bio-Rad, Hercules, USA) and real-time polymerase chain reaction (PCR) (GeneXpert, Cepheid, Sunnyvale, USA). Culture for mycobacterium species was done on a Mycobacteria Growth Indicator Tube system (MGIT; BD, Franklin Lakes, USA) and Lowenstein Jensen (LJ) media (Oxoid Co., Basingstoke, UK) after acid-fast staining using the Zeihl-Neelsen (ZN) stain. Identification of fungal infection was done using Saboraud's agar, and any isolated *Candida* species were tested using a germ tube test. Broncho-alveolar lavage was tested for the presence of *Pneumocystis jirovecii* (PCP) by a direct antigen detection test when clinically indicated using an immunofluorescence method (Bio-Rad). Cerebrospinal fluid (CSF) examination was done using India ink staining when *Cryptococcus neoformans* was suspected. Serological tests were conducted to confirm the presence of many pathogens. *Legionella pneumophila* was detected using an immunofluorescence technique; *Toxoplasma gondii* was determined by detection of IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA) techniques, especially when there was cervical lymphadenitis or CNS involvement; *Cytomegalovirus* (CMV) was determined by detection of CMV IgG and IgM antibodies using ELISA techniques, while CMV antigen was detected in the peripheral blood leucocytes by a CMV pp65 antigenemia assay (IQ Product, Rozenburglaan, Groningen, Netherlands). ELISA was also used to detect herpes simplex type II IgG and IgM antibodies

as well as antibodies to Epstein-Barr virus (Immulite Analyzer, Siemens, Erlangen, Germany).

Results

Table 1 shows demographic data, CD4%, absolute CD4 count, and CD4/CD8 ratio of HIV-infected patients with and without associated infections. There was no significant difference in age, age at HIV diagnosis, and sex between the two groups. There were 66 patients in the HIV-positive with associated infections group; 55 were male (83.3%) and 11 were female (16.7%) with a male-to-female ratio of 5:1. The mean age of this group was 48.3 ± 11.6 years when HIV was diagnosed, with the youngest age at diagnosis being 6 years and the eldest 83 years. The patients had a mean duration between HIV diagnosis and the current infection of 8.2 ± 6.5 years. There were 128 HIV-positive patients without any associated infections; 103 were males (80%) and 25 were females (20%), with a male-to-female ratio of 4:1, with no significant sex differences between the two groups. The mean age of the patients was 46 ± 12 years, and the mean age at HIV diagnosis was 35 ± 11 years of age; the youngest age at diagnosis was 1 year and the eldest was 83 years. Table 1 also shows a higher prevalence of HIV infection in males compared with females in both groups. There was a significant decrease in CD4% and absolute CD4 count in the HIV-positive with associated infections group compared with the HIV-positive without associated infections group ($p < 0.001$). However, there was no significant difference in CD4/CD8 ratio between the two groups.

There was a significant increase in the number of patients co-infected with hepatitis C in the group with OIs (21 cases; 32%) compared with the group without OIs (23 cases; 18%). There were no significant differences in the number of co-infections with hepatitis B or combined co-infections with both hepatitis B and C between the two groups (Table 1).

Table 2 shows a stratification distribution of absolute CD4 count in HIV-infected patients. The count was classified into four strata: CD4 count of less than 100 cells/μL, between 100 and 200 cells/μL, between 201 to 500 cells/μL, and more than 500 cells/μL. CD4 counts of less than 100 cells/μL were found in 22.7% of the total 194 HIV-infected patients; the ratio increased to 56% in the group of HIV-patients with OIs (out of 66 patients) and dropped to only 6% of 128 HIV-infected patients without OIs. On the other hand, CD4 counts of more than 200 cells/μL were found in 67.5% out of total 194 HIV-infected patients and in 86.7% of the group with HIV without OIs. There was a drop to 30.4% in the group of HIV patients with OIs (Table 2). This table also shows that the most common infection associated with absolute CD4 counts of less than 100 cells/μL were yeast (10), PCP (5), and EBV (5).

Table 1. Demographic data, CD4%, absolute CD4 count, and CD4/CD8 ratio between HIV-infected patients with and without associated infections

		HIV patients with infection (n = 66)	HIV patients without infection (n = 128)	t	P value
Age	Mean	48.3	46.6	0.78	0.43
	SD ±	11.6	12.3		
Age at diagnosis	Mean	35.2	34.5	0.227	0.78
	SD ±	13.8	10.7		
Male-to-female ratio		5:1	4:1		
Males		55 (83.3%)	103 (80%)	0.55	0.6
Females		11 (16.7%)	25 (20%)	0.5	0.6
CD4%	Mean	7.1	18.6	5.06	< 0.001
	SD ±	7.4	9.4		
Absolute CD4 count	Mean	157.4	470.9	4.03	< 0.001
	SD ±	295	284.7		
CD4/CD8 ratio	Mean	0.84	0.3	0.64	0.5
	SD ±	3.5	0.2		
Concurrent hepatitis B infection		3 (4.5%)	1 (0.7%)	1.79	0.7
Concurrent hepatitis C infection		21 (32%)	23 (18%)	2.2	0.03
Concurrent both hepatitis B & C infection		3 (4.5%)	2 (1.5%)	1.2	0.2

Table 2. Stratification of absolute CD4 count in HIV infected patients

CD4 number	Total number of patients (n = 194)	HIV patients with associated infection (n = 66)	HIV patients without associated infection (n = 128)	Common associated infections	Less common associated infections
< 100	44 (22.7%)	37 (56%)	7 (6%)	Yeast (10), PCP (5), EBV (5), *Acinto* (5), SA (4), MTB (4), MOTT (4), *E. coli* (4), *Kleb* (4)	*Pseud* (3), *Salmonella* (2), CMV (1), *S. maltophilia* (1), *S. epidermis* (1), HSV II (1)
100–200	19 (9.8%)	9 (13.6%)	10 (7.8%)	PCP (2), *Pseud* (2), SA (2)	CMV (1), yeast (1), enterococci (1), *Serratia* (1), *S. viridans* (1)
201–500	69 (35.5%)	7 (10.7%)	62 (48.4%)	*Aciento* (2)	*S. epidermis* (1), PCP (1), SA (1), HSV II (1), *E. coli* (1)
> 500	62 (32%)	13 (19.7%)	49 (38.3%)	SA (4), HSV II (2), yeast (2)	GBS (1), *S. epidermis* (1), *E. coli* (1)

Acinto: Acintobacter; CMV: *Cytomegalovirus*; *E. coli*: *Escherichia coli*; EBV: Epstein-Barr virus; GBS: group B streptococcus; HSV II: herpes simplex type II; *Kleb*: *Klebsiella*; MTB: *Mycobacteria tuberculosis*; MOTT: mycobacteria other than tuberculosis; PCP: *Pneumocystis carinii* pneumonia; *Pseud: Pseudomonas*; SA: *Staphylococcus aureus*; *S. epidermis*: *Staphylococcus epidermis*; *S. viridans*: *Streptococcus viridans*; *S. maltophilia*: *Stenotrophomonas maltophilia*

Table 3. CD4%, absolute CD4 count, and CD4/CD8 ratio in HIV-infected patients with each type of associated bacterial or fungal infection

Organism detected	Number	% in patients with infection (n = 66)	% in total HIV patients (n = 194)	CD4%	Absolute	Ratio	Site
S. aureus	19	28.7%	9.8%	14.3 ± 9.4	285.5 ± 313	0.26 ± 0.21	DTA 8, blood 5, sputum 2, pus 4, nose 2, ear 1
Yeast	18	27.2%	9.2%	9.42 ± 9.5	204.6 ± 425.3	1.3 ± 4.5	DTA 11, sputum 8, bronchial wash 1
S. epidermis	10	15.1%	5.1%	10.5 ± 9.8	111.8 ± 102.8	0.19 ± 0.25	Blood 10 (100%)
PCP	10	15.1%	5.1%	5.7 ± 5.4	85.9 ± 106.8	0.1 ± 0.13	Broncho-alveolar lavage 10
E. coli	9	13.6%	4.6%	10.1 ± 9	261.4 ± 428.6	0.16 ± 0.15	Urine 4, DTA 5 (2 ESBL)
MTB	7	10.6%	3.6%	12.2 ± 12.4	143.7 ± 243.6	0.24 ± 0.3	DTA 4, sputum 1, bronchial wash 2
Pseudomonas	7	10.6%	3.6%	10 ± 12.2	77.2 ± 68.9	0.25 ± 0.43	DTA 2, sputum 3, bronchial wash 1, pus 1, ear 1
Acintobacter	7	10.6%	3.6%	13.75 ± 9.7	178 ± 158	0.27 ± 0.2	DTA 3, bronchial wash 1, sputum 1, blood 1, urine 1, burn 1
Klebsiella	6	9%	3%	2.8 ± 3.3	18.7 ± 11.8	.03 ± 4.3	DTA 2, sputum 2, blood 1, urine 1, wound 1
MOTT	5	7.5%	2.5%	5.5 ± 6.9	155.8 ± 296.7	0.08 ± 9.7	DTA 2, sputum 2, bronchial wash 1
Salmonella	3	4.5%	1.5%	4.1 ± 5.1	9 ± 9	0.23 ± 0.25	Blood 2, urine 1, stool 1
Streptococci	Total: 7	10.6%	3.6%				
S. pneumoniae	1	1.5%	0.5%	11.5 ± 17.7	283 ± 440	0.53 ± 0.54	Blood 1
GBS	3	4.5%	1.5%				Blood 1, urine 1, pus 1
S. milleri	1	1.5%	0.5%				Pus 1
S. viridans	2	3%	1%				Pus 2
P. mirabilis	2	3%	1%	±	±	±	Wound 2
Enterococcus	2	3%	1%	12	139	0.16	Blood 2
S. maltophilia	1	1.5%	0.5%	2.05	56	0.02	Blood 1
***Citrobacter* sp.**	1	1.5%	0.5%	6	8	0.08	DTA 1, urine 1

DTA: deep tracheal aspirate; *E. coli*: *Escherichia coli*; ESBL: extended-spectrum beta-lactamase; GBS: group B streptococcus; MTB: *mycobacteria tuberculosis*; MOTT: mycobacteria other than tuberculosis; PCP: *Pneumocystis carinii* pneumonia; *S. aureus*: *Staphylococcus aureus*; *S. epidermis*: *Staphylococcus epidermis*; *S. milleri*: *Streptococcus milleri*; *S. pneumoniae*: *Streptococcus pneumoniae*; *S. viridans*: *Streptococcus viridans*; *S. maltophilia*: *Stenotrophomonas maltophilia*

Table 4. CD4%, absolute CD4 count, and CD4/CD8 Ratio in HIV-infected patients with each type of associated infection proved by positive serological tests

Organism detected	Number	% in patients with infection (n = 66)	% in total HIV patients (n = 194)	CD4%	Absolute	Ratio
CMV Ig G	41	62.1%	21.1%	15.1 ± 12.9	349.2 ± 414	0.95 ± 3.0
CMV IgM	1	1.5%	0.5%	±	±	±
CMV Ag	4	6%	2%	3.6 ± 1.6	55.6 ± 38.7	0.06 ± 4.35
CMV VL	1	1.5%	0.5%	2	10	0.03
EBV	18	27.2%	9.2%	9.91 ± 10.6	157.9 ± 216	1.3 ± 4.4
HSV II	6	9%	3%	13.6 ± 9.1	352.8 ± 290	0.24 ± 0.2
***Toxo* IgG**	16	24.2%	8.2%	14.6 ± 13.5	375 ± 383.8	0.33 ± 0.4
***Chlamydia pneumoniae* IgG (strong positive)**	1	1.5%	0.5%	20.3	177	0.34

CMV: *Cytomegalovirus*; EBV: Epstein-Barr virus; GBS: group B streptococcus; HSV II: herpes simplex type II; *Toxo*: *Toxoplasma*

Table 5. Comparison of data between a 2002 study and the current study

Organism detected	2002 study	Current study
Years of study	1997–1998	2009–2013
Age, years (Mean)	2–67 (26.7)	1–82 (48.3)
Number	67	194
Male	87.6%	81.4%
Female	12.4%	18.6%
CD4 < 100 cells/µL	31.3%	22.7%
CD4 100-200 cells/µL	7.5%	9.8%
CD4 201-500 cells/µL	37.3%	35.5%
CD4 > 500 cells/µL	23.9%	32%
Candidiasis	52.4%	27.2%
PCP	9.5%	15.1%
C. neoformans	4.8%	-
Pseudomonas	19.5%	10.6%
Salmonella	14.3%	4.5%
S. epidermis	14.3%	15.1%
Haemophilus influenzae	9.5%	-
Legionella pneumophila	4.8%	-
E. coli	4.8%	13.6%
S. aureus	-	28.7%

C. neoformans: *Cryptococcus neoformans*; PCP: *Pneumocystis carinii* pneumonia; *S. aureus*: *Staphylococcus aureus*; *S. epidermis*: *Staphylococcus epidermis*

Tables 3 and 4 show the different microbiological pathogens causing OIs in the HIV-infected patients. *S. aureus* was the most common bacterial pathogens observed among HIV patients (28.7% of all OIs and present in 9.8% of total HIV-infected patients), followed by *Candida albicans* (27.2% and 9.2%, respectively), *S. epidermis* (15.1% and 5.1%, respectively), *P. jirovecii* (15.1% and 5.1, respectively), *E. coli* (13.6% and 4.6% respectively) *Mycobacterium tuberculosis* (10.6% and 3.6%, respectively), and mycobacterium other than tuberculosis (MOTT) (7.5 and 2.5%, respectively). These results were slightly different from those of a previous study in Bahrain published in 2002, which showed rates of *Candida albicans*, *P. jirovecii*, and *Cryptococcus neoformans* to be 27.2%, 15.1%, and 4.8%, respectively. However, fungal infections were common infections in both studies [2].

The bacterial infections observed to be least frequently associated with AIDS patients were *Streptococcus pneumonia*, *Streptococcus milleri*, *Stenotrophomonas maltophilia*, and *Citrobacter* species. Herpes simplex II (HSV-II), known to cause genital disease, was isolated from 3% of the total HIV-infected patients and from 9% of HIV-infected patients with OIs. Previous infection with *Cytomegalovirus* was observed in 21.1% of the total HIV-infected patients and in 62.1% of the HIV-positive with OIs patients. However, *Cytomegalovirus* antigenemia was only present in 2% and 6% of patients, respectively.

There was no significant difference in CD4%, absolute CD4 count, and CD4/CD8 ratio between HIV-infected patients who had three or more associated infections and those who had two or fewer associated infections. Table 5 shows a comparison between the 2002 study and the current study.

Discussion

AIDS has become one of the leading causes of death since it was first recognized in 1981. The principal effect of HIV infection is the eventual destruction of the immune system, which renders the patients susceptible to OIs [2,12]. With increased and improved medical care of HIV-infected patients, the longevity and survival rate among those patients has markedly increased, and hence OIs can be detected more frequently [13].

In our study, OIs occurred in 66 out of 194 patients (34.3%). Male sex was a risk factor for both having HIV infection and for the occurrence of AIDS-associated OIs (male-to-female ratio was 5:1). This was not the case in other parts of the world, such as sub-Saharan areas of Africa [14]. This may be due to the increased incidence among males of unsafe sex and drug abuse compared to females in Bahrain and the late seeking of medical advice in male patients. Also, the risk of transmission of HIV from a male to a female through a legal relationship is low due to the implementation of a pre-marriage screening program that includes HIV. Another factor is that most of the foreign workforce in Bahrain is of male gender, which has a higher risk of HIV. However, this study focused only on Bahraini patients. At the same time, this male-to-female ratio was close to that found in nearby countries with similar social backgrounds, such as Oman, Egypt, Libya, Sudan, and Arab Maghreb [15,16].

In our study, there was a significant decrease in CD4% and absolute CD4 counts in HIV-positive patients with OIs compared to those without. The increased frequency of OIs with decreasing CD4 counts observed in our study indicates the predictive value of CD4 count for the development of AIDS and its important implication for acquiring OIs that usually complicates AIDS, as previously documented [17,18].

S. aureus infection was the most common OI observed in our study. It is one of the most common nosocomial infections and ranks among the most common causes of bacterial infections in HIV-positive patients. Most of the *S. aureus* bacterial isolates in our study were recovered from patients with invasive infections (68%). Eighteen out of 19 patients were male (94%), denoting the increased risk of *S. aureus* infection in males. Methicillin-resistant *Staphylococcus aureus* (MRSA) was present in only two cases (10.5% of total *S. aureus* isolates). Unlike the 2002 Bahraini study, which did not record any isolates with *S. aureus*, these bacterial isolates were the most prevalent bacteria in the current study [2]. This difference between the two studies could be related to the increased number of patients abusing drugs in the current study, the longer duration of HIV infection, or the change in antibiograms over the last 10 years. Senthilkumar *et al.* found that *S. aureus* infection among hospitalized HIV-positive patients was 16.5-fold greater than among HIV-negative patients [19]. However, Larsen *et al.* found that incidence of *S. aureus* infections among HIV-infected individuals declined but remained higher than that among HIV-uninfected individuals. *S. aureus* infection was more common among injecting drug users and patients with low CD4 cell counts [20]. *S. epidermis* was detected in 15.1% of our cases; all of them

originated from blood isolates. This could be the results of contamination, as *S. epidermis* is able to stick to intravenous feeding tubes and prosthetic devices so that it can get direct access to the bloodstream. Our findings match the results of the 2002 Bahraini study, which found that *S. epidermis* was present in 14.3% of cases. A similar finding was observed in a study done in Lagos between 2005 and 2006, where the researchers isolated *S. epidermis* from 5% of 140 HIV-infected patients [21].

E. coli caused 13.6% of the OIs in our study, compared to 4.8% in the 2002 Bahraini study. Forty-four percent of *E. coli* isolates came from urine samples, while 56% were isolated from deep tracheal aspirate (DTA). Twenty percent of *E. coli* isolates were extended spectrum beta-lactamase (ESBL) taken from DTA. Fifty percent of isolates received from patients with CD4 counts < 200 cells/μL. Chakraborty *et al*. showed that *E. coli* was isolated from 42% of HIV-infected patients [22]. In our study, ESBL *E. coli* were found in 20% of *E. coli* isolates, which may suggest their high multi-drug resistance (MDR) indices. This provides justification for continuous monitoring of bacterial susceptibility to antibiotics to reduce the emergence and spread of bacterial resistant strains [23].

In our study, *M. tuberculosis* and MOTT represented 10.6% and 7.5% of OIs, respectively. In the 2002 Bahraini study, all the isolated mycobacterium strains were from *M. tuberculosis* and represented 24% of the studied cases, all of them obtained from sputum culture. In 2011, there were 8.8 million new cases of TB, of which 1.1 million were among people living with HIV, with a risk 20–37 times greater than for healthy persons [24]. HIV infection is a particular risk factor for tuberculosis infection. Previous studies showed an increased rate of TB infection in HIV-infected patients that could reach up to 50%, and TB was considered to be one of the most common OIs associated with HIV infection [25]. HIV infection causes significant morbidity and mortality among patients with TB. Infection with HIV is the most powerful known risk factor predisposing for *M. tuberculosis* infection and progression to active disease, which increases the risk of latent TB reactivation 20-fold [26]. The observed relative decrease in TB incidence in HIV-infected patients in the current study compared to the 2002 Bahraini study may be related to the general decrease in TB incidence in Bahrain from 43/100,000 in 2004 to 18/100,000 in 2011. Also, the low incidence of TB in Bahrain when compared to other countries may be explained by low-intermediate TB endemicity in Bahrain [27].

In the current study, *Pseudomonas aeruginosa* was recovered from 10.6% of the isolates obtained from patients with OIs; 86% of them were recovered from respiratory samples. In the 2002 Bahraini study, there were fewer *P. aeruginosa* isolates, which were recovered from 19.5% of specimens. However, *P. aeruginosa* has been cited relatively infrequently as a respiratory pathogen in HIV-positive patients. Nevertheless, with improved medical care, earlier intervention, improved treatment, and improved survival among HIV-positive patients with increased longevity, the incidence of other respiratory pathogens including *P. aeruginosa* may increase [28,29]. *P. aeruginosa* infection is a complication of late-stage HIV disease with a mortality rate estimated to be between 22% and 36%. Neutropenia, indwelling catheters, and hospitalization may be associated risk factors of infection with *P. aeruginosa* [29-31].

In the current study; *Acinetobacter baumannii* was isolated from 10.6% of the specimens obtained from patients with OIs; 71% of them were recovered from respiratory samples, and 71% were associated with CD4 counts of less than 100 cells/μL. *Acinetobacter* is usually acquired as a nosocomial infection and may be accountable for a considerable morbidity risk in patients with HIV disease, especially in the presence of other risk factors. Hospitalization, other infectious complications, prior use of broad spectrum antibiotics, steroid treatment, neutropenia, indwelling intravascular catheter, and extended antimicrobial resistance patterns were probable risk factors [32,33].

In our study, *Klebsiella pneumoniae* was detected in 9% of cases with OIs; 66% of them were recovered from the respiratory tract. This is in contrast with the 2002 Bahraini study, which did not detect any *Klebsiella* species. *Klebsiella* is an important cause of pneumonia in more advanced HIV disease [34,35].

Salmonella species were detected in 4.5% of HIV-positive patients with OIs; 50% were detected in blood cultures, while 25% were detected in urine samples, and 25% were detected in stool samples. This was much less than what was detected in the 2002 Bahraini study, where the researchers detected *Salmonella typhimurium* in 14.3% of their cases. Recurrent *Salmonella* infection in an HIV-infected patient is considered to be diagnostic of AIDS. The decline in *Salmonella* from 14.3% in 2002 to 4.5% in our study may be the result of immune reconstitution and the direct bactericidal activity of the highly active antiretroviral therapy (HAART) on *Salmonella*

species; HAART had been provided to nearly 100% of our patients. The mean CD4 count was higher in our study than in the 2002 study [36]. Group B streptococci were recovered from 4.5% of cases with OIs. HIV infection is a well-known risk factor to increase the incidence of streptococcal infection in adults, pregnant women, and infants of HIV-infected mothers [37,38]. Other bacterial isolates infrequently recovered in our study included *Streptococcus pneumonia*, *Streptococcus milleri*, *Streptococcus viridans*, *Proteus mirabilis*, *Enterococcus* spp., *Stenotrophomonas maltophilia*, and *Citrobacter spp*.

In the current study, all the *Candida* specimens were isolated from respiratory tracts. *Candida* infection was present with different levels of CD4 reduction, even with normal CD4 counts (> 500 cells/μL). *Candida* infection can present even in early cases of AIDS. This is because HIV-1 infection of peripheral blood monocyte-derived macrophages (which are responsible for phagocytosis of opsonized *Candida albicans*) is unrelated to the level of CD4 expression on the surface of the cell [39]. Anwar *et al.* showed that the most common opportunistic fungal infection in HIV-positive patients was candidiasis, both mucocutaneous and invasive candidiasis [40]. The second most common type of fungal infection in the current study was *P. jirovecii.* Unlike candidiasis; *P. jirovecii* was present mainly in patients with low CD4 counts. About 62% of *P. jirovecii* infections were present in cases of CD4 counts of less than 100 cell/μL, which makes *P. jirovecii* a pathogen of continued interest and a public health threat to HIV-infected patients. Some studies showed that *P. jirovecii* was the most common fungal infection detected in HIV-infected patients. Jamaiah *et al.* showed that the most common HIV/AIDS related opportunistic infection among their studied Malaysian group was *P. jirovecii* (62.7%); 73% of those patients had CD4 counts of less than 200 cells/μL. This is because CD4 is essential for proper function of activated alveolar macrophages the eradication of *Pneumocystis* organisms [41]. In the current study, there were no *Cryptococcus neoformans* isolates, which was different from the 2002 Bahraini study [2].

Herpes simplex II (HSV-II), known to cause genital disease, was recovered from 9% of the studied cases with OIs. It was nearly about double that found in the 2002 Bahraini study (4.8%). Several epidemiological studies have demonstrated higher incidence and prevalence rates of HIV-1 among HSV-II-infected populations and vice versa [42]. Several studies have suggested a biological plausibility of HSV-II infection facilitating the acquisition and transmission of HIV-1 [43].

Previous infection with CMV was present in 62.1% of the studied cases with OIs, which is nearly the same percent found in the general population. However, CMV antigenemia were present in only 6% of cases with OIs. CMV antigenemia seems to be a useful diagnostic test for CMV-related diseases in HIV-infected patients [44]. CMV viremia is commonly found with advanced HIV disease and can present with severe HIV disease. Risk factors for death due to CMV-associated infection in HIV-infected patients include baseline CDC stage C, hemoglobin < 10 g/dL, lower CD4 count, and CMV viremia [45]. In our cases, CMV antigenemia was associated with CD4 counts of < 100 cells/μL.

When comparing the current pattern of OIs in Bahrain with the pattern in the 2002 study, there were considerable changes in the microbiogram. This could be explained by changes in demographic and social patterns, as well as by improving health care. The need for this study arose from the scarcity of information about OIs in HIV-infected patients in Bahrain as well as the Arab world. We hope that the results of this study will improve the awareness of physicians concerned with treatment of HIV-infected patients to develop an integrated and comprehensive medical care. However, this study has many limitations. The study was retrospective, with a wide age range in the studied group. The HIV viral load during the opportunistic infection was not documented. Also, we did not define the viral type of AIDS (HIV-1 or HIV-2) to see if there were any significant difference between the two types of infections and their relationship to OIs. There were no studies on the other risk factors that increase certain OIs in HIV patients, such as alcohol and drug abuse.

Conclusions

S. aureus infection was the most common OI observed in our study, followed by *Candida albicans*, *S. epidermis*, and *P. jirovecii*. Studying the pattern of OIs in HIV-infected patients in Bahrain is of paramount importance due to the scarcity of data in the Arab world. This information can help to improve the awareness of physicians concerned with treatment of HIV-infected patients.

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