Vitamin C Source Food Articles, Cure of Urinary Tract Infection in North India

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Different bacterial strains were isolated from hospitalized patients with Urinary Tract Infection and microscopically and biochemically characterized as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis Streptococcus sp., Staphylococcus saprophyticus and Alcaligenes faecalis. Klebsiella pneumoniae was the most common urine isolate and Staphylococcus saprophyticus was another common organism. The antimicrobial activity of vitamin C sources like cranberry, amla, lemon, roseberry and mango was determined against a total of forty eight isolated organisms by well plate method and sensitivity of antibiotics were also checked by Kirby-Baur disc diffusion method. On comparative analysis of vitamin C source food articles and antibiotic sensitivity against isolated organisms it was found that action of cranberry, amla, lemon, roseberry and mango have good effect as compare to different antibiotics. Among these sources cranberry was most effective against all urine isolates. Due to the emergence of drug resistant microorganisms, it is a need to search out more effective antimicrobial agents to cure the disease. Our studies suggest that high intake of vitamin C tends to increase the acidity of urine which is not well tolerated by the bacteria responsible for UTI.

Keywords: Urinary Tract Infection (UTI), Vitamin C, Cranberry Juice, *Klebsiella* pneumonia and Staphylococcus saprophyticus

1. INTRODUCTION

Urinary tract infection (UTI) is most common bacterial as well as fungal and viral infection in humans. All individuals are susceptible to UTI; however the prevalence of infection differs with age, sex and certain predisposing factors. It is reported that women are more susceptible to UTI as compare to males [1,2] because a women urethra is short, allowing quick access of bacteria to the bladder. It affects as many as 50% women at least once during their life time. Commonly prescribed antibiotic resistance among bacteria causing UTI is increasing both in developing as well as in developed countries [3]. Previous study reported that vitamin C can be helpful for infection affecting the urinary tract. A high intake of vitamin C tends to increase the acidity of urine; acidic urine is not well tolerated by bacteria [4]. Cranberries have long been the focus of interest for their beneficial effects in preventing UTI [5,6]. Since pre-historic times, man has used different natural sources against common illness prevailing in the society with varying degree of success. Natural substances are known to act synergistically with antibiotics, and resistance has not been reported against them. There are some advantages of using antimicrobial compounds of natural sources such as easily available, relatively less expensive and very effective, often fewer side effects, better patient tolerance, acceptance due to long history of use and being renewable in nature [7].

2. MATERIALS AND METHODOLOGY

A total of 30 clinical (urine) samples were collected. The midstream urine samples were collected in sterilized plastic containers from the Hospital of S.B.B. Dental College, Ghaziabad and Yashoda Multispecialty Hospital Ghaziabad. As soon as possible to prevent over growth of contaminating microorganisms and death of potential pathogens, thoroughly mixed urine samples were streaked on different culture media using triple streaking method and for quantitative estimation of bacteriuria, urine samples were serially diluted (tenfold) in physiological saline in sterile screw capped tubes to get dilution 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴. Each of these dilutions (100µl each) was poured and spread on the surface of nutrient agar and blood agar media and incubated at 37^oC for 24 hrs. for the growth of microorganisms. After incubation period colonies were counted on the plates. The isolates were sub cultured on different media for the identification of pure culture. The number of colonies per ml of urine was calculated by applying the formula:-

Bacteria / ml urine = No. of colonies/Amount plated X Dilution factor

Then isolated strains were identified on the basis of colony characteristics, microscopic examination and biochemical characterization (like catalase, oxidase and IMViC test). The isolated bacteria were preserved as pure cultures on slants and plates. A loop full of inoculam was taken from pure culture of the respective bacterial strain then inoculated into nutrient broth. The broth suspension was incubated at 37°C for 18-24 hrs. The growth thus obtained was used as inoculam for the sensitivity assay. Antimicrobial activities of various sources (cranberry, amla, lemon, roseberry and mango) of vitamin C and different antibiotics were tested by agar diffusion method. Nutrient agar plates were spread with 1 ml of the broth suspension of the isolated bacteria. Wells were cut from agar plates using a sterilized stainless steel borer and filled with 0.1 ml of the extracts and for the antibiotic sensitivity Kirby-Baur disk diffusion method was used. These plates were checked for zone of inhibition after 24 hrs of incubation period.

3. RESULTS AND DISCUSSION

As a result of the present scenario, out of 30 samples, 6 samples (20%) did not show any growth of microorganism, while rest 24 (80%) samples yielded one or more species of bacteria. From these 24 samples 7 different bacterial genera were isolated. It includes *Klebsiella pneumonia* (22.9%), *Staphylococcus saprophyticus* (22.9%), *Escherichia coli* (16.7%), *Pseudomonas aeruginosa* (16.7%), *Streptococcus* sp.(10.4%), *Proteusmirabilis* (6.2%), and *Alcaligenes faecalis* (4.2%) respectively. The data reveals that *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* were most common among all age groups and these were more common in females as well as previous findings [8] also illustrate *Klebsiella pneumoniae* was the most common urine isolate and *Staphlococcus saprophyticus* was another common organism.

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Some sources of vitamin C were used to check the antimicrobial activity against isolated bacteria by agar well diffusion method, all the bacterial isolates have strong inhibitory effect against cranberry, amla, lemon, roseberry and mango as shown in Figure 1.



Fig. 1: Comparative antibacterial activity of sources of vitamin C against the isolated bacteria.

Among these sources cranberry was most effective against all urine isolates. It was more inhibitory to *Streptococcus spp.*(23mm) and *Staphylococcus saprophyticus* (23mm) followed by *Escherichia coli* (21mm), *Klebsiella pneumonia* (18mm) and *Pseudomonas aeruginosa* (16mm). Our results of antibacterial activity of vitamin C confirms that Amla is the second source of vitamin C which was similarly sensitive to *S. saprophyticus* (20mm), *Streptococcus sp.*(18mm), *P. mirabilis* (16mm), *E. coli* (15mm) and *K. pneumonia* (15mm) followed by *P. aeruginosa* (14mm) and *A. faecalis* (14mm). Lemon was inhibitory to *Streptococcus* sp. (20mm), *A. feacalis* (17mm), *K. pneumonia* (16mm) and *P. aeruginosa* (16mm), followed by *E. coli* (13mm), *P. mirabilis* (12mm) and *S. saprophyticus* (12mm). Mango was inhibitory to all urine isolates but it was less effective than roseberry. The cranberry, amla and lemon showed strong antibacterial activity as compare to roseberry and mango.

Isolated strains were also checked for different antibiotic sensitivity using Kirby-Baur disk diffusion method. It was also found that *K. pneumoniae* and *Streptococcus spp.* were highly resistant bacterial genera to different antibiotics as shown in Figure 2.

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Fig. 2: Antibiotic resistance (%) of isolated bacteria (Gram's positive and Gram's negative).

According to previous study 73% and 84% strains of E. coli was resistance to ciprofloxacin and ampicillin, respectively [3,9], but in our study Ampicillin was most sensitive against S. saprophyticus, and E. coli and less effective for P. mirabilis, A. faecalis, and Streptococcus species, while it is resistant for K. pneumonia and P. aeruginosa. Ciprofloxacin and Cefotaxime were sensitive for all isolates. As described previously [10] our study also interprets Ciprofloxacin was highly sensitive for K. pneumonia and less sensitive for S. saprophyticus and E. coli. Tetracycline was highly sensitive to S. saprophyticus and resistant to Streptococcus species and E. coli. Our result are similar to earlier workers [11] who also found that E. coli showed low resistance to amikacin and gentamicin, while Klebsiella showed a high resistance to these agents despite being sensitive for many years. P. mirabilis strains were resistant to ampicillin, ciprofloxacin, gentamicin and chloramphenicol while it was resistant to cefotaxime [12], whereas in our findings P. mirabilis is less sensitive for ampicillin and tetracycline and highly sensitive for ceftizoxime, cefotaxime, ciprofloxacin and amikacin. Our study also supports the previous findings that ciprofloxacin (80%) has maximum sensitivity for Gram-negatives [13].

4. CONCLUSION

• The UTI causing microorganisms showed the prevalence of *Klebsiella* pneumonia and *Staphylococcus* saprophyticus to a tune 22.9% and other commonest organisms were *Pseudomonas* aeruginosa and *Escherichia* coli to a tune 16.7% and *Streptococcus* sp., *Proteus* mirabilis and *Alcaligenes* faecalis 10.4%, 6.2% and 4.2%, respectively.

- On comparative analysis of sensitivity against the sources of vitamin C (cranberry, lemon, amla, roseberry and mango) it was found that action of cranberry, amla and lemon is seen to have good effect as compare to roseberry and mango as well as Gram's positive bacteria were more sensitive as compare to Gram's negative.
- On comparative analysis of antibiotics it was found that *Proteus mirabilis* and *Alcaligenes faecalis* were sensitive against all the antibiotics except co-trimoxazole.
- *E. coli* showed less sensitivity against various antibiotics, while it showed maximum zone of inhibition against cranberry juice.
- Our studies suggest that high intake of vitamin C tends to increase the acidity of urine which is not well tolerated by the bacteria responsible for UTI.

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REFERENCES

- [1] S.R. Chaudhari, A.R. Thakur, P. Nandy and S. Samanta; "Urinary Tract Infection-A Survey of Local Population", Am. J. Inf. Dis., Vol. 4(2), pp. 117-23, 2008.
- [2] A.J. Schaeffer; "Recurrent Urinary tract infection in the female patient", Urology, Vol. 32(3), pp. 12-15, 1988.
- [3] J. Mandal, N.S. Acharya, D. Buddhapriya and S.C. Parija; "Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant Escherichia coli.", Indian J. Med Res., Vol. 136(5), November 2012, pp. 842-849, 2012.
- [4] D.R. Axelrod; "Ascorbic acid and urinary pH", JAMA, Vol. 254(10), pp. 1310-11, 1985.
- [5] R.G. Jepson G. Williams and J.C. Craig; "Cranberries for preventing urinary tract infections", The Cochrane Library. DOI: 10.1002/14651858.CD001321.pub5
- [6] E.N. Ruz, C.C. Gonzalez, S.L. Jaen L, P.G. Escoto, E.K. Urqiza, L.O. Rosenfield, C.S. Ortiz and P.V. Castellanos; "Cranberry juice and its role in urinary infections", Ginecol Obstet Mex., Vol. 77(11), pp. 512-517, 2009.
- [7] K. Vermani, S. Garg and L.J. Zaneveld; "Assemblies for in vitro measurement of bioadhesive strength and retention characteristics in simulated vaginal environment", Drug Dev. Ind. Pharm., Vol. 28(9), pp. 1133-1146, 2002.
- [8] L. Cernohorska and M. Votava; "Antibiotic resistance and biofilm formation in *Staphylococcus saprophyticus* strains isolated from urine", Epidemiol. Mikrobiol. Imunol., Vol. 59(2), pp. 88-91, 2010.
- [9] J. Mbanga, S. Dube and H. Munyanduki; "Prevalence and drug resistance in bacteria of the urinary tract infections in Bulawayo province, Zimbabwe", East Afr. J. Public Health, Vol. 7(3), pp. 229-232, 2010.

- [10] J. Palou, C. Pigrau, I. Molina, J.M. Ledesma and J. Angulo; "Etiology and sensitivity of uropathogens identified in uncomplicated lower urinary tract infections in women (ARESC Study): implications on empiric therapy", Med Clin (Barc)., Vol. 136(1), pp. 1-7, 2011. DOI: 10.1016/j.medcli.2010.02.042
- [11] C.R. Kiffer, C. Mendes, C.P. Oplustil and J.L. Sampaio; "Antibiotic resistance and trend of urinary pathogens in general outpatients from a major urban city", Int. Braz. J. Urol., Vol. 33(1), pp. 267-269, 2007.
- [12] L. Cernohorska and E. Chvilova; "Proteus mirabilis isolated from urine, resistance to antibiotics and biofilm formation", Klin Mikrobiol. Infekc. Lek., Vol. 17(3), pp. 81-85, 2011.
- [13] J. Iqbal, M.S. Kabir and M. Rahman; "Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Bangladesh", Jpn. J. Med. Sci. Biol., Vol. 50(6), pp. 241-250, 1997.