



Common bacteria and their antibiotic sensitivity among patients with acquired immunodeficiency syndrome presenting with acute diarrhea

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Abstract

Acquired immunodeficiency syndrome is still a major cause of death in developed and developing countries. In present descriptive cross sectional study, frequency of common bacteria and their antibiotic sensitivity among 125 patients with acquired immunodeficiency syndrome presenting with acute diarrhea were evaluated. Male to female ratio was 0.69:1. Average age was 34.82 ± 12.87 years. *Escherichia coli* was found in majority of cases which was 62 (49.6%) of the study followed by *Campylobacter jejuni* 50 (40%), *Shigella* and *Clostridium difficile* 48 (38.4%) each and *Salmonella* in 45 (36%) patients. Maximum number of *E. coli* and *Salmonella* were found in age group less than or equal to 30 years, *Shigella* in age group more than 46 years, *C. jejuni* and *C. difficile* in age group 31-45. *E. coli* was sensitive in 90.3% patients to Co-Amoxiclav, and Cefixime each, in 71.1% patients *Salmonella* was sensitive to Ampicillin, *Shigella* was sensitive to Co-Amoxiclav in 70.8% patients, *C. jejuni* was sensitive to Ceftriaxone in 82.0% patients and in 77.1% patients *C. difficile* was sensitive to Ampicillin and Metronidazol each. This study will not only assist the patients in proper treatment but also discourage the indiscriminate usage of antibiotics and stop additional bacterial drug resistance development.

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Introduction

In 1983, Human Immunodeficiency Virus (HIV) was first isolated in a patient with Acquire immunodeficiency syndrome (AIDS). Presently more than forty million people are HIV positive in the world. AIDS is still a major cause of death in parts of Africa, South East Asia and other developing countries as the treatment is very expensive and majority of people in these countries cannot afford it (Omar and Shakil, 2007). Since the diagnosis of the first HIV/AIDS case in 1987, Pakistan has progressed to 3000+ cases nationwide. However, these numbers are likely to a vast underestimation of the actual picture. United Nations Acquired Immunodeficiency Syndrome (UNAIDS)/National Acquired Immunodeficiency Syndrome Control Programme (NACP) estimated 74000 HIV/AIDS cases in 2006 with a prevalence of 0.1% (National AIDS Control Programme, 2006). Despite of global struggles and advances for AIDS control, prevention and treatment, the number of patients infected with HIV is rapidly increasing as the mortality rate associated to this disease. In 2006, 4.3 million children and adults were confirmed as newly infected around the world (UNAIDS - AIDS epidemic update 2006). Due to defective immunity superimposed infections are a key medical issue among HIV positive patients. Intestinal infection by pathogens, which is the basic health issue in tropical regions, is mutual in these patients. Among the clinical complications of AIDS, diarrhea characterizes a great concern (Kumar *et al.*, 2005), because they are susceptible to both classic and opportunistic pathogens, malnutrition and eventually death can be related to this co-infections (Attili *et al.*, 2006). Moreover, annoying this situation, antibacterial chemoprophylaxis is usually given to the patient that causes destruction of gastrointestinal flora. Thus, diarrhea is a major reason of morbidity and mortality among HIV/AIDS patients. This is second most common diagnosis and in developing countries, it affects 90% of AIDS patients (Rossit *et al.*, 2009) and among HIV infected persons, diarrheal disorders of 40-80% have a distinguishable cause and common bacterial etiology (Chhin *et al.*, 2006).

There is still uncertainty about the choice of treatment among HIV/AIDS patients presenting with diarrhea as there is not only regional variation but also changing trends of microbial causes of such diarrhea in same population (Uppal *et al.*, 2009).

In fact, in developing countries, it is the second chief cause of hospital visits and holds its position in the top ten globally. Studies specify that in developed countries, 30-60% of AIDS patients diarrhea occurs and about 90% of such patients in developing countries (Attili *et al.*, 2006) there is need for more research on this subject (Rossit *et al.*, 2009). While HIV/AIDS and water-borne infectious diseases, such as diarrhea, are the main causes of morbidity and mortality in developing countries, their link has received only brief attention. Identification of the diarrheal agent in an AIDS patient is very vital as it can help in a suitable treatment and drop of morbidity and mortality in these patients (Uppal *et al.*, 2009) as diarrhea is still a prevalent health issue in HIV patients (Chincha *et al.*, 2010). Commonly involved organisms in HIV infected patients with diarrhea include *Salmonella* 2.5-18% (Kownhar *et al.*, 2007; Uppal *et al.*, 2009), *Shigella* 11% (Rossit *et al.*, 2007; Kownhar *et al.*, 2009) and 2.5% (Uppal *et al.*, 2009), *Vibrio cholera* 5% (Uppal *et al.*, 2009), *C. difficile* 12.5% (Uppal *et al.*, 2009), *E. coli* 70% (Rossit *et al.*, 2009), (30%) (Uppal *et al.*, 2009), (42%) (Chincha *et al.*, 2010) and *C. jejuni* 3.8-13.1% (Kownhar *et al.*, 2007; Rossit *et al.*, 2009). Most of them were found resistant to nalidixic acid, trimethoprim-sulfamethoxazole, ampicillin, erythromycin and quinolones (Cárcamo *et al.*, 2005).

The rationale behind doing this investigation is to conclude the local trends of common bacteria with their antibiotic sensitivity among HIV/AIDS patients presenting with acute diarrhea. As mentioned above, there can be regional variation and changing trends of microbial causes of acute diarrhea among HIV/AIDS patients and there is no local study available in recent last 5 years on the same problem. Also the statistics mentioned above are quite variable and the idea behind doing this study is to generate local statistics and disseminate the results among local health

professionals to aware them about regional trends of bacteria involved and planning the treatment strategy accordingly. The purpose of this study is to determine the frequency of common bacteria and its antibiotic sensitivity among patients with Acquired immunodeficiency syndrome presenting with acute diarrhea.

Materials and methods

Total of 125 consecutive patients with HIV/AIDS and acute diarrhea were studied in department of medicine, Lady Reading Hospital, Peshawar, Pakistan. Investigation of sequential patients was planned to escape any prejudice due to assortment of patients. Inclusion criteria of patients for this study were: (1) All HIV/AIDS patients presenting with acute diarrhea; (2) Adult patients above 18 years of age; (3) Either gender. The exclusion criteria included (1) History of antibiotic use in the past two weeks; (2) Patients with body mass index (BMI) of $> 25 \text{ kg/m}^2$; (3) Diabetics (on history and medical records). These mentioned conditions act as confounders and in case of inclusion, bias was introduced in the results.

Procedure of data collection

This study was conducted after approval obtained from hospital ethical and research committee. Patients meeting the inclusion criteria (i.e. presenting with HIV/AIDS and acute diarrhea) were included in the study through Out-Patient Department. The purpose and benefits of the study were explained to all patients and informed consent in written was obtained from all patients.

All patients were subjected to detailed history and clinical examination. From all patients a specimen of the stool were obtained in a sterile container and were

immediately sent to hospital laboratory for inoculation under various agar to detect the commonly involved bacteria i.e. *E coli*, *Salmonella*, *Shigella*, *C. jejuni* and *C. difficile*. The detected bacteria were subjected to antibiotic sensitivity testing against Co-Trimaxazole, Ampicillin, Ciprofloxacin, Co-Amoxiclav, Metronidazole, Ceftriaxone and Cefixime. The entire information with name, gender, age and address were recorded in a pre-designed proforma. All the laboratory investigations were done by single expert microbiologist. To control confounders and bias in the study, strictly exclusion criteria were followed.

Data analysis procedure

Data collected were analyzed in SPSS version 10.0. Mean + SD were calculated for quantitative variables like age. Percentage and frequencies were computed for categorical variables like gender and common bacteria (*E coli*, *Salmonella*, *Shigella*, *C. Jejuni* and *C. difficile*). Multiple response tools were utilized to stratification of common bacteria among gender and age to see the modification effect.

Results

In this study, 125 patients with AIDS presenting with acute diarrhea were studied, in which 51(40.80%) were male and 74(59.20%) were female. Male to female ratio was 0.69:1. Patients age was divided into three groups, out of which most presented with age of less than or equal to 30 years were 56(44.8%) while 40(37.9%) patients were in the age range of 31-45 years and 29(23.2%) were of age range of more than 50 years of age. The study included age ranged from 18 up to 65 years. Average age was 34.82 years + 12.87SD (Table 1).

Table 1. Age-wise distribution of patients.

AGE GROUP	FREQUENCY	PERCENT	CUMULATIVE PERCENT
≤ 30.00	56	44.8	44.8
31.00 - 45.00	40	32.0	76.8
46.00+	29	23.2	100.0
Total	125	100.0	

Distribution of common bacteria shows that *E. coli* was found in majority of cases which was 62(49.6%), followed by *Shigella* and *C. jejuni* in 48(38.4%) each and *Salmonella* in 45(36%) of patients (Fig. 1). Age wise distribution of common bacteria shows that *E. coli* was found in majority of the patients having age

less than or equal to 30 years which was 55.4% followed by 47.5% patients having age 31-45 years and more than 45 years of age with 41.4%. While almost all the other organisms were present in majority of patients having age less than or equal to 30 years (Table 2).

Table 2. Age-wise distribution of common bacteria.

Bacteria	Presence	Age (in years)		
		<= 30.00 %	31.00 - 45.00 %	46.00+ %
<i>E. coli</i>	Yes	55.4%	47.5%	41.4%
	No	44.6%	52.5%	58.6%
<i>Salmonella</i>	Yes	39.3%	32.5%	34.5%
	No	60.7%	67.5%	65.5%
<i>Shigella</i>	Yes	39.3%	35.0%	41.4%
	No	60.7%	65.0%	58.6%
<i>Campylobacter Jejuni</i>	Yes	33.9%	47.5%	41.4%
	No	66.1%	52.5%	58.6%
<i>Clostridium difficile</i>	Yes	37.5%	40.0%	37.9%
	No	62.5%	60.0%	62.1%

The majority of females i.e. 37(59.7%) presented with AIDS presenting with acute diarrhea have *E. coli* while 25 (40.3%) *E. coli* were found in male patients. Similarly 21(46.7%) *Salmonella* found in male and 24(53.3%) were found in female. There were 22(45.8%)

Shigella have found in male and 26 (54.2%) have found in female patients. There were 25(50.0%) *C. jejuni* have found in male and 25(50.0%) have found in female patients. At last *C. difficile* were found in male patients which were 20(41.7%) while female have 28 (58.3%) (Table 3).

Table 3. Common bacteria-wise distribution of gender.

Bacteria	Presence	GENDER			
		MALE		FEMALE	
		COUNT	%age	COUNT	%age
<i>E. coli</i>	Yes	25	40.3%	37	59.7%
	No	26	41.3%	37	58.7%
<i>Salmonella</i>	Yes	21	46.7%	24	53.3%
	No	30	37.5%	50	62.5%
<i>Shigella</i>	Yes	22	45.8%	26	54.2%
	No	29	37.7%	48	62.3%
<i>Campylobacter Jejuni</i>	Yes	25	50.0%	25	50.0%
	No	26	34.7%	49	65.3%
<i>Clostridium difficile</i>	Yes	20	41.7%	28	58.3%
	No	31	40.3%	46	59.7%

The antibiotic sensitivity of common bacteria shows that Co-Amoxiclav was more sensitive while Co-Trimaxazole was more resistant in different micro-organism. *E. coli* were sensitive in 90.3% to

Co-Amoxiclav, followed by Cefixime in 90.3% patients. The rest of antibiotics sensitivity and resistant has given in (Table 4).

Table 4. Antibiotic sensitivity of common bacteria.

Antibiotics	Sensitivity	<i>E. coli</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>Campylobacter Jejuni</i>	<i>Clostridium difficile</i>
	S=sensitive					
	R=Resistance					
		%age	%age	%age	%age	%age
Co-Trimaxazole	S	33.9%	37.8%	35.4%	40.0%	41.7%
	R	66.1%	62.2%	64.6%	60.0%	58.3%
Ampicillin	S	71.0%	71.1%	60.4%	66.0%	77.1%
	R	29.0%	28.9%	39.6%	34.0%	22.9%
Co-Amoxiclav	S	90.3%	64.4%	70.8%	78.0%	58.3%
	R	9.7%	35.6%	29.2%	22.0%	41.7%
Ciprofloxacin	S	51.6%	62.2%	62.5%	62.0%	66.7%
	R	48.4%	37.8%	37.5%	38.0%	33.3%
Metronidazol	S	66.1%	62.2%	58.3%	64.0%	77.1%
	R	33.9%	37.8%	41.7%	36.0%	22.9%
Ceftriaxone	S	59.7%	60.0%	60.4%	82.0%	60.4%
	R	40.3%	40.0%	39.6%	18.0%	39.6%
Cefixime	S	90.3%	57.8%	60.4%	74.0%	54.2%
	R	9.7%	42.2%	39.6%	26.0%	45.8%

Discussion

The problem of acute diarrhea in case of an immune-compromised host, who are given chemotherapy or immunosuppressive agents, or victims of HIV infection, malignancy, especially hematologic malignancy, autoimmune diseases, and acute graft-versus-host condition are a special entity (Asplund and Gramlich, 1998; Van Kraaij *et al.*, 2000). Occurrence of acute diarrhea in such patients can easily lead to septicemia, therefore, premature antibiotic usage during their course of diarrhea should be started, irrespective of the diarrhea type or severity (Yeomans *et al.*, 1991; Kraus *et al.*, 1991; Poulos *et al.*, 1994). Along with hemodynamic support, parenteral antibiotics are frequently required. Cytomegalovirus enteric infection may also cause bloody diarrhea in immune-compromised patients. Occurrence of both acute and chronic diarrhea in an HIV infected individual must give special care in terms of examination and management (Merlin *et al.*, 1996; Weber *et al.*, 1999).

HIV infected individual, who is not immune-compromised yet or his CD4 count >500 cell/mm³, may be managed as in the proposed algorithm. However, HIV infected individual who is already immune-compromised or having CD4 counts are <500 cell/mm³, along with routine stool culture and examination, modified trichrome staining, stool staining for AFB, modified AFB and assay for *C. difficile* cytotoxin must also be performed. Also enteropathogens and *M. avium* often cause bacteremia in immune-compromised individuals, therefore, blood cultures should be performed (Pont and Marshall, 1995). Empiric cure may be measured, if the causative enteropathogens nature is known (Ramakrishna, 1999).

Water is the basic need of human beings. It is considered as the source of life. But, sometime this water could cause infections, principally among immune-compromised individuals, such as HIV and AIDS victims (Arvelo *et al.*, 2010). Studies conducted in the Vhembe region of South Africa revealed that

the water reservoirs utilized by the local community were contaminated by both viral and potential enteric bacteria (Obi *et al.*, 2007). But, several people among them they do not have substitute water sources; therefore, they use the water with big risk involved. Additionally, the hygiene level among these people are very poor (Potgieter *et al.*, 2005).

These studies also indicated the importance of good quality drinking water to these HIV and AIDS infected individuals (Hall *et al.*, 2004; Obi *et al.*, 2006). Since low quality of water can cause several diseases, such as cholera, hepatitis, typhoid fever, giardiasis, dysentery and many more gastrointestinal infections in rural societies.

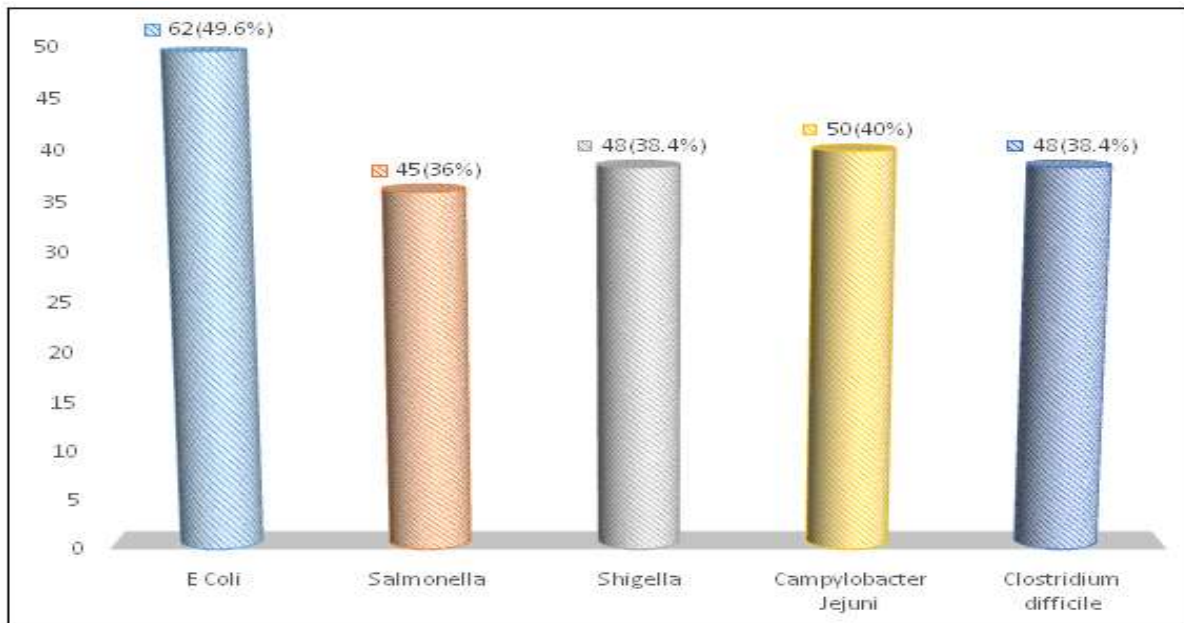


Fig. 1. Distribution of microorganisms in patients.

The scope of bacterial organisms isolated in this study was broad, *E. coli*, *Shigella*, *Salmonella*, *C. jejuni* and *C. difficile* accounting for most isolates. *E-coli* organisms were the most commonly-isolated organisms in the present investigation. Current studies have revealed the fact that these microorganisms were the cause of diarrhea outbreaks in an urban part of India (Saha *et al.*, 2009).

The study conducted in Nigeria, *E. coli* was the common organism isolated from the water source (22.7), followed by *Shigella* and *Klebsiella* (19.3%) each, *Proteus* spp. (18.5%), *Salmonella* spp. (13.3%), *P. aeruginosa* (4.2%) and *Enterobacter aerogenes* (2.5%) (Oluyeye *et al.*, 2009). In Italy, *Shigella* has been reported is the most common causative agent diarrhea outbreaks (Blasi *et al.*, 2008). Presence of *E. coli*, *Shigella*, *V. cholerae*, *A. hydrophila*, *Plesiomonas*, and *Campylobacter* spp. were also reported by Obi *et al.*, (2004).

Diarrhea is the characteristic of HIV/AIDS (Prasad *et al.*, 2000). In Africa, approximately 90% of HIV/AIDS victims are facing chronic diarrhea (Janoff and Smith, 1998).

The significant morbidity and mortality level due to diarrhea in developing countries, particularly in Africa, is compounded by the epidemic of HIV/AIDS. Although diarrhea is specifically linked with HIV infection, there is scarcity of data on the relationship of HIV infection and enteric bacterial pathogens in rural societies of the Limpopo Province of South Africa, in spite of the acknowledgement of HIV/AIDS as a major health issue (South Africa Department of Health, 2000). This diarrheal issue as a mutual complication of HIV infection, is additional complicate by the absence of drinkable water and absence of good hygienic practices in most rural societies of this Province. Local people these areas get the drinking-water from ponds, rivers, wells, and streams (Nevondo and Cloete, 1999).

These water reservoirs are contaminated faecally and lacking treatment, therefore, assist as a significant source of waterborne diseases transmission exemplified by diarrhea (Esru *et al.*, 19990; Nevondo and Cloete, 1999). About 30-90% HIV/AIDS patients experienced diarrhea, and variation in isolation frequency of aetiologic agents occurs with time and geography reportedly from 40-83% (Wasfy *et al.*, 2000). Acute diarrhea causes by bacteria are less common. Among the invasive bacteria, *Salmonella* is the common diarrheal cause in the United States (Laney and Cohen, 1993).

In case of infants (younger than 6 months) the *Salmonella* infections is more common (Cohen, 1991) and also among AIDS victims, reticuloendothelial dysfunction and sickle cell anemia, and it is cathead from contaminated foods products, especially dairy and poultry products and meat (Northrup and Flanigan, 1994).

Sero-positivity for HIV in this study was much more often in females (73.3%) as compared to males (26.7%), more women are infected than men (Ickovicas *et al.*, 1996). Women mostly infected with HIV through sexual contact (Ickovicas and Rodin, 1992), and it could be linked with imbalanced power between women and men, such as in the case of rape women are victims, and also it is very difficult for a woman to resist the unprotected sex by the male partner. This greater prevalence among females is serious because it upsets maternal morbidity and mortality rate and also the higher childbearing responsibility and rearing rests (Ross and Clark, 1996; Webb, 1996; Obi *et al.*, 1997a; Obi *et al.*, 1997b). Potential enteric bacterial agents were identified, *E. coli* 62 (49.6%) *C. jejuni* 50 (40%), *Shigella* and *Clostridium difficile* 48 (38.4%) each and *Salmonella* in 45 (36%) patients the study cohort. Maximum number of *E. coli* and *Salmonella* were found in age group less than or equal to 30 years, *Shigella* in age group more than 46 years, *C. jejuni* and *C. difficile* in age group 31-45. No attempt was made for fungal, viral and parasite isolation and the primary focus was on bacteria in this study.

Antibiograms determination in this study exhibited that the most susceptible isolate was *E. coli* to Co-Amoxiclav and Cefixime (90.3%). Degree of resistance and susceptibility of the enteric bacterial pathogens towards other antibiotics were highly variable. Same variability for enteric bacteria was documented previously (Black, 1993; Obi *et al.*, 1998). This susceptibility of the enteric isolates towards different antibiotics might be highly valuable in the diarrheal management especially in HIV patients having acute diarrhea. However, this study examined the potential pathogens for *in-vitro* susceptibility towards different antibiotics, and susceptibility of this kind may not essentially associated with real clinical situation.

The bacterial diarrhea management along with its complications require therapy with antibiotics in cethese antibiotics may contract their durations, reduction of stool output and revoke some problems (Black, 1993). The bacterial susceptibility profile towards antibiotic may be differ region to region and time to time (Obi *et al.*, 1998), therefore, it needs the episodic antibiotic susceptibility profiles updates and prevalence of enteric pathogens. These pathogens include species of *Plesiomonas*, *Campylobacter*, *Yersinia*, *Aeromonas*, *Vibrio*, *Shigella* and *Salmonella* (Black, 1993; Obi *et al.*, 1998; Obi *et al.*, 1997c).

Conclusion

Bacterial diarrhea and its complications in HIV/AIDS patients need antibiotic base therapy since antibiotics can condense the period of diarrhea, decline stool output, and repeal some complications. Susceptibility of several enteric pathogens isolated from AIDS victims having acute diarrhea towards several antibiotics were checked in this study. The pattern of resistance and susceptibility was variable of all the isolates towards different antibiotics. The bacterial sensitivity profile towards antibiotic may be vary from region to region and time to time, and regular updates about this sensitivity and prevalence of pathogens are very necessary.

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