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ANTI-BACTERIAL EFFECT OF INDIVIDUAL DRAVYAS OF MUTRAVIRECHANIYA MAHAKASHAYA ON E. COLI, KLEBSIELLA AND STAPHYLOCOCCI VIS A VIS URINARY TRACT INFECTION – IN VITRO STUDY.

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Abstract

An infection in any part of urinary system i.e., Kidney, bladder or urethra is called as urinary tract infection. UTI may be asymptomatic or symptomatic. Thus, the term Urinary tract infection encompasses a variety of clinical entities, including asymptomatic bacteriuria, cystitis, prostatitis and pyelonephritis. The Most common pathogenic organism in UTI is **E. Coli (in 75-90 % of cases)** followed in decreasing frequency of **Klebsiella, staphylococci.** Most of the bacteria enter the urinary tract from the fecal reservoir via ascent through the urethra in to the bladder.

Objectives - To evaluate the efficacy of individual Dravyas of Mutravirechaniya Mahakashaya on isolated samples of E. coli, Klebsiella and staphylococci by **disc diffusion method**.

Methods - The urine samples of patients having the pus cells more than five including other inclusion criteria have been collected for culture test and among that isolated sample of E. Coli, Klebsiella,

Staphylococci were taken for sensitivity test by **disc diffusion method,** with disc prepared by Kashaya of individual Dravyas of Mutravirechaniya Mahakashaya.

Result – The Sensitivity test conducted with Mutravirechaniya Mahakashaya Dravyas individually on isolated samples of E. coli, Klebsiella and Staphylococci were **statistically not significant.**

Conclusion – The present study the Kashaya of Mutravirechaniya Mahakashaya Dravya has prepared by general rule of Kashayavidhi i.e., boiling the Kwatha Dravyas in sixteen times of water and reducing it into one by eighth. Each disc has made to absorb 10 mMl of Kashaya and allowed to dry. These discs were taken for sensitivity test. By changing the concentration levels of Kashaya preparation, the better result may obtain.

Key words – E. coli, Klebsiella, Staphylococci**,** Mutravirechaniya Mahakashaya, UTI, Disc diffusion method.

Introduction

Infectious diseases have increased to a great extent during the recent years, and they are the second leading cause of death across the world and third leading cause of death of economically developed countries.[1] Urinary tract infections (UTIs) have plagued mankind long before bacteria were recognized as the causative agents of disease and before urology became an established medical specialty. The discovery of microorganisms as the etiological agents of infectious diseases in general and inflammation associated with urinary diseases in particular provided an impetus for physicians critically to examine management approaches and develop evidence-based strategies for UTI treatment. Most of the bacteria enter the urinary tract from the fecal reservoir via ascent through the urethra in to the bladder. These organisms usually originate in the bowel flora, and it makes way to urinary tract. E. coli is the most common causative organism for the urinary tract infection.[2]

Escherichia coli [3]

E. coli is the most common causative organism for the urinary tract infection. In fact, 75% to 90% of urinary tract infections are caused by E. Coli. Women are particularly at risk for UTI because of their urethra lies close to anus where E. coli present. Common ways that bacteria enter Urinary tract include,

Wiping back to front can carry E. Coli from the anus to the urethra.

Birth control measures: - contraceptive like, diaphragms and spermicidal condoms can kill the healthy bacteria in body that protect from bacteria like E. coli. The bacterial imbalance will cause susceptible to UTI. Nonoxynol -9 in spermicides spermicide is a toxic to the normal vaginal lactobacilli and thus is likewise associated with an increased risk of E. Coli vaginal colonization and Bacteriuria.

Klebsiella[4]

The Klebsiella/ Enterobacter proteus species from heterogeneous group of gram-negative lactose, fermenting, encapsulated, non-mobile bacilli. It normally lives in intestine and faeces. These bacteria are harmless if they reside in intestine. But if they spread to another part of, they can cause infection. Typically, K. pneumonia cause urinary tract infection in women.

- Factors influence Infection by Klebsiella,
  - Taking antibiotic for a long time.
  - Taking corticosteroids
  - Being hospitalized
  - Using an intravenous or ureter catheter

Staphylococcus bacteria [5]

Staphylococcus saprophyticus is a gram positive, coagulase, non- hemolytic coccus that is the common, cause of uncomplicated urinary tract infection. Particularly in young sexually active females. Less commonly it is responsible for pyelonephritis, urethritis, epididymitis and prostatitis. It is the part of normal human flora that colonize the perinium, rectum, urethra, cervix and gastrointestinal tract. It has also been found that S. saprophyticus is a common gastrointestinal flora in pigs and cows and thus may be transferred to human through eating the respective food.

MATERIALS AND METHOD

The urine sample of patient who is having pus cells more than 5 was collected and send it for culture text in microbiology department of KR hospital for the identification of microorganism causing the infection.

Among that 10 isolated samples of each E. Coli, Klebsiella, and Staphylococci has taken for sensitivity test, and the sensitivity test was conducted in laboratory of Government Ayurveda College and Mysuru.

Drug source Materials

The *Kashaya* of individual drugs and *Mutravirechaniya Mahakashaya* has prepared and made to absorbs in a filter paper and later it was cut in to the shape of discs. These discs were used in determining sensitivity towards the isolated samples of bacteria.

The ingredients of *Mutravirechaniya Kashaya* are,

Vrikshadani, Swadamstra, Vasuka, Vasira, Pashanabheda, Darbha, Kusha, Kasha,Gundra And Ithkatamoola

B) Methodology

Study design - In Vitro

Sampling method – Purposive sampling

Sample size – 10 isolated culture media of each E. Coli, Klebsiella, Staphylococcus has taken.

Sample collection Method: - The urine samples of patients having the pus cells more than five including other inclusion criteria have been collected for culture test and among that isolated sample of E. Coli, Klebsiella, Staphylococci were taken for sensitivity test with *Mutravirechaniya Mahakashaya* in disc diffusion method.

Disc diffusion method -Culture and sensitivity method adopted in this study

Disc diffusion method – Definition [6]

Disc diffusion method**,** also known as Agar diffusion test. In diagnostic laboratories the test is performed by inoculating the surface of agar plate with bacteria isolated from patient infection. Antibiotic containing paper disc are then applied to this agar plate and incubated for twenty-four hours.

Method of Disc Diffusion

If an antibiotic disc stops the bacteria from growing or kills the bacteria, there will be an area around the disk, where the bacteria not grown enough to be visible. This is called zone of inhibition. The susceptibility of the bacteria isolated to each antibiotic can then be semi quantified by comparing the size of these zone of inhibition it has been estimated that the antibiotic is Moderately susceptible, Antibiotic suspectable, Resistant suspectable. In this study instead of Antibiotic disc, Disc prepared out of individual Dravyas of *Mutravirechaniya Mahakashaya* has used.

Steps that have followed during the study

Step 1 -Preparation of culture media.

Simple media (basal media)[7]

It consists of peptone, meat extract, sodium chloride and water. Nutrient agar, made by adding 2% agar to nutrient broth is the simplest and most common medium in routine diagnostic laboratories. If the concentration of agar is reduced to 0.2 – 0.5%, semisolid or sloppy agar is obtained which enables motile organisms to spread. Increasing the concentration of agar to 6% prevent spreading or swarming by organisms such as proteus.

Step 2- Streak culture [8]

This is also known as “surface plating”. It is the most commonly employed method for the isolation of bacteria in pure form from any specimen.

One loopful (Nichrome wire) of the specimen is transferred onto the surface of a suitable medium in a petri plate and spread evenly over a small area. This is known as primary inoculation.

A series of parallel lines are drawn or streaked from this primary inoculation and from there on

in different segments.

The loop is flamed and cooled in between the segments.

Plates are incubated at 37º C.

Growth is confluent at the site of primary inoculation, become streaks thinner and as discrete colonies on the final streaks.

Step 3- Sensitivity test with disc of Mutravirechaniya Mahakashaya

The *Kashaya* with individual Dravyas of *Mutravirechaniya Mahakashaya* has made to absorbs in disc of filter paper. These discs were used in determining sensitivity.

Discussion on observation

Discussion on Bacteria identified in the culture test

Table 01 - Distribution based on bacteria identified		
Bacteria identified	**Frequency**	**Percentage**
E. coli	10	33.3%
Klebsiella	10	33.3%
Staphylococcus	10	33.3%
Total	30	100,0%
PEARSON CHI- SQUARE TEST - .001 SIGNIFICANT		

In the present study E. Coli (33.0%) Klebsiella (33.0%) and staphylococci (33.0%) these are the main micro-organisms observed with *p* value of 0.001. It indicates that, these are the common causative micro-organism for UTI. Hence these sample were selected for the sensitivity study.

Discussion on Sensitivity of individual *Dravyas* of *Muttravirechaniya Mahakashaya*, towards the isolated samples of Bacteria.

Table No. 02 – Sensitivity of Individual *Dravyas* of *Mutravirechaniya Mahakashaya* on isolated samples of Bacteris

Mutravirechaiya Dravyas	**Observation on Sensitivity (%)**			**Result**
	E. Coli	**Klebsiella**	**Staphylococci**	
Vrukshadani	30.0%	20.0%	10.0%	*P* = .731
Swadamstra	40.0%	30.0%	10.0%	*P* = .574
Vasuka	40.0%	20.0%	10.0%	*P* = .720
Vashira	20.0%	20.0%	10.0%	*P* =.720
Pashanabheda	10.0%	20.0%	20.0%	*P* = .519
Darbha	20.0%	20.0%	10.0%	*P* =.519
Kusha	10.0%	10.0%	10.0%	*P* = .252
Kasha	10.0%	10.0%	10.0%	*P* = .252
Gundra	10.0%	10.0%	10.0%	*P* =. 630
Itkatamula	20.0%	30.0%	20.0%	*P* = .521

Out of 10 isolated samples of E. coli, 3(30.0%) samples were sensitive to *Vrukshadani*, 4(40.0%) samples were sensitive to *Swadamstra*, 4(40.0%) Samples were sensitive to *Vasuka*, 2(20.0%) samples were sensitive to *Vashira*, 1(10.0%) sample was sensitive to *Pashanabheda*, 2(20.0%) samples were sensitive to *Darbhha*, 1(10.0%) sample was sensitive to *Kusha*, 1(10.0%) sample was sensitive to *Kasha*, 1(10.0%) sample was sensitive to *Gundra* and 2(20.0%) samples were sensitive to *Itkatamula*.

This indicate that out of 10 drugs of *Mutravirechaniya Mahakashaya*, E. coli is more sensitive to *Swadamstra*(40.0%), *Vrukshadani*(30.0%) and Vasuka (40.0%). This suggests that these drugs will be more effective in diagnosed cases of UTI with E. coli infection.

Out of 10 isolated samples of Klebsiella, 3 samples of Klebsiella were sensitive to *Vrukshadani,* 3(30.0%) samples were sensitive to *Swadamstra,* 2(20.0%) samples were sensitive to *Vasuka, Vashira, Pashanabheda,* and *Darbha*. 1(10.0%) sample was sensitive to *Kusha, Kasha* and *Gundra*. And 3(30.0%) samples were sensitive to *Itkatamula*.This suggests that Klebsiella is more sensitive towards *Swadamstra* and *Itkatamula* (30.0%). And these drugs will be more effective in diagnosed cases of UTI with Klebsiella infection.

Out of 10 isolated samples of Staphylococci, only 2(20.0%) samples showed sensitivity to *Pashanabheda* and *Itkatamula.* Remain all drugs were showed sensitivity to only 1(10.0%) sample of staphylococci.This suggests that *Mutravirechaniya Dravyas* are having reduced sensitivity over the Staphylococci bacteria compared to E. coli and Klebsiella

Conclusion

The present study the Kashaya of individual Dravyas of Mutravirechaniya Mahakashaya has prepared by general rule of Kashayavidhi i.e., boiling the Kwatha Dravyas in sixteen times of water and reducing it into one by eighth. Each disc has made to absorb 10 mMl of Kashaya and allowed to dry. These discs were taken for sensitivity test. The result obtained was statistically not significant. By changing the concentration levels of Kashaya preparation, the better result may obtain. Evidence based medicine gains prime importance. *Acharya Charaka* has told that, an intelligent physician will treat the disease with the help of single drug and for physician with less intelligence (*Alpabudhi*) use of multi drug for treating the disease is ideal. Considering the same, culture and sensitivity test has done in urine samples having pus cells more than five, to identify and isolate the organism causing UTI. The sensitivity test was conducted with individual as well as all the ten drugs together of *Mutravirechaniya Mahakashaya* towards the isolated sample of bacteria. By that the physician can easily asses the most effective single drug from out of these ten.

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