6. DISCUSSION

Urinary tract infections (UTIs) are a leading cause of morbidity and health care expenditures in persons of all ages. Sexually active young women are disproportionately affected, but several other populations, including elderly persons and those undergoing genitourinary instrumentation or catheterization, are also at risk.

Urinary Tract Infections are a serious health problem affecting millions of people every year. The purpose of present study was to find out the existence of uropathogens and their susceptibility and resistance profile pattern. The prevalence of UTI varies by age, race, sex and temperature (Bachur and Haper, 2001).

According to Foxman, (2010) about 40% of women and 12% of men experience at least one symptomatic UTI during their lifetime, and approximately 25% of affected women show recurrent UTI (RUTI).

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans all the way through their life span. Urinary Tract Infections are more common in females than in men. Incidence in women in the age of 20—40 years ranges from 25 to 30% whereas in older women above 60 years of age it ranges from 4 to 43% (Jarvis and Martone, 1992; Kunin C, 1987 and Williams and Schaeffer, 2004). In our study similar findings were obtained in people of Namakkal Dt. and also reported that the same incidence in women in the age groups of 21 to 30.

In this study, totally 64% of individuals found to be positive for the significant urinary tract infection from patients attending Govt. hospital, Namakkal District, Tamilnadu, India. Standard biochemical tests were performed to identify the bacterial isolates that cause UTI from the collected urine samples. Characterization and identification of the isolates was done using the methods of Cowan and Steel (1985), Fawole and Oso (1988), Cheesbrough (2004) and Senthilkumar et al. (2013).
Our findings suggest that the relevant clinical epidemiological data which identify the prevalence of UTI patients. Namakkal is fast growing developing town with a dense urban population. Many patients do not undergo regular diagnosis for UTI unless they showcase the symptoms. Many UTI patients in the urban slums face unhygienic conditions, malnutrition and no proper sanitation making the situation worse.

*E. coli* was the commonest bacteria (63.44%) in UTI patients, but with a different rate obtained from the populations in U.S.A. studies (75.5% - 87.0%) (Ghedira Besbes *et al.*, 2004 and Mangiarotti *et al.*, 2000), and the number are 68.69% and 83.0% in general population of India (Rayan *et al.*, 1978). On the contrary, *Klebsiella* spp. and *Proteus* spp. were responsible for 16% and 11% of all urinary tract infections, respectively (Noor *et al.*, 2004). Likewise, Truls *et al.* (2006) reported *Escherichia coli* (31%), followed by species of *Pseudomonas* (13%), *Enterococcus* (10%), *Klebsiella* (10%), *Enterobacter* (6%) and *Proteus* (6%) as the most commonly reported pathogen. Rupinder Kaur *et al.*, (2012) reported that *E. coli* (71.7%) was most common in UTI followed by *K. pneumoniae*. In this present study, UTI causing bacterial causative agents screened from the patients were *Escherichia coli* (43.86%), *Pseudomonas aeruginosa* (24.56%), *Staphylococcus aureus* (19.3%) and *Klebsiella pneumoniae* (12.28%). Suman kumar Maji *et al.*, (2013) reported that the percentage of the Bacterial causative agent’s variation may be attributed to different life style, poor healthcare system, lack of education, and inadequate availability of water, and also may be due to geographical variations. From this finding, the predominant bacteria were *Escherichia coli* in different life style and geographical variations.

Microbiologically, urinary tract infection exists when pathogenic microorganisms are detected in the urinary tract. The infection is considered significant and requires treatment when more than $10^5$ microorganisms per milliliter of urine are present in a properly collected specimen. Gram-negative bacteria such as *E. coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. and *Pseudomonas* spp. are usually detected in recurrent infections, especially in association with stones, obstruction, urologic manipulation (Modarres and Oskoii, 1997).
In this present study, UTI causing bacterial causative agents screened from the patients were *Escherichia coli* (28.09%), *Pseudomonas aeruginosa* (15.73%), *Enterococcus faecalis* (14.61%), *Staphylococcus aureus* (12.36%), *Streptococcus pyogenes* (12.36%), *Klebsiella pneumonia* (8.99%) and *Protease vulgaricus* (7.87%). Truls et al. (2006) reported *Escherichia coli* (31%), followed by species of *Pseudomonas* (13%), *Enterococcus* (10%), *Klebsiella* (10%), *Enterobacter* (6%) and *Proteus* (6%) as the most commonly reported pathogen. Rupinder Kaur et al. (2012) reported that *E. coli* (71.7%) was most common in UTI followed by *K. pneumoniae* (15.3%), *S. aureus* (4.3%) and *P. aeruginosa* (4.3%). *E. coli* is the predominant pathogen in UTI and there is a high chance of recurrent infection by *E. coli* within first 6 months (Foxman et al., 2000). Akortha and Filgona, (2009) studied 300 urine samples with significant bacteriuria collected from 3 hospitals in Mumbai and analysed for presence of Enterobacteriaceae bacteria. 187 urine samples comprising 68.9% female and 31.1% male yielded Enterobacteriaceae bacteria growth. The isolates include *Escherichia coli* 51.5%, *Klebsiella pneumonia* 24.4%, *Klebsiella oxytoaca* 3.1%, *Enterobacter aerogenes* 9.7% and *Citrobacter freundii* 10.9%. Several studies revealed that UTI is associated with *E. coli* in 80% of cases (Kasper et al., 2005); 90% of all UTIs (Kunin, 1997); *E. coli* are often occur in both community and hospital acquired UTI (Gruneberg, 1994; MacGowan et al., 1993 and Barret et al., 1999). Urinary tract infection is more likely to occur in women and 50-60% of adult women experience UTI during their lifetime (Foxman et al., 2000 and Czaja and Hooton, 2006). From the present study too it is clear that the predominant organism in UTI samples is *Escherichia coli*.

*P. aeruginosa* is the third most common pathogen associated with hospital-acquired catheter associated UTIs (Jarvis and Martone 1992). But Kolawole et al., (2009) studied in Three hundred (300) patients not clinically diagnosed as having UTI were involved (150
males and 150 females and aged between 15-30 years) of these, 180 isolates obtained, gram negative bacteria had a higher frequency of occurrence than gram positive constituting 140 (77.7%) of the total isolates. These included: *E. coli* 55 (30.56%); *Pseudomonas aeruginosa* 42 (23.33%); *Proteus mirabilis* 29 (16.11%) and *Klebsiella aerogenes* 14 (7.78%). Gram positive bacteria accounted for 40 (22.22%) of the isolates. They include *Staphylococcus aureus* 27 (15%); *Staphylococcus saprophyticus* 13 (7.22%). It was also found that the rate of isolates of *E. coli* and *P. aeruginosa* were higher in isolates exclusively from females. Similarly in this present study also reported the *E. coli* and *P. aeruginosa* was higher causative agent for UTI in Namakkal Dt, Tamilnadu, India.

In early childhood, enterobacteria and enterococci are part of the normal periurethral flora. *Escherichia coli* is the dominant gram-negative species in young girls, whereas *E. coli* and *Proteus* spp. predominate in boys (Naylor, 1984; Modarres and Oskoii, 1997; Anis-ur-Rehman et al., 2008 and Shaikh et al., 2008). This is true because the percentage of *E.coli* is higher.

*K. pneumoniae* also found in bowel but in low number than *E. coli*. Although *K. pneumonia* common in nosocomial infections it cause community-acquired UTI with bacteremia. At risk patients *K. pneumoniae* require long course of antibiotics (Department of Health and Hospitals, 2010). *S. aureus* is an important cause of serious infections in both nosocomial and community acquired (Lowy, 1998). In our study too the presence of *Staphylococcus aureus* was 19.3% and *Klebsiella pneumoniae* 12.28%.

The prevalence of urinary tract infection varies markedly with sex and age. Symptomatic UTI occur in about 1.4 per 1000 newborn infants, with a slight male preponderance (Anis-ur-Rehman et al., 2008). It is assumed that the short urethra in girls predisposes them to ascending infection, because, for e.g. *E. coli* serotypes from bowel flora are the same as those that infect the urinary tract. However, some factors other than the
proximity of gut flora to the short urethra are likely because the female to male ratio in urinary tract infection varies directly with age (Jodal and Winberg, 1987 and Fischer, 2010). In this study, we noted a similar trend that the sex wise distribution showed inclination towards women. The UTI prevalence is found to be highly dependent on age and gender. The higher incidence of UTI was recorded in female (65.67%). In female among 6 types of age groups, the highest prevalence was recorded in the age group of 21-30 years (78.26%) and followed by 0-10 (75%) and lowest in 11-20 (40%) age groups. In case of male, the highest prevalence was recorded in 41-50 years (80%) followed by 31-40 (71.43%) and lowest in 0 – 10 (0%). In general, according to age wise, the highest occurrence was observed in 31 - 40 age groups peoples (67.54%). Similar observations were made by Obi et al., 1996 and Shahab and Nassirioskii, 1997. Even higher incidence of UTI causing E.coli was observed in young women who were sexually active of age group 20-29 years (Thomas Hooton et al., 1996). Our reports correlated with the above findings.

The most useful antibiotics used for previous study were fluroquinololones (Gatifloxacin,lefloxacin), erythromycin, and linezolid (in gram positives) because they inhibit most commonly isolated UTI pathogens. These drugs are relatively expensive with compared to most antibiotics frequently used. Therefore make the organisms susceptible to it. Nitrofurantoin, ampicillin and nalidixic acid which are commonly used antibiotics were poorly effective against majority of the organisms isolated by Kolawole (2009) and also in India, Suman kumar Maji (2013) findings were obtained in tribal people (Lodha, Kheria , Sabar, Munda, Santal, Kohl, Oraon, Mahali, and Bhumij )of West Medinipur zone, West Bengal, India that females are more susceptible to UTI than males and that the most effective drug is Amikacin (from amino-glycosides group) for the tribal people. The usage of antibiotics differs in countries and regions. The global percentages of first-choice, alternative-choice and inappropriate antibiotic prescriptions were: 42.4% (95% CI: 40.8-
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43.9), 44.1 (95% CI: 42.5-45.7) and 13.6% (95% CI: 12.5-14.7), respectively. The most commonly used antibiotics were fluoroquinolones (35%), cephalosporins (27%), penicillins (16%), aminoglycosides (15%), and co-trimoxazole (9%). Differences between countries and regions were highly significant. In this present investigation, antibiotic resistant among the E. coli isolates was found to be 100% to Cefpodoxime (CPD) and Novobiocin (Nv); 96% to Vancomycin (Va); 88% to Ceftizoxime (CZX), 84% to Ampicillin (A), 80% to Erythromycin (E), Tetracycline (T) Bacitracin (B), and Nitrofurantoin (NF) and 28% to Cefamandole (Cef). Moreover, the most sensitivity rates were reported for 72% to Cefamandole (Cef). ESBL-producing isolates to have a higher level of resistance towards antibiotics that are routinely prescribed against urinary tract infections as compared to non-ESBL-producing isolates (Aruna and Mobashshera, 2012) E. coli isolates producing ESBLs are significantly more frequently found to be resistant to other antibiotics, in particular fluoroquinolones (Lautenbach et al., 2001).

When Akortha and Filgona, (2009) conducted studies on 300 urine samples with significant bacteriuria collected from 3 hospitals in Mubi and obtained Antibiotic resistance profile. It revealed high resistance of isolates to ampicillin 37.5%, ciprofloxacin 36.4% and co-amoxyclov 21.3%. Streptomycin, nalidixic acid,cephalexin and gentamicin highly inhibited growth of the organisms tested. Gentamicin resistance rate of 17% was obtained while curing of selected donor isolates showed that gentamicin resistance in 75.8% of the isolates were plasmid mediated or located on mobile genetic element. Transfer rates of 34.8% and 41.1% respectively were obtained for inter-generic and intra-species transfer of gentamicin resistance genes (Gmr) among the Enterobacteriaceae isolates.

The extensive use of antibiotics in the treatment of bacterial infections led to development of multi-resistance strains. Ampicillin/amoxicillin and co-trimoxazole continued to lose their sensitivity so it is better to avoid it in empiric treatment of UTI
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(Winstanley et al., 1997 and Renuart et al., 2013). Some studies revealed that the prevalence of resistance among community of E. coli strains ranged from 5-60% for these drugs (Alon et al., 1987; Gruneberg, 1994; Maartens and Oliver, 1994; Hooton and Stamm, 1997 and Gupta et al., 1999).

According to Joseph DiPersio and Michael Dowzicky (2007), 131 (5.9%) Escherichia coli, 174 (10.1%) Klebsiella pneumoniae, 4 (1.2%) Klebsiella oxytoca, 24 (4.9%) Enterobacter aerogenes, 126 (9.5%) Enterobacter cloacae and 20 (2.6%) Serratia marcescens isolates were MDR. Four isolates (two K. pneumoniae and two E. cloacae) were resistant to nine antimicrobials. Tigecycline performed well against MDR E. coli (MIC90 0.5µg/mL, 0% resistant) and K. pneumoniae (MIC90 4µg/mL, 9.2% resistant). A MIC90 of 8µg/mL was reported for tigecycline against the other MDR organisms studied here, notably lower than those of most comparators.

E. coli isolates producing ESBLs are significantly more frequently found to be resistant to other antibiotics, in particular fluoroquinolones (Lautenbach et al., 2001). In the present study, the most resistance rates of E. coli detected from urine culture were found to 100% resistance to Cefpodoxime (CPD) and Novobiocin (Nv) and 28% to Cefamandole. Kenneth Todar (2008-2012) reported strains that are resistant to all available clinically useful antibiotics except vancomycin.

Variety of antibiotics increasing more resistance due to emergence of multi drug-resistance (MDR) bacterial strains (Arora and Kaur, 1999; Tadhani and Subhash, 2006; Atefl and Erdo, 2003 and Dash et al., 2005). This enhances the use of more antibiotics (Ibezim et al., 2006). The use of combination of more antibiotics is useful in preventing or delaying the emergence of resistance microbes. Hence the combination treatment if useful in case of resistant-bacteria (Zinner et al., 1981).
Present study showed 100% of the isolates were beta lactamase producers. In case of cell surface hydrophobicity analysis, the highest percentage was obtained in female (88.54%) in 37 years of age and lowest (75.23%) in 27 years of age. In male, highest percentage (86.95%) was observed in 53 years of age and lowest (75.29%) in 35 years.

In this current study next parameter was biofilm formation with Congo red method. Totally 23 (92%) isolates were in vitro positive for the biofilm production. Highest incidences (positive) for slime production were recorded in female (60.87%) followed by male (39.13%). In female, among 6 types of age groups, the highest prevalence (100%) was recorded in the age groups of 0-10, 11-20, 31-40, 41-50, above 50 and lowest in the age group between 21-30 years (80%). In case of male, 100% of slime producers were observed in the age groups of 11-20, 21-30, 41-50 and above 50 and lowest incidence observed in 31-40 years (66.67%) and no production in 0-10 years. Biofilm causing isolates was very difficult to treat because highly resistant to antibiotics. It’s a serious global threat and challenge to health care professionals (Saravana Murugan et al., 2011). During the production of biofilm, isolates was express several virulence factors and an increased resistance against phagocytosis and other host defence mechanisms (Costerton et al., 1999). Number of authors suggested that biofilm is responsible for antibiotic resistance (Saravana murugan et al., 2011; Dunne, 2002). At the same time, our reports evaluate argument with other reports for growing resistance of UPEC. Marhova et al (2010) reported that biofilm isolates are not significantly associated with antibiotic resistance.

Our isolates were exhibiting resistance to various commonly used antibiotics with the strains producing strong positive, moderate positive. Nevertheless a few of the strong biofilm forming isolates were sensitive to some of the antibiotics. This could be due to the ability of the antibiotics to penetrate the biofilm, and thereby inhibiting the growth. Biofilm assays may be helpful in selecting patients who require a therapeutic approach to eradicate persistent biofilm-forming E. coli strains (Soto et al., 2006).
One of the main mechanisms of resistance to antibacterial agents is the appearance of β-lactamase enzymes. In most cases, β-lactamase causes bacteria to get resistant to a broad spectrum of antibiotics like fluoroquinolones, aminoglycosides and trimethoprim. According to Ambler classification, these enzymes, most of which are called ESBLs, are divided into four main groups from A to D. ESBL enzymes of CTX-M, TEM and SHV, from group A, have been widely reported to be produced by *E. coli*.

ESBL producing bacteria spreading among *Enterobacteriaceae* is common one. In *E. coli* which causing community – acquired infections CTXm is the prevalent one among *bla-SHV*, *bla-TEM* and *bla-CTXm*. CTXm enzymes hydrolyse extended – spectrum cephalosporins and are inhibited by clavulanic acid. Extensive use of antibiotic is one of the essential factors for raised of ESBL-expression. ESBL producing bacteria showed antibiotic resistance against various antibiotic classes, including cephamsynincs, fluoroquinolones, aminoglycosides, tetracyclines and trimethoprim/sulfamethoxazole. The treatment for ESBL-associated infections is limited by drug resistance. Carbapenems may be the therapeutic option for UTI because of their more effectiveness than fluoroquiunolones (Falagas and Karageorgopoulos, 2009).

A total 250 clinical isolates of *E. coli* were collected from 3 university hospitals in Tehran. The phenotypic tests for confirmation of ESBL were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Presence of *bla-CTXm* genes were determined by multiplex PCR which is the fast and rapid method to detect sequencing the genes. In primary phenotypic tests among 250 isolates 140 (56%) were ESBL producers. But in confirmatory tests using clavulanic acid, only 135 isolates were considered as ESBL producers. In 135 isolates the genotypic results indicated the presence of *bla-CTXm-1* in 50 isolates, *bla-CTXm-9* in 5 isolates and *bla-CTXm-25/26* in one isolate of test *E. coli* (Mohsen Mirzaee *et al.*, 2009).
In this study ESBL genes were detected among the *E.coli* isolates. The result revealed that presence of *TEM* in 56%, *CTXm* in 64%, *SHV* in 40% and *OXA* in 60%. Of these *CTXms* are the most prevalent ESBLs in *E.coli* isolates. Further, *CTXm* subgroups containing 16 *E.coli* isolates were detected by multiplex PCR. Among these 2 subgroups were observed in the *CTXm*. The highest percentage of *CTXm* subgroup was II (68.75%) followed by I (62.5%). Other groups of *CTXm* (subgroup III and IV) were not observed in *E.coli* isolates.

In this study, ESBL genes were amplification was done with multiplex PCR. The multiplex PCR assay has been shown to have the advantage of rapidly screening large numbers of clinical isolates in addition to the fact that the isolated DNA would be suitable for further molecular epidemiological studies when required (Monstein *et al.*, 2007), in this PCR with only one target was effectively used for the detection of different ESBL encoding genes.

Bacteria may manifest resistance to antibiotic through a variety of mechanisms such as low permeability of microbial cell wall, has a genetic capability to express resistance character, mutation of regulating gene which responsible for resistant and acquire resistance gene from resistance organism via plasmid, transposons and bacteriophages (Lambert, 2002).

Peoples in Namakkal Dt. are having more antibiotic resistance. This may occur due to use of more antibiotics poultry feed to enhance productivity has also led to increased rate of resistant species dissemination among the Entero-bacteriaceae. The same antibiotic resistance may leads to human beings.
Prevalence rate of *E. coli* was found to be the most commonest organism for UTI in all age groups and showed resistance to commonly used antibiotics especially, 100% resistant to cefpodoxime and novobiocin. Tested *E. coli* isolates were positive for β-lactamase, slime production and higher percentage in cell surface hydrophobicity, also showed high antibiotic resistance. Thus, the highest incidence of virulence factors, recorded by the *E. coli* strains became more resistance to several antibiotics because they help the organism to overcome host defenses and colonize or invade the urinary tract. Virulence factors are very often responsible for causing disease in the host because they are often responsible for converting non-pathogenic bacteria into dangerous pathogens. However, the prevalence of antimicrobial resistance can vary according to geographical and regional location. Hence, finally we recommend the selection of antibiotic should be based on knowledge of local prevalence and we suggest to mitigate the problem of antibiotic resistance is development of new antimicrobial drugs for UTIs.