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Research Article

PREVALENCE AND DISTRIBUTION OF BACTERIA AND FUNGI ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS IN PATTUKKOTTAI, TAMILNADU, INDIA

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ABSTRACT

Urinary tract infections (UTI) cause a many morbidity and mortality. The aim of this study was to study the risk factors and uropathogens of urinary tract infection in all patients. In the present study 2400 patients with clinical symptoms and suspected to UTI were examined. Clean-catch midstream urine was collected. Urine samples were inoculated, isolated and identified using standard bacteriological methods. Over all 650 (27.1%) uropathogens isolated belonging to 12 species with four Gram-positive, seven Gram-negative bacteria and *candida* spp. during the span of 12 months. The overall prevalence of UTIs in women was 69.8 % and men was 30.2%. Age-wise distribution of uropatogens was predominant in the age groups of 20-30 years both women (105, 16.2%) and men (40, 6.2%). In almost of patients (60.9%) was associated with common urinary tract infection. Urinary cystis was the second (9.7%) leading risk factor causing urinary tract infection followed by urithritis (7.9%), catheterization (7.7%), pyelonephritis (4.3%) and suspected cancer (3.1%) almost equally contributed. In the gram Negative bacteria *Escherichia coli* were the predominant pathogen in both the groups (38.09%). *Klebsiella Sps* (11.2%) *was* the second common organism in hospital acquired infection followed by *Pseudomonas sps*. (6.8%), *Proteus sps*. (3.7%), *Enterobacter sps*. (0.6%), *Citrobacter sps*. (0.3%) and *Acinetobacter sps*. In the gram positive bacteria the main organism identified was *Coagulase Negative Staphylococcus* (4.1%), *Enterococcus* (3.7%), *Staphylococcus saprophyticus*, (2.3%), *Staphylococcus aureus* (1.1%), and *candida* species. In the present study, overall incidence of UTIs were observed in females. High rate of UTI was observed in female of 21-40 years age. It is concluded that Gram-negative bacilli were responsible for UTI infections in our patients. The common isolated bacteria from urinary tract infections were *E.coli.* In the Indian setting, routine urine cultures may be advisable, since treatment failure is likely to occur with commonly used antimicrobials. This study provides valuable laboratory data to monitor the status of uropathogens and to improve treatment recommendations in a specific geographical region.

Keywords: Urinary tract infection, pyuria, risk factors, age, gender, bacterial isolates.

INTRODUCTION

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits (Ronald, 2002). Urinary tract infections (UTIs) are more common among women than men, although the prevalence in elderly men and women is similar. Most of the research on UTI has focused on young, sexually active women who are at high risk for developing an infection (Harrington and Hooton, 2000). The predominant UTI risk factors in young women are sexual intercourse and the use of spermicidal contraceptives. Worldwide, about 150 million people are diagnosed with UTI each year (Gupta, 2001). Urinary tract infections (UTI) are one of the common infectious diseases, and nearly 10% of people will experience a UTI during their lifetime (Foxman *et al.*, 2003; Feld and Mattoo, 2010).

Urinary tract infection may involve only the lower urinary tract or both the upper and the

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lower tracts. The term cystitis has been used to describe the syndrome involving dysuria, frequency, and occasionally suprapubic tenderness. Acute pyelonephritis (Lane and Takhar, 2011) describes the clinical syndrome characterized by flank pain or tenderness, or both, and fever, often associated with dysuria, urgency, and frequency (Mandell *et al.,* 2005). More than 95% of urinary tract infections are caused by a single bacterial species.

Under normal circumstances, the urine is sterile until it reaches the distal urethra. Various defense mechanisms of body prevent the infection of urinary tract. One of the most important defense mechanism is the flow of urine that washes bacteria out of the body. In men prostrate gland produces secretions that prevent bacterial growth. The acidic pH (5.5) and low osmolarity of urine also discourage the bacterial growth (Acharya, 1992). Similarly there are a number of factors that increase the risk of developing urinary tract infection. Some of these are: sex, age, pregnancy, catheterization, kidney stones, tumors, urethral strictures, neurological diseases, congenital /acquired anomalies of bladder, vesico-ureteric reflux, suppressed immune system, diabetes mellitus, enlarged prostrate, ureteric stresses, etc. (Sklar *et al.,* 1987).

Urinary system infections are usually bacterial, however, fungal etiology, particularly Candida pp. are encountered in about 10% of these infections (Nayman Alpat *et al.,* 2011). *C.albicans* is still the most frequently isolated species in candiduria. Candiduria, the presence of Candida species in urine, is a common clinical finding, particularly in hospitalized patients.

E. coli is the most frequent infecting organism in acute infection (Jellheden *et al.,* 1996; Ronald, 2002; Joshi *et al.,* 2011). *Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas, and Enterococci species* are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an outpatient population (Monali *et al.,* 2013; Manikandan *et al.,* 2011) Coagulase Negative *Staphylococcus* are a common cause of urinary tract infection in some reports (Mandell, *et al.,* 2005) *Staphylococci saprophyticus* tends to cause infection in young women of a sexually active age (Schneider and Riley, 1996). This study was aimed to identify the risk factors of bacteriuria, candiduria and to determine species distribution which cause urinary tract infections in Pattukkottai area hospitalized patients and outpatients.

MATERIAL AND METHODS

Source of data: Pattukkottai is located along the southeast coast of India in the East-central region of Tamil Nadu. The coast of the Bay of Bengal is just 12 km away from this town. A total of 2400 urine samples from January 2012 to December 2012 of suspected UTI patients attending in various departments of Medicine, Surgery, Obstetrics and Gynaecology and Paediatrics were included in this study.

Specimen collection: Midstream urine sample from male and female patients were collected in a wide mouthed universal container with a secure lid. A proper instruction was given to the patient regarding the method of collection of midstream urine sample.

Wet film examination: Urine sample was mixed carefully and about 0·05 ml. of urine was placed in the middle of a microscopic slide. At once a No 1 coverslip was placed over it, taking care to avoid air bubbles. The preparation was observed under high power dry objective (40×) of light microscope. Number of pus cells per high power field was recorded. Observation was also done for the presence of epithelial cells, red blood cells, bacilli, casts and crystals, parasites, yeasts and bacteria. All the findings were recorded.

Gram's staining: A drop of well-mixed uncentrifuged urine was air dried over a microscopic slide, heat fixed and Gram's Staining was carried out. The stained smear was examined under oil immersion (×1000) and the number of bacteria per oil immersion field was recorded.

Culture: In the laboratory, each sample was inoculated on McConkey agar, Nutrient agar, blood agar and UTICHROMagar media. The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop. The culture plates were incubated at 37°C for 24

hours and observed for growth through the formation of colonies. The culture inoculated on UTICHROMagar media, growth was observed after 24 and 48 hours of incubation. Isolates were identified by colony's colour and morphology. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Sharma (2008).

RESULTS

A total of 2400 midstream urine samples were processed from patients having clinically suspected Urinary Tract Infection (UTI) attending various Hospitals in pattukkottai area. During January 2012 to December 2012, 650 (27.1%) of the 2400 specimens, were culture positive and 1750 (72.9%) specimens showed no growth (Table 1). The age and sex-wise distribution among male and female patients is given in Table 1. Among them 980 (40.8%) were males and 1420 (59.2%) were female patients.

Out of the 1420 samples collected from females 454 (69.8%) showed growth. Of the 980 male urine samples 196 (30.2%) grew uropathogens in culture. Among females, children of 0-10 years age group had 15.5% UTI infection, 11-20 years age group had 10%, 21-30 years age group had above 16.2%. 31-40 years age group had 10.8%. 41-50 years age group had above 7.7%. 51-60 years age group had above 4.3%. 61-70 years age group had above 3.0%. Above 71 years age group had above 2.3% infection (Table 2).

Male children of 0-10 years age group had 9.2%, 11-20 years age group had 2.8, Similar to females, males of above 21-30 years of age group showed approximately, more than 6.2% infection, 31-40 years age group had 3.4%, 41-50 years age group had above 2.6%, 51-60 years age group had 2.0%. 61-70 years age group had 2.2%. Above 71 years age group had 1.8% infection (Table 2).

Table 3 showed that the sources of the isolated strains from 650 patients with different diseases of urinary tract infection. Urinary tract infection (396) with the percentage 60.9%, Urinary Cystitis (63) with the percentage 9.7 %, Urethritis (51) with the percentage 7.9%. Catheter (50) with the percentage 7.7%, Pyelonephritis (28) with the percentage 4.3%. Suspected Cancer (25) with the percentage 3.8%, Prostatitis (20) with the percentage 3.1%, Stone (10) with the percentage 1.5 %, Ureteric Stone (7) with the percentage 1.1%.

Microscopy to detect pyuria: Among 650 samples showing bacterial growth in culture, microscopy of wet mount revealed 260 (40%) samples with 0 – 3 pus cells/HPF, 170 (26.2%) samples with 4–6 pus cells/HPF and 220(33.8%) samples with >6 pus cells/HPF. The details of wet mount microscopy findings along with results of Gram's staining are given in Table 4.

Gram's stain and microscopy: Microscopy of 1750 Gram's stained uncentrifuged urine samples did not show any bacteria. These samples also failed to show any growth in culture. Another 205 samples also were negative for bacteria by microscopy, but grew bacteria in culture. However, 445 samples were positive both by microscopy and culture. 125 (19.2%) culture positive samples showed >6 pus cells/HPF but no bacilli on Gram's staining. 95 (14.6%) of culture positive samples showed both >6 pus cells/HPF and bacilli in Gram's staining. All the samples which showed bacilli in Gram's staining were culture positive. 50 (12·08%) culture positive samples showed 4 – 6 pus cells / HPF, but no bacilli were observed upon Gram's staining. 30 (40·58%) culture positive samples had 0 – 3 pus cells /HPF and no bacilli seen on Gram's staining (Table 4).

The colony characteristics and colour of the different microorganisms detected are described in Table 5. *E. coli*, *Proteus* spp., and *Enterococci* grow on this medium in typical differentiated colonies. *Acinetobacter* spp. were also easily differentiated and distinguished from *Pseudomonas* isolates. The similarity of colours produced by *Klebsiella*, *Enterobacter* and *Citrobacter* spp. prevents differentiation among them, and additional biochemical tests were done for final identification. The results showed that overnight incubation is optimal for the growth response of microorganisms on HiChrome UTI agar medium. Longer incubation of up to 72 h confirmed the results and deepened the colony colours.

Table 1. Result of urine culture among study population (n=2400).

Growth	Number (n)	Percentage
No growth	1750	72.9
growth	650	27.1
Female	1420	59.2
Male	980	40.8

Table 2. Distribution of urine pathogens according to age groups and gender. Data are reported as number of isolates and percentages of total patients in each age group.

Age groups in years	No of Females infected	%	No of males infected	%
0-10 (Children)	101	15.5	60	9.2
11-20	65	10.0	18	2.8
21-30	105	16.2	40	6.2
31-40	70	10.8	22	3.4
41-50	50	7.7	17	2.6
51-60	28	4.3	13	2.0
61-70	20	3.0	14	2.2
Above 71	15	2.3	12	1.8
Total	454	69.8	196	30.2

Table 3. The sources of isolated strains and percentage.

Clinical Diagnosis	No of isolated uropathogens	Percentage %
Urinary tract infection	396	60.9
Urinary Cystitis	63	9.7
Urethritis	51	7.9
Catheterization	50	7.7
Pyelonephritis	28	4.3
Suspected Cancer	25	3.8
Prostatitis	20	3.1
Stone	10	1.5
Ureteric Stone	7	1.1

Table 4. Correlation between pyuria, Gram's stain and culture in UTI.

Gram's stain	Growth in culture	Number of pus cells/HPF			Total
		0-3/hpf	4-6/hpf	>6/hpf	
Negative	Nil	1558	140	52	1750
	Present	30	50	125	205
Positive	Present	230	120	95	445

Table 5. Urine isolates presumptively identified on HiChrome UTI agar according to pigment reactions.

Organism	Morphology and/or colour(18–36-h incubation)
E. coli	Small, pink-purple
K. pneumoniae	Mucoid, *a* metallic blue
Proteus sp.	Pale brown
Pseudomonas	Green
Enterobacter sp	Metallic blue
Citrobacter sp	Metallic blue
Acinetobacter sp.	Nontransparent, cream, white
CONS sp	Colourless, small, undifferentiated
Enterococcus sp	Tiny blue, dry
Staph. saprophyticus	Small translucent; opaque
Staphy. aureus	Small, colourless
Candida species	Creamy, wet convex

Table 6. Distribution of bacterial isolates from urine samples (n = 2400). Data are reported as number of isolates and percentages of total.

Microorganisms	Frequency	Percentage
Escherichia coli	355	54.6%
Klebsiella pneumoniae	72	11.2%
Pseudomonas aeruginosa	68	10.5%
Proteus sp.	44	6.8%
Enterobacter sp.	10	1.5%
Citrobacter sp.	4	0.6%
Acinetobacter sp.	2	0.3%
GNB Total	555	85.4%
CONs	27	4.1%
Enterococcus sp.	24	3.7%
Staph. saprophyticus	15	2.3%
Staph.aureus	7	1.1%
GPC Total	73	11.2%
Candida sp.	22	3.4%
Total	650	100%

A total of 2400 urine samples yielded 650 (27.1%) strains of pathogens belonging to 12 species with four Gram-positive, seven Gram - negative bacteria and *candida* spp. The most common isolates in this study have been the Gram negative bacilli which accounts for 85.4% of the total positive isolates. In the gram negative bacilli, the predominant isolate from UTI were 355 strains of *E.coli.* The frequency of other uropathogens in descending order were 72 strains of *K.pneumoniae*, 68 strains of *P. aeruginosa*, 44 strains of *Proteus* sp., 10 strains of *Enterobacter* spp., 4 strains of *Citrobacter* spp., 2 strains of *Acinetobacter* spp. In the gram positive bacteria the main organism identified was 27 strains of *CONs*, 24 strains of *Enterococcus* spp., 15 strains of *Staphy.saprophyticus*, 7 strains of *S. aureus* and 22 *candida* sp. Thus, *E. coli* (54.6%) was the maximally isolated UTI causing bacterium, followed by, *K.pneumoniae* (11.2%), *P.aeruginosa* (10.5%), *Proteus* spp., (6.8%), *CONs* (4.1%), *Enterococcus* spp., (3.7%), *Staphy.saprophyticus* (2.3%), *Enterobacter* spp., (1.5%), *S. aureus* (1.1%), *Citrobacter* spp., (0.6%), and *Acinetobacter* spp., (0.3%). Table 6 and Figure 1 shows the detailed frequency of all the isolates identified.

Candida species were isolated from 22 patients (Table 7). The yeast cells were identified according to morphology and color of colonies on CHROMagar Candida. The color of colonies on CHROMagar Candida was similar as given by the manufacturer, i.e. green colonies of *C.albicans,* steel blue colonies of *C.tropicalis*

accompanied by purple pigmentation which diffuses into surrounding agar by growth, and large, fuzzy, rose colored colonies with white edges of *C.krusei*, the smooth white to light pink colonies of *C. glabrata* which later became pink. Therefore, the prevalence candiduria in critically ill patients in our study was 3.4 percent. *C. albicans* (45.5%) was the commonest species isolated, followed by *C. glabrata* (22.7%), *C. tropicalis* (18.2%), and *C. Krusei* (13.6%). Females were affected predominantly (81.8%).

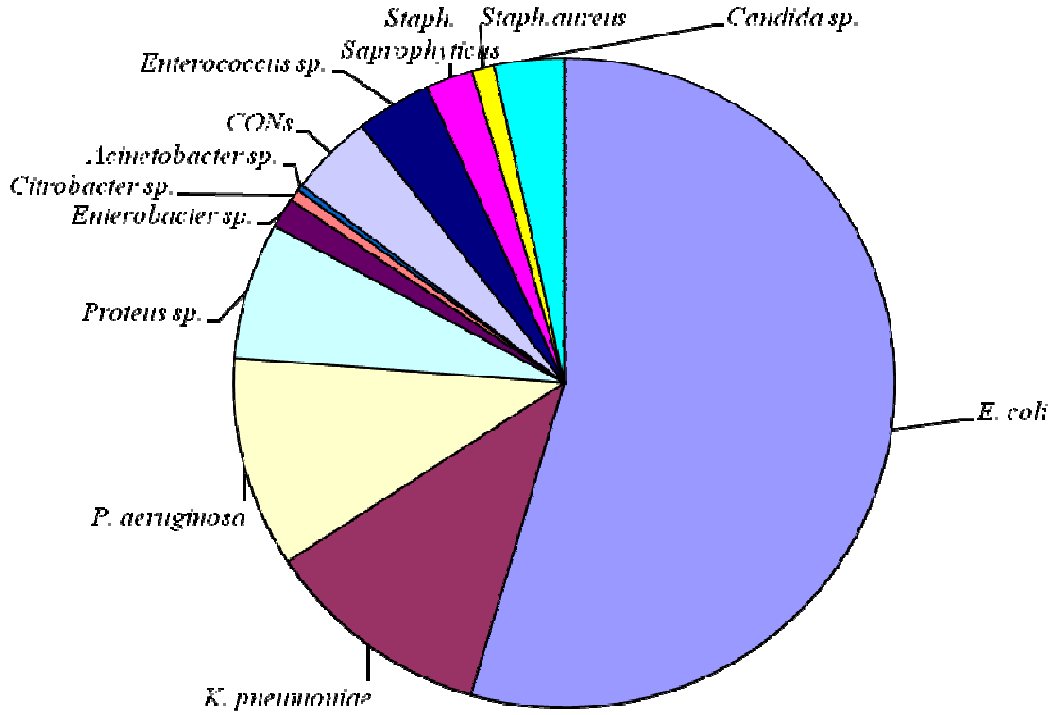

Figure 1. Percentage of organisms involved in UTI.

Table 7. Distribution of isolated *Candida* species (n=22).

Species	No. of identified organisms	Male	Female
C. albicans	10 (45.5%)	2	8
C. glabrata	5 (22.7%)	1	4
C. tropicalis	4 (18.2%)	0	4
C. krusei	3 (13.6%)	1	2
Total	22 (100%)	4 (18.2%)	18 (81.8%)

DISCUSSION

Infection of the urinary tract is one of the most common infectious diseases and it would affect all age groups peoples including men, women and children in worldwide (Arjunan *et al.*, 2010). The present study describes the relationships between sex, gender, source and isolated bacterial agents of UTIs. In the United States, UTIs account for seven million office visits and 100,000 hospitalizations yearly, making them the most common bacterial infections in outpatient settings (Foxman, 2003).

According to the census of India, Pattukkottai has a population of 2,73,097. Both Males and Females constitute 50% each of the population. The population under 6 years of age is 13.54%. Pattukkottai is located along the southeast coast of India in the East-central region of Tamil Nadu. The coast of the Bay of Bengal is just 12 km away from this town. It is surrounded by paddy and coconut field area inhabited by agriculturists. Majority of the patients attending Hospital are rural people. In the present study, we collected midstream urine samples (MSU)

from 2400 patients belonging to both sexes and all ages ranging from 4 months to 95 years, in whom UTI was suspected on clinical grounds. Among them 650 (41.4%) samples were diagnosed in the laboratory to be suffering from UTI. This correlates with the finding by Tambekar *et al*. (2006) wherein 39.1% of urine samples showed growth.

A total of 650 (27.1%) strains of uropathogens were isolated belonging to 12 species with four Gram-positive, seven Gram - negative bacteria and *candida* spp. during the span of 12 months. The overall prevalence of UTI in women was 69.8 %. This result is similar to those reported from many other centers (Abu Shaqra, 2000). The elevated incidence of infection among females is related to differences between the male and female genitourinary systems in anatomy and microflora (Strom *et al.,* 1987).

The different microorganisms isolated during the study period were shown in Table 6. It is clear that *E. coli* was the predominant uropathogen (38.9%) causing UTI. This is comparable to a retrospective study done at Bombay hospital by Sonavane *et al,* (2008) in which *E. coli* was isolated from 41.31% of UTI cases. The major isolate in most of Indian studies of UTI was *E.coli* (Kothari and Sagar, 2008; Taneja *et al.,* 2010). The second major uropathogen isolated in our study was K. *pneumoniae* (11.2%). In majority of other Indian studies showed (Sonavane *et al.,* 2008; Taneja *et al.,* 2010), *Klebsiella* spp. is the second major uropathogen. We isolated *P. aeruginosa* from patients who were either old, suffering from chronic diseases such as diabetes, tuberculosis or most of them had a history of recent catheterization. *P. aeruginosa* is an opportunistic pathogen causing infections mainly in debilitated or immunocompromized patients. Hospital acquired UTIs are common due to *P. aeruginosa* (Forbes *et al.,* 2007; Tambekar *et al*., 2006). The other uropathogens were *Proteus* sp, followed by *Enterobacter* spp., *Citrobacter* spp., *Acinetobacter* spp. *CONs,, Enterococcus* spp., *Staphy.saprophyticus, S.aureus* and *candida* sp. However, CONs, *Enterococcus* and *Staphy. saprophyticus* were the least dominant uropathogen causing UTI strains. The findings of this study similar to Akortha and Ibadin (2008) and Mansour *et al.,* (2009). A large number of micro-organisms were isolated from female urine samples, especially *Escherichia coli*. The frequency of UTI is greater in women as compared to men (Schaeffer *et al.,* 2001) and our results were similar to these reports; 69.8% of all patients were female. This might be owing to anatomic and physical factors (Aiyegoro *et al.,* 2007).

In the present study, HiChrome UTI agar was used for identification of uropathogens. 650 urine samples were tested by inoculation on HiChrome agar media. From the 650 "Positive" samples showed growth were unimicrobial and poly microbial. In agreement with other studies on chromogenic media, our data proved HiChrome agar as an excellent medium for the isolation of uropathogens (Leela Rani *et al.,* 2012). A total of 22 Candida isolates from urine clinical specimens were included in our study, of which *C. albicans* showed the highest number of isolates (45.5%), followed by *C. Glabrata* (22.7%), *C. Tropicalis* (18.2%) and *C. Krusei* (13.6) respectively. According Patel *et al.,* (2012), *Candida* species is the seventh most common nosocomial hospital wise pathogen, which caused 25% of all the urinary tract infections.

In our study we found that girls in the 0-10 years age group had 15.5% UTI. Generally UTIs are infrequent among girls aged 2-10 years, but some experience multiple repeated episodes of recurrent cystitis or pyelonephritis (Koff *et al.,* 1998). The present finding on UTI among girls of 0-10 years is quite high (24.7%). The presence of bacteriuria in childhood defines a population at higher risk for the development of bacteriuria in adulthood (Sobel and Kaye, 2005). Higher rate of UTI was seen in females in age groups from 30 years and above. Risk factors for high incidence of community acquired UTI among females are school aged girls, sexual activity, vaginal colonization (Stamy and Sexton, 1975) and antibiotic therapy that alters the normal vaginal flora. The increased risk of UTI in sexually active women has been attributed to mechanical effect of introducing uropathogens into the bladder (Schneider and Riley, 1996; Hooton, 2000).

The overall incidence of UTI observed among males in the present study was 30.2%. Though apparently this appears marginally lower than that was observed in females (69.8%). We have also observed a high rate of UTI in male children of 0-10 years of age (9.2%).

Generally infections are reported to be rare in boys except in association with anatomic or functional abnormalities in first year of life (Koff *et al.,* 1998). In the present study, most of these boys were being investigated for febrile illness.

Generally low prevalence of UTI is observed among men. This is attributed to greater distance between the anus and urethra, the drier environment surrounding the urethral meatus, the greater length of male urethra and the antibacterial activity of the prostatic fluid (Lipsky, 1989). UTI may be present even in the absence of these factors . We have also observed that more than 70% infection was seen among men of below 40 years of age.

In the present study we could grow bacteria only from 650 (41.4%) samples out of 2400 MSU samples examined, though almost all samples showed pus cells. Therefore, presence of pus cells may not be a reliable indicator of UTI. In our study, the samples showing bacteria in Gram's stained smear, also grew bacteria in culture in significant numbers (10^5 CFU/ml). However 31.5% of the samples negative for bacteria in Gram's stained smears, also showed growth in cultures revealing low sensitivity of Gram's stain microscopy compared to culture. In a few of the studies microscopic bacteriuria was detected in Gram's stained, uncentrifuged urine in over 90% of UTIs with colony counts of 10^5 CFU/ml or more (Stamm, 1982 and Jenkins *et al.,* 1986). Tilton and Tilton (1980) and Muray *et al.* (1987) found that Gram's stain microscopy is of low sensitivity in detecting UTIs.

CONCLUSION

This study concludes that understanding the effect of the different factors on community-acquired urinary tract infections and Gram-negative bacilli were responsible for UTI infections in our patients shall aid the proper management of this disease. Moreover, this study shows that, *Escherichia coli* were the predominant pathogen in both the groups. *Klebsiella Sps was* the second common organism in hospital acquired infection followed by *Pseudomonas sps*. The most common isolated bacteria from urinary tract infections were *E. coli*. Our data proved HiChrome agar as an excellent medium for the isolation of uropathogens. This is the first study conducted to determine the prevalence of UTI, the effect of gender and age on its prevalence and bacterial profile in a rural community of Pattukkottai area, Tamil Nadu state, India. This study provides valuable laboratory data to monitor the status of uropathogens and to improve treatment recommendations in a specific geographical region.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interests associated with this article.

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