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Study of urinary tract infections in patients on antiretroviral therapy

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Abstract

Introduction: India is estimated to have around 1.16 lakhs annual new HIV infections among adults and around 14,500 new HIV infections among children in 2011. The main site of attack of HIV is the immune system, especially the CD4 T lymphocytes (CD4 cells). People living with HIV are likely to be more predisposed to UTI due to suppression of their immunity. UTI in HIV positive individuals can lead to life threatening diseases and thus accurate and prompt detection of the uropathogens from the urine specimens of HIV positive individuals is expedient to enhance the health status. Thus this study was undertaken to determine the occurrences of different microbes in the aetiology of urinary tract infections in HIV positive patients on ART.

Material and Methods: Total 250 urine specimens were collected from HIV positive cases of clinically diagnosed to have urinary tract infection presenting to the ART center at GMCH Nagpur. Samples were processed and isolates identified by standard techniques.

Results: Out of the 250 urine specimens, 132(52.8%) were from the female patients whereas 118(47.2%) were from male patients. Out of the 250 urine specimens from cases having UTI taking ART 200(80%) urine specimens showed significant growth (Colony count ≥ 100 CFU/ml). In 50 (20%) the growth was not significant.

E. coli was the commonest organism isolated from HIV positive patients with UTI on ART. This was followed by *Klebsiella pneumoniae* 19.5%, *Pseudomonas aeruginosa* 13%, *Citrobacter freundii* 5%, *Acinetobacter baumannii* 12.5% among the gram positive organisms, 12% *Enterococcus faecalis*, 2% Coagulase Negative Staphylococci (CoNS) and 4% *Candida spp* were isolated.

In the control group, Gram negative bacilli were the commonest cause of UTI. Gram positive cocci were found in 20% of the controls. *E. coli* was the commonest organism accounting for 35% followed by *Klebsiella pneumoniae* accounting for 29%, *Pseudomonas aeruginosa* accounting for 16% and CoNS accounting for 20%.

Conclusion: In present study, HIV positive patients with low CD4 counts are infected with varied bacterial urinary pathogens compared with HIV seronegative with UTI. HIV positive patients with low CD4 counts were more frequently infected with bacterial urinary pathogens as compared to HIV positive patients with low CD4 counts.

Keywords: Urinary tract infection, antiretroviral-therapy

Introduction

India is estimated to have around 1.16 lakhs annual new HIV infections among adults and around 14,500 new HIV infections among children in 2011. The total number of people living with HIV in India is estimated at 21 lakhs in 2011 [1]. HIV infection results in the depletion of CD4 cells in the peripheral blood which has a pivotal role in cell mediated and humoral immunity. Once infected, the virus gradually and silently overpowers the host's defence mechanisms, resulting in opportunistic infections and cancers that are otherwise rare [2].

UTI is one of the secondary bacterial infections that occurs in HIV positive individuals with low CD4 counts. UTI is prevalent in patients with Human Immunodeficiency Virus (HIV) when compared to those without HIV, also people living with (HIV) are likely to be more predisposed to urinary tract infections due to the suppression of their immunity. Untreated UTI accounting for 7-60% of the opportunistic infections could be a source of ascending UTI and septicemia in immunocompromised hosts [3]. Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV.

Thus, this study was undertaken to determine the prevalence of UTI and the occurrence of different microbes in the aetiology of UTI in HIV seropositive patients on ART.

This pilot study aimed at studying urinary tract infections in patients on Anti-retroviral therapy. The objectives of the study was to isolate the bacterial pathogens from the urine specimens of the symptomatic patients attending the ART clinic of GMCH Nagpur, to identify the isolated organisms bacterial uropathogens causing UTI and to study the correlation between urinary tract infection and CD4 count in patients attending ART clinic.

Material and Methods

This is a descriptive study carried out in the department of microbiology at GMCH Nagpur over a period of 2 years. Institutional and ethical approvals were obtained.

Inclusion criteria

1. HIV positive patients with UTI who are on ART and willing to participate in the study.
2. HIV seronegative patients with UTI and willing to participate in the study.
3. Patients on ART having CD4 count less than $\leq 300/\mu\text{l}$ of blood.

Exclusion criteria

1. Patients less than 12 years.
2. Patients who are not taking ART regularly (Non-compliant).
3. Patients who are unwilling to register.

Collection of urine specimens ^[4,5]

Clean catch mid-stream technique was used to collect the urine specimens. The method is neither invasive nor uncomfortable. It is simple and can be performed in almost all clinical setting.

Transport ^[6]

Urine specimens were examined within 1 hour of collection. The well mixed samples were divided into 2 parts one which was used for the quantitative cell count and the other for bacteriological studies.

Methods of urine examination ^[6]

Screening tests

- **Gross examination of the urine:** Each urine specimen was noted for the quantity, colour, odour, clarity or turbidity ^[7].
- **Wet film examination (Uncentrifuged):** The finding of 1 leucocyte per 7 high power fields corresponds to 10^4 leucocytes per ml and the finding of clearly larger number than this indicates significant pyuria ^[6].
- **Gram stain ^{5a,5b}:** the presence of ≥ 1 or 5 bacteria per oil emersion field (OIF) correlates with colony count of 10^5 CFU/ml.
- Triphenyl tetrazolium chloride test ^[8]

Semi-quantitative culture: Standard loop method ^[9]

Identification of the uropathogens ^[9,10]

- Culture of the urine sample.
- Demonstration of motility by hanging drop preparation
- Gram stain
- Biochemical tests

Results

A Total of 250 cases comprising of HIV seropositive patients on ART having UTI and 100 controls comprising of HIV seronegative patients having UTI were included in the study.

A total of 250 urine samples from HIV seropositive patients on ART having UTI were included in the study group. Maximum number of urine samples were from the age group of 21-30 years followed by 31-40 years.

Maximum number of urine samples among males was from the age group of 21-30 years accounting for 48.30%, 31-40 years accounting for 22.88%, 41-50 years accounting for 18.64% and 51-60 years accounting for 5.93%. Among the females, maximum urine samples were from the age group of 31-40 years accounting for 40.90%, followed by 21-30 years accounting for 30.30% and 41-50 years accounting for 17.42% (Table 1).

Out of 250 urine specimens from cases of UTI receiving ART, 200 (80%) urine specimens showed significant growth i.e. colony count of >100 CFU/ml. However, in 50 (20%) of the urine specimens the growth was not significant (Table 2, Figure 1).

In present study, gram negative bacilli were found to be commonest cause of urinary tract infections 79% (158/200) in HIV patients on ART. Gram positive cocci was found only in 17% (34/200) of the cases and in 4% of cases *Candida spp* were isolated. *E. coli* was the commonest organism isolated 29% from cases of urinary tract infections on Anti-retroviral therapy. This was followed by *Klebsiella pneumoniae* 19.5%, *Pseudomonas aeruginosa* 13%, *Citrobacter freundii* 5%, *Acinetobacter baumannii* 12.5% (Table 3).

Amongst the Gram-positive organisms, 12% Enterococcus spp, 2% Coagulase Negative Staphylococci (CoNS), 3% *Staphylococcus aureus* and 4% *Candida spp* were isolated (Table 3).

In the present study, in the control group, Gram negative bacilli were the commonest cause of UTI. Gram positive cocci were found to be 20% of the controls.

E. coli was the commonest organism accounting for 35%, followed by *Klebsiella pneumoniae* accounting for 29%, *Pseudomonas aeruginosa* accounting for 16% and CoNS accounting for 20% (Figure 2, Figure 3, Figure 4).

Table 1: Age & sex wise distribution of cases of urinary tract infections in HIV seropositive patients on Anti-retroviral therapy (ART)

Age (Years)	Gender		Total (%)
	Males	Females	
≤ 20	5	5	10 (4%)
21-30	57	40	97 (38.8%)
31-40	27	54	81 (32.4%)
41-50	22	23	45 (18%)
51-60	7	10	17 (6.8%)
Total	118 (47.2%)	132 (52.8%)	250 (100%)

Table 2: Distribution of significant growth in urine specimens of HIV seropositive cases of UTI receiving ART therapy (N=250)

Total no of urine specimens	Urine specimens showing significant growth	Urine specimens showing insignificant / no growth
250 (100%)	200 (80%)	50 (20%)

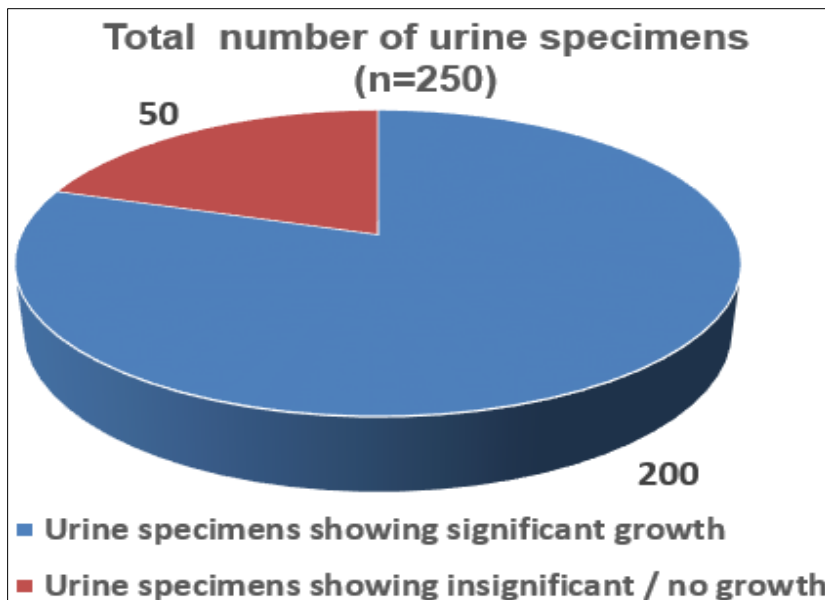


Fig 1: Total number of urine specimens of HIV positive patients on ART showing significant growth (N=250)

Table 3: Distribution of the uropathogens in HIV seropositive patients with UTI on ART therapy

Bacterial isolates	Number of isolates n (%)	Number of isolates in the control group (N=100)
Gram-negative isolates		
<i>Escherichia coli</i>	58 (29%)	35 (35%)
<i>Klebsiella pneumoniae</i>	39 (19.5%)	29 (29%)
<i>Pseudomonas aeruginosa</i>	26 (13%)	16 (16%)
<i>Citrobacter freundii</i>	10 (5%)	-
<i>Acinetobacter baumannii</i>	25 (12.5%)	-
Gram-positive isolates		
<i>Enterococcus faecalis</i>	4 (2%)	20 (20%)
<i>Coagulase negative Staphylococci</i>	6 (3%)	-
<i>Staphylococcus aureus</i>	8 (4%)	-
Fungus		
<i>Candida spp</i>	20 (100%)	100 (100%)
Total	200 (100%)	100 (100%)

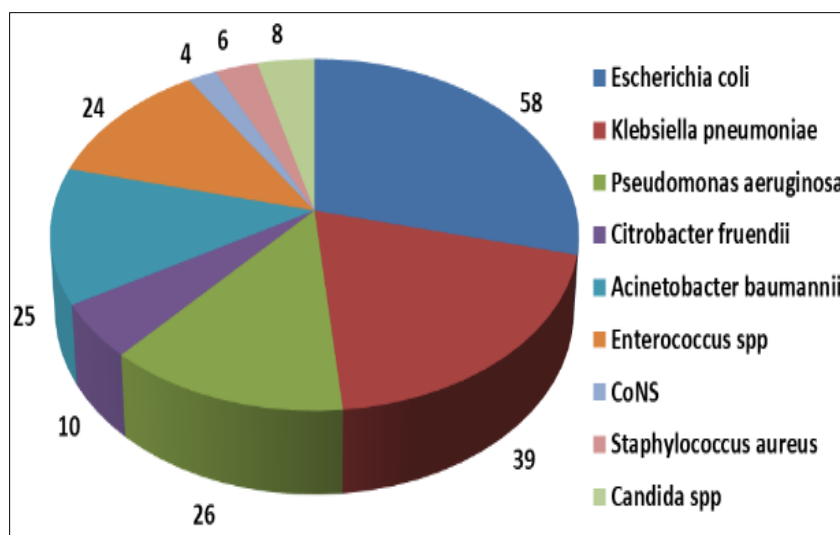


Fig 2: Distribution of the uropathogens in HIV seropositive patients with UTI on ART

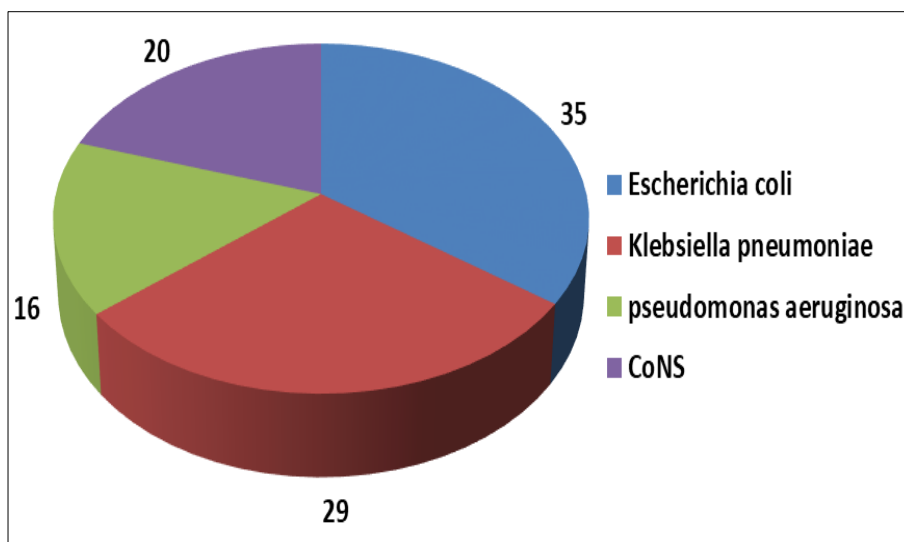


Fig 3: Distribution of the uropathogens in HIV seronegative patients having UTI

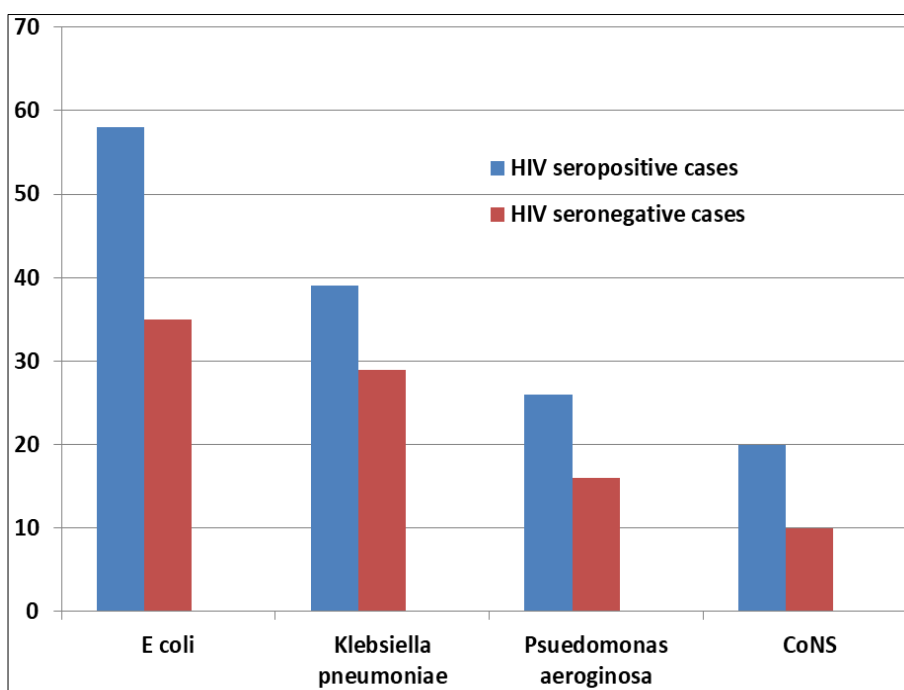


Fig 4: Number of uropathogens in HIV seropositive (Cases) and HIV seronegative patients (Controls) having UTI

Discussion

In the present study a total of 250 urine specimens from clinically diagnosed UTI cases who were HIV seropositive on ART were studied, out of these 118 (47.2%) were from males and 132 (52.8%) were from females.

The reason for this are the factors that predispose to bacterial adherence in females (compared to males) including a dry urethral meatus, a shorter urethrae, its proximity to the anus and its closeness to the external genitalia (i.e. the vagina) makes contamination of the female’s urinary tract system from the external genitalia almost unavoidable [11, 12].

Maximum number of urine samples was from the age group of 21-30 years followed by 31-40 years.

Among males, maximum number of urine samples was from the age group of 21-30 years accounting for 57 (48.30%), 31-40 years accounting for 27 (22.88%).

Among the females, maximum urine samples were from the age group of 31-40 years accounting for 54 (40.90%), followed by 21-30 years accounting for 40(30.30%).

In a similar study, Debalke *et al.* [13] (2013) found that the maximum number of HIV positive patients on ART having UTI were females in the age group of 25-34 years and males were in the age group of 15-25 years.

In a study by Samuel S.O, *et al.* [14] (2012), the mean age for having UTI in male HIV patients on ART was 35±9.5 years and 25±6.8 years for female HIV patients on ART.

Xavier *et al.* [15] (2015) reported that the number of males as well as female HIV patients on ART having UTI were in the age groups of 31-40 years.

In a study conducted by Kemajou, *et al.* [16] (2016), maximum number of HIV seronegative controls were in the age group of 24-30 years (30%) followed by 31-37 years (25%) which is similar to our findings.

In our study, out of the 250 urine specimens received, 200 (80%) showed significant growth. 50 samples showed insignificant or no growth.

In the study conducted by Kemajou, *et al.* [16] (2016) reported culture positivity in 57.31% urine specimens from HIV Bigwan EI, *et al.* [17] (2013), Rashmi KS *et al.* [18]

(2013), reported a culture positivity of urine specimens in HIV positive patients 23.5% and 14.44% respectively.

In present study *E. coli* was the most commonly isolated organism i.e. 58 (29%), followed by *Klebsiella pneumoniae* (19.5%), *Pseudomonas aeruginosa* 26 (13%), *Citrobacter freundii* 10 (5%), *Acinetobacter baumannii* 25(12.5%), *Staphylococcus aureus* 6(3%), Coagulase negative Staphylococci (CoNS) 4(2%), *Enterococcus faecalis* 24 (12%) in HIV positive patients having UTI on ART.

Similarly, studies conducted by Iwerboirm *et al.* [19] (2012) (70%), Samuel, *et al.* [14] (2012) (58.3%), found the predominance of Gram-negative organisms as compared to Gram positive organisms among the uropathogens isolated from HIV positive patients on ART.

In similar studies, by Inyang *et al.* [20] (2009), Iwerboir *et al.* [19] (2012), Gundala, *et al.* [21] (2017) the most common organism isolated was *E. coli* which was 84%, 17.92%, 66.7% respectively followed by *Klebsiella pneumoniae* which was 17.92% reported by Iwerboir *et al* and 16.7% reported by Gundala *et al.*

E. coli was demonstrated as the most common organism by Agersew *et al.* [22] (2013) (56.1%), Bigwan *et al.* [17] (2013) (9.6%), Debalke *et al.* [13] (2014) (54.3%), Fenta *et al.* [23] (2016) (49%), Banu *et al.* [24] (2017) (41.7%), Yadhav *et al.* [25] (2017) (22.5%), Olowe O.A *et al.* [26] (2015) (31.4%).

In present study *Klebsiella pneumoniae* constituted 19.5% of the uropathogens isolated, which was similar to the results in studies done by Iwerboir *et al.* [19] (2012) (17.92%), Ifeanyichukwu *et al.* [12] (2013) (22.67%), Gundala *et al* [21] (2017) (16.7%), Kemajou *et al.* [16] (2016) (13.9%).

In our study, we found *Pseudomonas aeruginosa* to be 13%, which was similar to the findings of Peterside *et al.* [27] (2013) who reported 9.1%, Banu *et al.* [24] (2013) who reported 13.1%, Gundala *et al.* [21] (2017) who reported 8.3% and Yadhav *et al.* [25] (2017) who reported 7.5% of the *Pseudomonas aeruginosa* isolates. In present study, *Citrobacter freundii* was 8 (4%). Similarly, in study conducted by Iwerboir *et al.* [19] (2012), Fenta *et al.* [23] (2016) reported 9.7% and 3.9% of the *Citrobacter spp* respectively from the urine specimens of the HIV seropositive patients on ART.

In present study, *Acinetobacter baumannii* were found to be 25 (12.5%). However, in studies conducted by Murugesh K *et al.* [28] (2014) and Fenta *et al.* [23] (2016) the isolated *Acinetobacter spp* from the uropathogens were less as compared to our study i.e. 1.66% and 1.9% respectively.

In our study, we found 6 (3%) *Staphylococcus aureus* isolates from the urine specimens of the HIV seropositive patients having UTI on ART.

However, in contrast to our study, Omoregie *et al.* [29] (2009), Etoh Inyang *et al.* (2009), Peterside *et al.* [27] (2013), Ifeanyichukwu *et al.* [12] (2013), Rashmi *et al.* [18] (2013), Murugesh K *et al.* [28] (2014) reported *Staphylococcus aureus* as the predominating uropathogen isolated from the urine specimens of HIV positive patients with UTI on ART i.e. 28.73%, 87.2%, 63.6%, 45.33%, 40%, 56.66% respectively. In present study, 4 (2%) CoNS were isolated which was similar to the findings of Debalke *et al.* [13] (2014) (4.4%) and Murugesh *et al.* [28] (2014) (1.66%).

In present study, 24 (12%) *Enterococcus faecalis* were isolated which was similar to the findings of Omoregei *et al.* [29] (2009), Murugesh *et al.* [28] (2014), Fenta *et al.* [23] (2016) who reported 10.34%, 15% and 13.7% of the *Enterococcus*

isolates respectively from the urine specimens of the HIV patients having UTI.

In present study, 8 (4%) of the *Candida spp* were isolated which is similar to the findings of Murugesh *et al.* [28] (2014), Esseini *et al.* [30] (2015), Samuel *et al.* [14] (2012) who isolated 20%, 10.6%, 7.7% of the *Candida spp* respectively. The reason for *Candida spp* isolation from urine specimens of patients on ART is that *Candida* is a common opportunistic pathogen during the course of human immunodeficiency virus (HIV) disease progression. Infections with *Candida* appear when the CD₄ count is 200-500 cells/ml and may be the first indication of immunodeficiency [31,32].

In present study, maximum number of isolates was Gram-negative organisms with a preponderance of *E. coli* (35%), followed by *Klebsiella pneumoniae* (29%) and *Pseudomonas aeruginosa* (16%) in the control group.

Among Gram-positive organisms 20% CoNS were isolated. In similar study by Kemajou *et al.* [16] (2016), *E. coli* (20.83%), *Klebsiella pneumoniae* (12.5%), *Pseudomonas aeruginosa* (29.16%) and *Staphylococcus aureus* (37.5%) were isolated from the control group. However, in this study, *E. coli* was predominating organism in the study group while *Staphylococcus aureus* was predominating organism in the control group. However, in present study, in both study group and control group most common organism isolated was *E. coli*.

Many different bacterial isolates were obtained from the urine specimens in the study group as compared to the control group because patients with immunosuppression have very high chances of developing bacteriuria. HIV positive individuals are at increased risk of opportunistic and common bacterial infections [16].

Conclusion

In present study, HIV seropositive patients with low CD₄ counts are more frequently infected with varied bacterial urinary pathogens compared with HIV seronegative with UTI. HIV positive patients with low CD₄ counts were more frequently infected with bacterial urinary pathogens compared with HIV positive patients who had higher CD₄ counts.

Urinary tract infection in HIV positive patients tend to recur, requiring longer treatments and it is suggested that treatment should be culture-specific. According to the findings of this study, the need for appropriate health education to reduce self-medication and drug abuse is very imperative and desirous.

Thus regular monitoring of uropathogens in HIV positive patients and their prompt treatment with ameliorate the problem in HIV positive patients with various opportunistic infections.

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