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# Isolation of pathogens from urinary tract infection in HIV patient and effect of turmeric extract as an anti-bacterial agent on it

**Dhananjay Dabir[1], Hamde Venkat[2]**
Department of Microbiology, Yogeshwari Mahavidyalaya, Ambajogai, Maharashtra, India

**Abstract**
HIV and Opportunistic infection are the two words which go parallel. HIV patients are immune-suppressed patients, so they are prone to many opportunistic infections like Tuberculosis, Meningococcal infections, Parasitic & Bacterial infections of stool, etc. Urinary Tract Infection is an important opportunistic infections amongst the HIV patients. Study include infection prevalence and cause of infection. To rule out Urinary Tract Infection, urine culture along with antibiotic sensitivity are important investigations. In selected HIV positive patients, we carried out urine culture and isolated organism in pure culture. Identification and antibiotic sensitivity test is performed by manual method. Total 115 specimens were tested and noted the following result; 55 (47.82%) of them shown bacterial growth while 58 (50.43%) no bacterial Growth while 2 (1.74%) are yeast. Organisms isolated are: *E. coli* in twelve cases (21.81 %) *Pseudomonas sp.* in five cases (9.09%), *Enterococcus sp.* in sixteen cases (29.09%) *Staphylococcus sp*. in six cases (10.90%) *Klebsiella sp.* twelve (21.81%) *Enterobacter spp.* four (7.27%). Antibiotic sensitivity of all bacteria done according to gram character HIV patients are already immuno compramised and treatment with antibiotics may further lower it. So we tried herbal, turmeric extract extracted by crude method, in different solvent to obtain its sensitivity pattern so it can be used to replace antibiotics.

**Keywords:** urinary tract infection, HIV, PUO, herbal, turmeric

## Introduction
Human immunodeficiency virus (HIV) cause an acquired immunodeficiency syndrome (AIDS) in human being, this is a spectrum of condition [1, 2, 3]. Initially symptoms are mild like a mild influenza [4]. A lag phase of no symptoms is followed by initial mild symptoms [5], this is window period. In further progress of disease it affect the immune system of patient and suppressed, and the individual becomes immuno compramised. This increase risk of opportunistic infection which include tuberculosis, as well as other opportunistic infection which do not affect a normal individual [4]. The spread of this disease is due to unprotected sex, contaminated body fluids, blood collection materials, from mother to child during delivery & breast feeding [6]. Saliva can also spread this disease [7]. To prevent maternal spread anti-retrovirus treatment is recommended [4]. Anti-retroviral treatment slow down the course of disease, there is no cure or vaccine for the disease, a normal life is experienced by the patient for a long time [5, 8]. Treatment should start immediately after confirm diagnosis. Eleven year survival period is observed without treatment with treatment one can leave for many years [9].
According to UNAIDS The global HIV & AIDS statistical update till 2020 shows the following facts; Globally 38 million peoples were living with HIV in 2019, 26 million were on antiretroviral therapy at the end of June 2020. New 1.7 million peoples were diagnosed with HIV in 2019. Approximately 6, 90,000 people died with AIDS related disease in 2019. 75.5 million Infected since start of epidemic. Till 2019, 32.7 million were died due to disease. Approximately 38 million people living with HIV, out of which 36.2 million adults & 1.8 million children with age upto 14 years. New HIV cases reduced by 40% since peak of 1998 & deaths reduced by 60 % since peak of 2004. In 2019 about 48% of all new case are women & girls. Tuberculosis is remain main cause of death in HIV patients, approximately one in three death.
In HIV & AIDS the common threat is opportunistic infection which include; Tuberculosis & other opportunistic infections like meningitis, GI tract infection, other parasitic infection. One most skipped common infection by clinicians is Urinary tract infection in these patients. A Urinary tract infection means presence of pathogenic microorganisms in urine of an individual, with greater or equal to $10^5$ colony forming units/microliter. In community Urinary tract infection is most common in world with E.coli is most common bacteria.
An antibiotic sensitivity (Susceptibility) is testing of antibiotic for its sensitivity against the pathogenic microorganism, in this case uropathogens, which gives idea of treatment to clinicians. In this study Urinary tract infection in HIV patients are diagnosed and tested for antibiotic sensitivity to help clinician & patients. As patient is immunosuppressed, the use of antibiotics still further effect immunity. Many researcher tried extract different herbs & plant parts against urinary tract infection successfully. On medicinal plants world's 80% population relies, India have a rich tradition of herbal medicine against various infectious diseases, injuries, inflammation, & other diseases, treated with herbal medicine in India. A good efficacy of plant & herbal medicine is

noticed, also these medicines are much cheaper than modern medicines available [12]. We also used crude extract of turmrric In different solvent and tested against the different bacterial uropathogens isolated, for its sensitivity, and to replace antibiotics if possible. Turmeric is a common food ingredient in world which is available in every Kitchen. It is mostly used for its aroma & included in spice. For every preparation like vegetable, baking, curry, serials, fast foods, rice, non-vegetarian food cooking, almost in all Type of cooking it is used widely. It belongs to ginger family Zingiberaceae and product of Curcuma longa. Turmeric tuber is a juicy tuber from which juice can be extracted easily (Fig 1). The extract of turmeric is curcumin which is used in many herbal medicine as an anti-microbial & anti-inflammatory. We also tested turmeric tuber extract (juice) to check its usefulness against uropath isolated from HIV patients.

## Method and Material

Confirm diagnosed HIV patients selected for study and details of the patient noted. A morning midstream urine sample of patient is collected in a sterile container with proper aseptic precautions. Collected sample inoculated on Nutrient agar, Mac Conkey's agar, for isolation and primary identification by colony characters, Gram's stain, and motility. Isolated bacteria identified by streaking on HI chrome UTI agar, different biochemical like IMViC, Catalase, Oxidase, Urease, different sugar fermentation.

Sensitivity test is carried out on isolated strains with selected antibiotics with reference to CLSI & clinician's choice. Sensitivity is carried out by preparing a 0.5 Mc farmland suspension of isolate & spreading evenly it on Muller Hinton agar by sterile cotton swab. Selected pre-soaked sterile antibiotic discs for Gram positive, Gram negative & pseudomonas spp. are used (Kirby Bauer method). Results of sensitivity noted next morning.

As we earlier mentioned these patients are immunosuppressed & antibiotic still lower the immunity, for that we tried turmeric a common ingredient used in food, to replace antibiotics We prepared extract by dissolving the powder of turmeric in Distilled water (10% w/v) by heating to boil till reduced to half quantity & then continuous agitating at 150 rpm for 24 hrs. Also methanol, ethanol, acetone solvent is used in which 10% w/v powder of turmeric dissolved by continuous agitation at 150 rpm for 24 hrs. In a screw tight bottle. A fresh juice of turmeric tuber (Fig1) is also prepared, for this first the tuber is washed with 0.5% mercuric chloride to disinfect it and followed by sterile distilled water wash, then we crushed this tuber to extract the juice. These all then filtered through Whatman filter paper No 1 to recover final solution. In these solutions sterile discs from Hi-media is socked & dried at $60^0$C. These discs were then used instead of antibiotic to obtain sensitivity pattern. Representative sample from each strain is used & also MDR strains. Observation noted & compared.

**Fig 1:** Turmeric tuber

## Results

### Isolation of pathogens from UTI Patient.

Total 115 pathogens isolated from HIV patients suffering from Urinary Tract Infection. Morphological, cultural and some biochemical characters were studied and tentatively identified up to genus level. These cultures periodically subcultured on Nutrient Agar slants.

### Preparation of turmeric extract.

About 10.00 g of dried powdered was mixed 100 ml each of distilled water, acetone, methanol, and ethanol in flasks separately and kept at room temperature for 24 hrs. During this period, shaking of the flasks was performed regularly. The solvent soluble compounds were filtered using double Whatman filter paper (Whatman™).

Tumric tuber is disinfected with 0.5% mercuric chloride & then washed with sterile

Distilled water. This washed tuber then crushed &extracted. Extracted tuber juice then filtered & used

### Antimicrobial activity of turmeric extract by disk diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of turmeric was carried by disc diffusion assay as described in Bauer *et al* [12]. Briefly, for disc diffusion assay, Mueller Hinton agar plates evenly inoculated all over area of plate with a sterile cotton swab with the respective test microorganisms. Antibiotc disc from Hi media & discs prepared from turmeric extract & turmeric tuber are placed in differnt plates aseptically. The plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zones (in mm) were measured. Going through all method & observation w noted following Results. 3.4 Total 115 HIV patients tested for Urinary tract infection 55 (47.82%) patients shown positive for bacterial growth while 58 (50.43%) Negative for growth. In two patient Candida spp. growth Detected (3.63%). As shown in Chart No.13.5 out of 55 positive isolates we identified *Enterococcus spp*. 16 (29.09%), *E.coli* 12 (21.81%), *Klebsiella spp*. 12 (21.82), *Staphylococci spp*. 06 (10.90%), *Pseudomonas spp.* 05 (9.09%), *Enterobacter spp*. 04 (7.27%). As shown in chart No. 23.6 All strains shown good sensitivity except one MDR *Staphylococcus aurius.*

3.7. Gram negative shown good sensitivity to Amoxycillin/Clauvanic acid, Meropenum, Amikacin, Piperacillin/Tazobactum. Gram positive sensitive to Meropenem Amoxycillin/Clauvanic, Nitrofurantoin.

3.8. To avoid administration of antibiotics to these immunosuppressed patients we tried representative sample from each stain for sensitivity against turmeric extract in different solvent; Water, Acetone, Ethanol & Methanol along with blanks. Also extracted juice from fresh turmeric tuber (Un dried) & used. Unfortunately we did not get any sensitivity to any bacteria tested. We found resistance in all isolates tested with these extracts. (Fig 2).

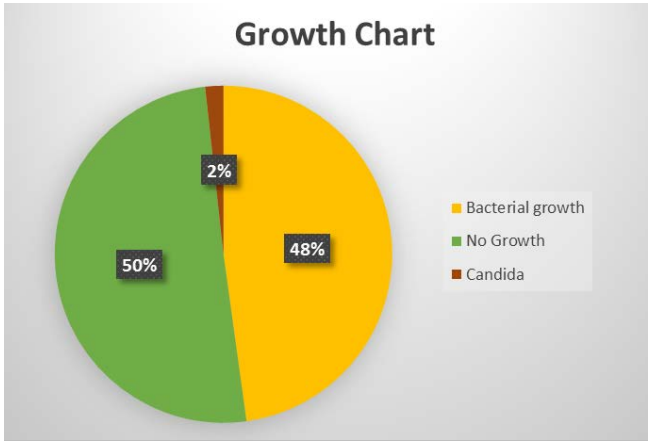


**Fig 2:** Showing Growth distribution

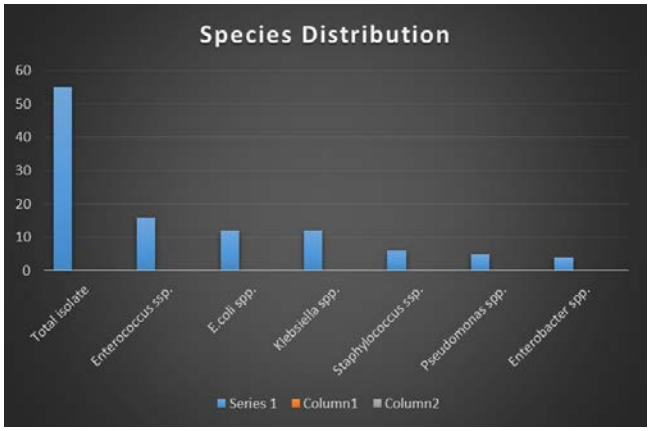


**Fig 3:** Showing species identified

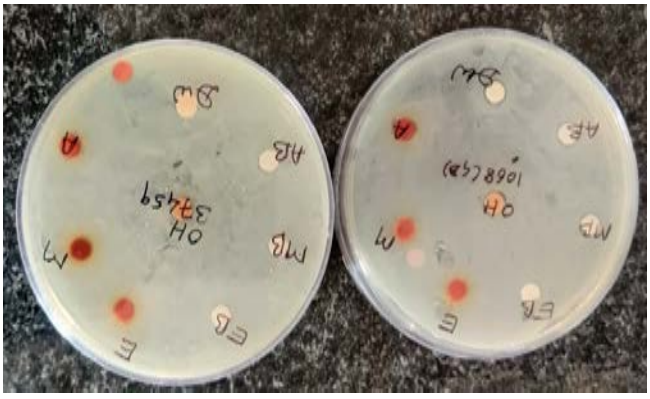

**Fig 4:** MH agar plates showing resistsnt to turmeric extract.

The letter on plate denotes E; Ethanol extract of Turmeric, M; Methanol extract of turmeric, A; Acetone extract of turmeric, DW; turmeric extract in distilled water OH; turmeric tuber EB; Ethanol blank, MB; Methanol blank, AB; Acetone blank.

## Discussion

Tchounga S Kemajou, Anslem O Ajugwo *et al* tested 246 HIV patient for UTI & out of these 141(57.3%) shown positive report [10] while study by Schonwald *et al*. observe 41% Positive infection among 96 patients. Francis Xavier, *et al* carried out semilar study at Karur District of Tamil Nadu, India, Out of 40 HIV positive patients they detected 31positive for UTI.[11] In this project we also tested 115 HIV patients for UTI and the observation is; out of which 55 (47.82%) shown positive growth while 58 (50.43%) no growth, 2 (3.62% ) sample shown growth of Candida spp. *Enterococcus spp* is prominent of all (29.09%) while *E.coli*,& *Klebsiella spp*. (21.82% each) *Staphylococcus spp*.(10.90%), *Pseudomonas spp.* (9.09), *Candida* (3.63%). All these shown good sensitivity to the antibiotics tested, except *Staphylococcus aureus.* Even after good sensitivity we used turmeric extract to check sensitivity against it. We selected representatives sample strain of each species & checked sensitivity against turmeric extract in different solvent. Unfortunately not a single strain shown sensitivity against turmeric extract, all shown resistance. The extract in, water, acetone, ethanol, methanol is prepared by Simple dissolving turmeric powder in solvent as mentioned earlier, is a crude method applied which may have affected the results. A fine scientific method of extraction or extraction of curcumin may produce better results. So our trials will be continued with better extraction method & also with some other foods of medicinal value

## Conclusion

The HIV patients shown a significant no. of urinary infection in them with or without any symptoms. They are sensitive to antibiotics for which they were tested. The crude turmeric extract & turmeric tuber extract do not have any anti-bacterial effect on the isolated uropathogens, so, the extract turmeric & turmeric tuber cannot replace antibiotics when they are extracted by a crude method. Our research does not end here, but it opens the scientific window to try for a more scientific methods of extraction curcumin in much more pure & concentrated form. Further studies are needed using different food ingredient with medicinal values.

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