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Original Article

Antibacterial Potentials of Human Urine at Acidic pH 5

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Abstract

Background: To identify factors determining susceptibility of individuals to urinary tract infections (UTIs).

Methods: In this descriptive study , 55 hospitalized patients' urine samples were analyzed. Presence of red blood cells , pus cells, epithelial cells, casts and crystals were observed and counted under per high power field (HPF) by microscopy. While pH, specific gravity, protein, leukocytes, nitrites, glucose, ketones, urobilinogen, blood and bilirubin, were analyzed using dipstick method. All the samples were streaked on CLED agar for isolation of bacteria; and SDA for yeast.

Results: Twenty urine samples were found culture positive, of which 15 were from females and 5 from males. Cultures were isolated and identified as E. coli (11), Enterococcus (4), Klebsiella (3), Pseudomonas (1) and yeast (1). Interestingly, organisms were mainly isolated from urine samples having pH ≥5.5. In all the culture positive samples, pus cells were ≥20-40 /HPF. No patient with culture negative had urine pH 6.5 or above in the present study.

Conclusion: The probability of bacteriuria (UTI) and pyuria (increase pus cells in urine) increases with rise in urine pH. Persons with urine pH5 are generally protected from UTIs. Thus mechanism/s needs to be elucidated.

Key Words: Urinary tract infections, pH, Uropathogens, Escherichia coli

Introduction

Infectious diseases are thought to be serious concerns for the entire world as infections are accountable for the death of large number of people since centuries. Urinary tract infection is present both in community and hospital patients.[1] Females are more affected in comparison to males. Structural abnormalities are considered as recognized predisposing factor of UTI. [2]

Diagnosis and appropriate treatment of UTI is of paramount importance in any developing countries with only 0.6% of GNP and the per capita income of only US$ 2. This makes the treatment of UTI beyond the reach of any patient in a developing country particularly when 40% of population is below the poverty line. Health-care should be the responsibility of the state but 0.6% of GNP cannot look after any patient properly and adequately. The importance of UTI is being highlighted because the long standing / chronic UTI can lead to permanent damage of the kidneys requiring renal replacement therapy (RRT) in the form of intermittent urinary dialysis/ continuous ambulatory peritoneal dialysis (CAPD) and transplantation. Both modality of treatment cost Pak. Rs.300,000/- to 500,000/- per anum. Any attempt to reduce the burden of patients requiring the above mentioned expensive treatment should be understood by researcher. [3,4]

Escherichia coli is predominate organism causing UTI in both community and hospital environment . It has been observed that extremes of ages i.e. children and old age are more vulnerable to UTI. [5,6]Ideally the urine should be cultured to find out the causative organisms so that effective treatment can be advised, however, the procedure is time consuming and expensive in Pakistan particularly in rural areas where most of the family physician prescribed treatment of UTI on the basis of symptoms of the patient where facilities of culture is not available. Keeping the cost and relevance of diagnosis, this study was designed for simple, inexpensive diagnostic test for diagnosis of UTI which is relevant to affordability of patient.

In a normal healthy individual, rate of formation of urine is 800-2000 ml/day. However, various factors determine urinary output including water intake, weather conditions, etc. Urine has up to 95% of water and rest is organic (urea,creatinine, uric acid) and inorganic (sodium, potassium, calcium, chloride, sulphate, magnesium, ammonium, chloride, sulphate and phosphates) substances. A very unique property of urinary tract is sterility, in spite of its close proximity to gastrointestinal system and parts of large intestine.[7]Despite of constant exposure to microbes, nature has provided multiple barriers and different

systems to combat invading offenders.[8] Collectively all the barriers and mechanisms provided for defence of human body are termed as immune system. This system comprises of two arms; innate and adaptive. Innate immunity is inborn, rapid and nonspecific. In contrast adaptive immune response is specific, delayed and required pathogen for activation. Components of innate immunity include physical agents (skin, hairs, mucous membranes, pH etc.) and chemical barriers and immune cells (body secretions, phagocytic cell, enzymes, natural killer cells, etc.).

The barricade apparatus of urinary tract, cells of epithelium and lining of the tract, along with continuous flushing contribute immensely towards pathogens.[9] In addition, expression of certain receptors by host allows the immune system to sense and identify the intruder.[10] After recognition, several arms and ammunition are employed by the immune organization to expel the foreign invader. Similarly chemokine, cytokines and antimicrobial peptides, iron sequestering protein, and several others are constantly providing protection by innate and adaptive immunity.[7] The adaptive immunity is delivered by lymphocytes; B lymphocytes for humoral immunity and T lymphocytes for cell-mediated immunity.11Acidic pH of the urine also contributes toward controlling microbes. Present study was designed to identify the pH at which most microbes are controlled.

Patients and Methods

This descriptive study includes school going children, young adults and grownups , suffering from acute or chronic urinary tract infection, catheterized or un-catheterized hospitalized patients were included. Infants, toddlers, menstruating women and those suffered from tumours of urinary and genital tract of either sex were excluded. Urine of 55 hospitalized patients was obtained from Civil Hospital Karachi during January to December 2015. Samples were analyzed within 6 hours of collection. Microscopy was performed after centrifugation of urine samples for the presence of RBCs, pus cells, epithelial cells, casts and crystals. While pH, specific gravity, protein, leukocytes, nitrites, glucose, ketones, urobilinogen, blood and bilirubin were analyzed. All the samples were streaked on Cysteine Lactose Electrolyte Deficient (CLED) (Oxoid) agar by standard calibrated technique and incubated at 37°C for 24-48 h for isolation of bacteria; and Saborauds Dextrose Agar (SDA) (Oxoid) for yeast. Bacterial isolates were identified on the basis of cultural characteristic and gram staining. The isolates were further confirmed by biochemical tests using the standard methods For yeast direct microscopy and germ-tube formation test was performed.[12-17]Antibacterial activity was determined by well diffusion method. Briefly, Escherichia coli ATCC 25922 was grown overnight in Muller Hinton broth (Oxoid) and its turbidity was adjusted to McFarland 0.5 standard. 0.5 ml of adjusted culture was seeded to 20 ml melted MacConkeys agar and poured into sterile petri plate. After solidification of agar, 8 mm wells were punched with a sterile borer. 0.1 ml of urine samples of different pH (5- 7) were added in respective wells. Similarly, antibiotics discs were added in respective wells and 0.1 ml of PBS or citrate buffer or urine was poured in each well, and plates were then transferred to 37^{o}C incubator. Zones of inhibition were measured in mm. DR and co-efficient correlation of Pearson and statistical significance of variables were studied.

Results

In all the culture positive samples, pus cells were found to be $\geq$20-40 /HPF. No other significant finding was observed (Figure-1).While the analysis of pH, specific gravity, protein, leukocytes, nitrites, glucose, ketones, urobilinogen, blood and bilirubin revealed no significant difference between culture positive and culture negative samples. It is noteworthy to mention that cultures were isolated from urine samples at pH $\geq$5.5 (Figure-1).The urine DR data were subjected to correlation analysis to estimate the Pearson's correlation coefficient (r) and statistical significance (p-values) among variables using **s**tatistical package IBM SPSS statistics 20**.** Pearson's correlation indicates a predictive linear relationship (positive or negative)**.** A strong positive correlation was observed between urine pH and bacteriuria ($r = 0.551$, $p < 0.001$).The probability of bacteriuria increases with the rise in urine pH and pus cells. Nevertheless, there is strong relationship between pus cell count and bacteriuria with culture negative urine (p 3.644E-08).On testing the correlation between urine pH and pus cells, a statistically significant ($p < 0.001$) positive correlation of 0.659was observed. The Urine parameters such as pus cells and bacteriuria were appraised under acidicpH$\geq$5.5 (Figure- 1).Twenty urine (36%) samples were culture positive . Majority (75%) were females.. Escherichia coli was the commonest bacteria (55%) in urine samples (Table-1). Urine samples with pH 5 didn't show any growth. Organisms were mainly isolated from urine samples having pH $\geq$5.5 (Figure-2).Growth was not detected in 63.64% urine samples of

hospitalized patients with UTIs (Table-2). The reason for the negative culture could be prior antibiotic therapy or may have some physiological problems/ some lateral injuries or recovery from infection.).

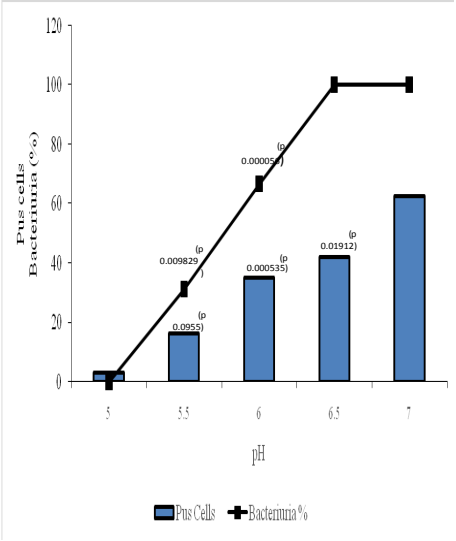

Figure-1: Relationship between bacteriuria (urine culture positive) and pus cells with urine

Table-1: Distribution of organisms in culture positive urine samples

Organisms isolated	Percentage	Male%	Female%
Escherichia coli	55	18.18	81.82
Klebsiella	15	33.33	66.66
Enterococcus	20	25	75
Pseudomonas	5	100	0
Yeast	5	100	0

Table-2: pH and growth of microorganisms in urine of hospitalized UTIs patients

pH	Culture Positive	Culture Negative
5.0	0	19
5.5	05	11
6.0	10	05
6.5	04	0
7.5	01	0
Total	20(36.36%)	35(63.64%)

All culture negative urine samples having pH 5 to 6 are probably from the patients moving toward recovery from infections. No patient with culture negative had urine pH 6.5 or above. Antibacterial activity of human urine pH5 was observed against E. coli ATCC25922, but no activity was seen in urine pH5 obtained from old peoples aged 69 and 83 (Table-3). Little antibacterial activity was observed at pH5.No activity was detected in urine samples tested having pH6 or above. Furthermore, ampicillin and norfloxacin activity was found slightly higher with the urine sample pH5 against E. coli ATCC 25922 (Table-4)

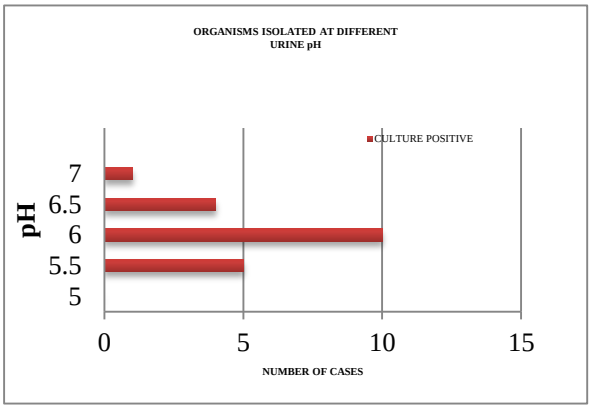

Figure 2: Relationship between urinary pH and organisms isolated

Table-3: Effect of Urine samples on Escherichia coli ATCC 25922

Urine sample with	Zone of inhibition in mm	S .No	Urine sample with	Zone of inhibition in mm
pH 5	10	9	pH 5.5	Nil
pH 5	11	10	pH 6	Nil
pH 5	11	11	pH 6	Nil
pH 5	12	12	pH 6.5	Nil
pH 5 (age 69Years)	Nil	13	pH 7	Nil
pH 5 (age 83Years)	Nil	14	pH 7	Nil
pH 5.5	7	15	pH 7.5	Nil
pH 5.5	6	16	Citrate buffer pH5	Nil

Table-4: Effect of antibiotics on microorganisms in the presence of urine pH5 Zone of inhibition in mm

Treatment	Escherichia coli ATCC 25922	
PBS alone pH 7	Nil	Nil
Citrate buffer pH5	Nil	Nil
Urine pH5	10	11
PBS+Ampicillin	26	26
Citrate buffer+Ampicillin	24	26
Urine+ampicillin	28	28
PBS+Norfloxacin	28	28
Citrate buffer+ Norfloxacin	30	30
Urine+ Norfloxacin	32	34

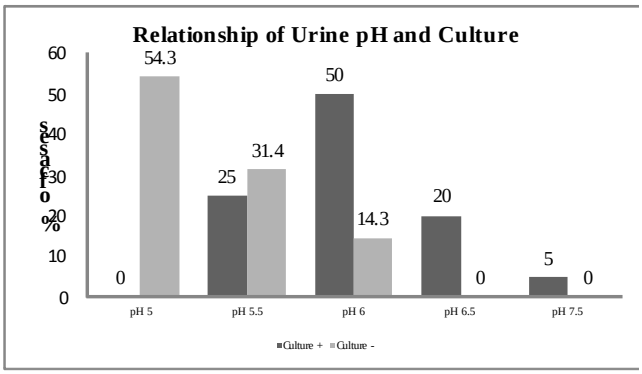

Figure-3: Culture negativity increases in urine of hospitalized UTIs patients with relation to pH of urine.

Discussion

Prevalence of urinary tract infection is unfortunately ignored and improperly treated both in community and hospital settings. This is leading to increase morbidity and mortality[18]. In the current study, it is observed that urinary pH, like stomach pH, is an important barrier for many pathogenic invaders in urinary tract.In a healthy person the urine pH ranges from 4.5 to 8. A number of factors contribute to determine the urine pH such as acid-base balance by kidneys, diet, water intake etc. Vegetables, legumes and citrus fruit increase pH and cause alkalinity, while non-vegetarian diet contributes to acidic urine.

Urine sterility is maintained by the multiple barriers and factors of immune system to combat invading organisms.[8] pH is an important barrier of innate immune system and this study reflects antimicrobial potential of urinary pH in the control of infectious diseases. In early 19th century bactericidal potential of dog urine was demonstrated against gram negative bacteria.[19] Another study suggested a role of ketogenic urine with bactericidal activity.[20,21] Reduction in growth of microorganisms due to accumulation of metabolic acid is also observed.[22] Antibacterial activity is also noted in young adults' urine pH5; and little higher activity of antibiotics was observed when tested with urine. However, urine samples pH5 from old peoples above age 69 did not show any activity.

Carlsson and his colleagues (2001 & 2003) established a link between nitrites and acidification of urine.[23, 24] Nitrate, a metabolic product, excreted in urine acts as bactericidal for nitrate-reducing bacteria at low pH. Recently, Shields-Cutler et al. (2015), pointed out an antibacterial protein,siderocalin, produced naturally in response to infection.[25] This protein binds iron and deprives bacteria from iron, required for bacterial growth,but this protein works better at neutral pH. However, body also produces aryl metabolites that control activity of Siderocalin. Similarly urine also contains small metabolites called aromatics, which vary depending on a person's diet.Urine samples that have more aromatic compounds restrict bacterial growth.Sarah et al. (2015)demonstrated that urine contains substances, like mannosides, that inhibit UropathogenicE. coli type-1 pilus mediated colonization and invasion of the bladder epithelium.[26]

In the present study a strikingly important observation that all the urine samples having pH 5 do not have detectable microbes, forcedus to believe that urine at pH 5 control infections owing to its acidic nature or presence of certain factor or components which are either produced in response to infection or become active at pH 5 or low. In view of above experience a hypothesis that urine at pH 5 or below possesses strong antimicrobial potentials is developed. Recovery from UTI could be high if urine is acidic. However, the mechanism needs to be elucidated.

Pyuria, is a prime index of bacteriuria requiring antibiotic therapy.[27,28] In the present study all culture positive urine samples were found to have high count of pus cells(pyuria). This shows that after the pathogen overcome first-line of defence mechanism the other line is present to fight against intruders.

In a latest study conducted in USA, Cunha et al., (2016)demonstrated the importance of renal insufficiency and urine pH. [29] During this study, UTI hospitalized patients with decreased renal function and acid / alkaline urinary pH were treated with ertapenem (antibiotic) and the time of bacteriuria eradication to negative urine cultures estimated. The study revealed that ertapenem eliminates bacteriuria efficiently in <3 days in patients with acidic urinary pH compared to patients with urine pH towards alkaline side. The results further strengthen and firmly affirm the proposed hypothesis that there is a very strong correlation between pH and antimicrobial potential of urine. Glen et al., (1996)described minor difference in the efficacy of ampicillin in microbiological media and human urine against bacteria[30]. Similar results were recorded in present study. On the other hand, Hohl and Felber¿(1988) indicated that urine pH influence the activity of quinolones, such as urine pH 5 markedly reduces the activity of norfloxacin compared to pH 6 and 7.[31] Similarly, Anandkumaret al.(2003)demonstrated that the activity of norfloxacin decreases four folds at pH 5.0 and 2000 mg/dL sugar concentration under in vitro condition.[32]Nevertheless, in severe diabetic individuals, norfloxacin may not be a drug of choice.

pH of urine can be used as important diagnostic tool for UTIs in patients with clinical manifestations together with other specific tests; and for selecting appropriate therapy. Acidification of urine both via diet modification and by other means can reduce the treatment period and hence prompt recovery. Furthermore, UTIs patients with acidic urine generally respond better to therapy. This procedure can reduce the cost of treatment in UTI patients with a reduction in therapy duration and doses.

Conclusion

Acidic urine pH5 play a pivotal role in controlling UTIs. The probability of bacteriuria (UTI) increases with rise in urine pH; and severity of UTI is proportional to the number of pus cells present in the urine.

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