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In Vitro Study Of Antibacterial Properties Of Shatvaryadi Kwatha In Urinary Tract Infection By Culture And Sensitivity Method.

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Abstract: Now a day due to sedentary lifestyle health status of the individual is rapidly going down which resulting in low immunity that's why there is more tendency of individual to get infected. For to get relief from infectious diseases and prevention, antibiotics are used frequently so that its indiscriminate use may lead to resistance or tolerance. That's why the need to evaluate the equally effective new antibiotics. Due to the world modernization life become faster and very difficult for human being to achieve a good health that's way people suffering from many diseases like Diabetes mellitus, Obesity, Hypertension, Hypotension and many more. Urinary Tract Infection are among the most common bacterial infection. The urine culture and sensitivity test is important in the diagnosis of Urinary Tract Infection for determination of the identity of the infecting microorganism and for antimicrobial susceptibility testing. This is particularly true because of the increased incidence of antimicrobial resistance. According to modern science, bacteria are main causative factors for many infectious diseases. In Ayurvedic literature a lot of drugs have been mentioned which are useful in Urinary Tract Infection Shatvaryadi Kwatha is one of them and used in Mutravaha strotas dushti. In which I have focus the Shatvaryadi Kwath as mutral, pittashamak, Antibacterial 4 (krumighna) property related to urinary tract Infection, hence I have selected this topic for research.

Key Words: Urinary Tract Infection, Shatvaryadi Kwatha, Krumighna, Antibacterial, Mutral, Pittashamak.

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Introduction: Due to the world modernization life become faster and very difficult for human being to achieve a good health that's way people suffering from many diseases like Diabetes mellitus, Obesity, Hypertension, Hypotension and many more. Urinary Tract Infection are among the most common bacterial infection. It has been estimated that symptomatic Urinary Tract Infection result in as many as 7 million visits to emergency department and 100,000 hospitalizations annually [1]. The urine culture and sensitivity test is important in the diagnosis of Urinary Tract Infection for determination of the identity of the infecting microorganism and for antimicrobial susceptibility testing. This is particularly true because of the increased incidence of antimicrobial resistance. In Ayurvedic literature a lot of drugs have been mentioned which are useful in Urinary Tract Infection Shatvaryadi Kwatha is one of them and used in Mutravaha strotasdushti. In which I have focus the Shatvaryadi Kwath as mutral, pittashamak, Antibacterial 4 (krumighna) property related to urinary tract Infection, hence I have selected this topic for research[2].

Burning sensation during micturation, Dysuria with increased frequency has close resemblance with the signs and symptoms of Urinary Tract Infection one of the most common condition. People are not taking proper diat on time not drinking sufficient amount of water. Do not eating fruits in day to day life, people not doing the exercise. Eating fast food. While deciding the management this infectious disease pinpoint approach in the treatment and drug selection is needed. This could be done by detecting the organism on the basis of urine culture method and finding out the sensitivity of the Shatvaryadi Kwatha.

Materials and Methods :

Material required for urine examination[3]

1) Morning midstream urine
2) Urine container with 25 ml urine sample
3) 15ml plastic centrifuge tube
4) Centrifuge machine
5) Glass test tube
6) Glass slide
7) Microscope
8) Cover slip
9) pH paper
10) Dropper
11) Bunsen burner

Physical Examination [4]

Examined the urine color under good illumination and noted the color and transparency (appearance) Check the pH of urine by pH paper dipped in urine sample.

Chemical Examination [5]

Protein test

- Take 2 ml of a centrifuged (clear) urine specimen in test tube
- Add equal amount of sulphosalicylic acid 5%
- Shake the test tube gently and let it stand for 10 min.
- Noted the degree of turbidity by looking at the illuminated tube

against a dark background as per in the text.
- Other tests are done as per mentioned in text.

Microscopic Examination [6]

Procedure

- Collection of morning mid stream urine in a labelled container of UTI patient.
- Mix the urine and pour 10 ml urine in a plastic centrifuge tube and centrifuge it at 1500 rpm (revolution per minute) for 5 min.
- After centrifugation removed the supernatant fluid by keeping 0.5 ml of volume of residual fluid & sediment.
- Place one small drop of the suspended sediment on a clean slide and place a cover slip on it without formation of air bubbles.
- Then observed all constituents under high power objective. Count the various formed elements like pus cell, RBCs, epithelial cell, bacteria etc. in about 10 fields and reported the average number in each high power field.

Shatvaryadi Kwatha Preparation[7]

In ayurvedic text, different pharmacological methods of preparation of drug have been mentioned. These depend upon different form of drug and presence of active principal in that particular part of plants.

Material

1. Weighing machine
2. Measuring cylinder
3. Water
4. Raw drug (bharad churna of Shatavari, Kush, Kash, Gokshur, Vidarikanda, Kaseruka each of 10 gms)
5. Marked steel container
6. Thermometer
7. Bunsen burner

Procedure Of making Shatvaryadi Kwatha

- Total 60 gm of bharad churna is taken in a marked steel container.
- 480 ml of water is added in to the container
- Mixed well and boiled on low and constant flame.
- 1/8th remnain of mixture i.e. around 60 ml is obtained.

Observation Of Shatvaryadi Kwatha

The obtained kwatha is observed on panchbhautic pariksha ayurvedic test. It is observed based on Quantity - 1/8th of mixture i.e 60ml

- Rasa (taste) - Madhur
- Rupa (color) - Brownish
- Gandha (odour) - Madhur (Sweet smell)

Preparation Of Shatvaryadi Disc

Material

- Whatman paper No .2
- Shatvaryadi Kwatha
- Punching machine
- Petri dish
- Forcep

Procedure:

- Cut paper disc from Whatman No.2 filter paper with the help of paper punching machine (8 mm in diameter)
- Kept the paper disc in Shatvaryadi Kwatha for 10 min.
- Placed the disc in petri dish by sterilized forcep and allow to dry.
- The shatvaryadi disc dried for 45 min.
- Placed the shatvaryadi disc in a petri dish and sterilized them in a hot air oven at 1210C for 1 hours.
- After cooling, store the Shatvaryadi disc in a refrigerator (40C) and used for sensitivity.

Urine culture [8]

Urine is usually cultivated in MacConkey"s agar and blood agar plates directly from the centrifuged deposit of a sample of urine by streak method. Any growth of the organism, namely Escerichia coli, Klebsiella (lactose fermenting, pink colonies) Staphylococcus, Pseudomonas and other organism should be investigated for identification.

Material required for urine culture[9]

- Autoclave
- Sterile Petri dishes
- Sterilized MacConkey agar
- Nichrome loop wire
- Sterilized forceps
- Disposable gloves
- Spirit lamp
- Urine sample

Procedure[10]

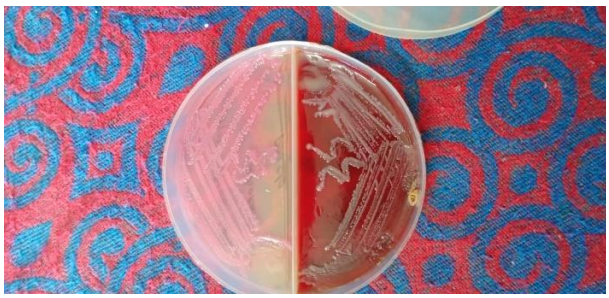
Preparation of culture plate

- Sterilized the medium (MacConkey agar & Nutrient agar) by autoclaving test tube holding 20 ml of medium.
- Cooled the agar to 550C.
- Poured the cooled melted agar into sterilized Petri dishes under aseptic condition.
- Allowed the medium to solidify in a flat position and leave the Petri dish overnight at room temperature in an inverted position; the lid of Petri dish which is bigger in size is placed at the bottom.
- Stored the plates in the refrigerator or in a cool place in inverted position.

Transfer of inoculum to Petri dishes[11]

- Sterilized loop by flame sterilization method
- Cooled the loop in the air so that the hot loop does not cremate the living bacterial cells.
- Picked up the specimen to be plated with the sterilized loop in right hand, open the lid halfway with left hand and streak the plate (by streaking technique) without exposing the agar surface more than necessary.
- While flaming the loop during streaking, kept the lid fully closed.

Streaking technique[12]



- Placed a small amount of inoculums near the periphery of the plate by loop.
- Spread the inoculums over zone of inoculums by to and fro movement of the loop.

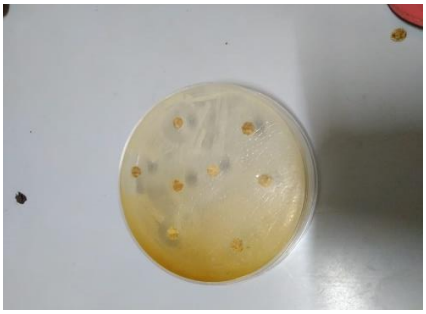
- Turned the plate at 450C, overlap the previous streak on the second zone area.
- Turned the plate again to 450C, flame the loop; allow the loop to cool.
- Start streaking and overlap the previous streak at several points.
- Finally, lift the loop and streaked the centre of the plate with zigzag motion.
- Placed the plate in incubator in an inverted position for 24 hours.
- Bacteria are grown within 24 hour.

Observation of colony character

Colonies are circular, raised, smooth and emit a fecal odour.

- E.coli Colonies are pink i.e. lactose fermenters.
- Klebsiella forming large, dome shaped, mucoid colonies.
- Staphylococcus colonies are large, circular, convex, smooth and opaque.
- Pseudomonas form non lactose fermenting colonies.

Disc Diffusion Method For Sensitivity



Dip the forcep in alcohol and sterilized on flame

- Keep the sterilized Shatvaryadi disc with the help of forcep on nutrient agar plate 4cm apart from each other.
- Press the disc firmly into the agar to ensure complete contact. The discs are distributed so that they are no

closer than 15mm from the edge of the Petri dish.

- After preparing culture on macConkey‟s agar, pick up colony by a sterilized loop and spread on sterilized nutrient plate in the center.
- Place the inoculated plate with the shatvaryadi discs, in the incubator and incubate at 370C.
- Following incubation measure the diameter of the zone of inhibition by scale.

Sample Size

The prevalence rate of UTI is 33.3%

According to Daniel formula

Sample size (n) = z2 p (1-p)/e2

Where,

n= sample size

z=standard normal variable (1.96)

p=prevalence of disease (33.3%)

e =error of margin 10%

n= (1.96)2 X 0.333 X (1- 0.333) / (0.1)2

n= 3.8416 X 0.333 X 0.667 / 0.01

n= 0.85 / 0.01 n= 85

For the statistical point of view total 80 patients to be taken.

Patient will be randomly selected in OPD & IPD of hospital attached to home institute.

Duration Of Study- 18 Months

Study Population

I. Patients coming to our college OPD.
II. Patients from medical health checkup camp organized by College and Hospital authorities.
III. Volunteers from College and Hospital premises.

Sampling Technique

Simple Random Sampling (SRS)

Study Design

I. Experimental Randomized controlled open Clinical Trial.
II. It was comparative clinical study.
III. 80 patients were randomly selected on which study was carried out.

Selection Of Study Criteria

I. In Vitro Experimental priclinical study.
II. Total 80 no. of patient will be selected randomly irrespective of religion, economic status, education and occupation.

Gradation Parameters

A) Subjective parameters

1. Kruchha mutrapravruti
2. Sadah mutrapravruti
3. Saruj mutrapravruti
4. Sarakta mutrapravruti

B) Objective parameters

1. Urine Routine and microscopic examination
2. CBC (Differential count)

3. Urine culture and sensitivity test.

Observation and Result :

Bacteria found in UTI :

Isolated Organism	Frequency	Percentage
Escherichia coli	59	73.75
Klebsiella	4	5.00
Pseudomonas	9	11.25
Staphylococcus	8	10.00
Total	80	100.00

Sensitivity and Resistance of bacteria :

Sensitivity	Frequency	Percentage
Resistance	**22**	**27.50**
Sensitive	**58**	**72.50**
Total	**80**	**100**

Isolated Organism	Sensitive	Resistance
Escherichia coli	49	10
Pseudomonas	9	0
Staphylococcus	0	8
Klebsiella	0	4
Total	58	22

Discussion

we had taken 20 – 60 age group for my research work related to Mutravaha strotas vyadhi (urinary tract infection).The transmission of diseases is often influenced by poor sanitation, less water intake, bad eating habit like excessive fast food, spicy, oily. My research work is related to Invitro Study of Shatvaryadi Kwatha in urinary tract infection to check used as Antibacterial properties or not.

Out of 80 patients 31 were male and 49 were female who suffering from urinary tract infection. After the study of urine culture I found the bacteria Escherichia Coli in 59 patients, Klebsiella in 4 patients, Pseudomonas in 9 patients, Staphylococcus in 8 patients.

After the study of urine culture and sensitivity of Shatvaryadi Kwatha sensitivity were observed in 58 patients and resistance was observed in 22 patients. Shatvaryadi Kwatha shows the antibacterial properties against the urinary tract infection so in future we can use it for the invivo study in the urinary tract infection.

Conclusion

Out of 80 patients, Escherichia Coli was observed sensitive in 49 patients and Pseudomonas was observed sensitive in 9 patients. Escherichia Coli resistance in 10 patients, Pseudomonas resistance in 0 patients, Staphylococcus resistance in 8 patients. patients Klebsiella resistance in 4 patients. We can use the Shatvaryadi Kwatha in urinary tract infection. In future we can do the invivo study of Shatvaryadi Kwatha in urinary tract infection.

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