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Extended Spectrum Beta -Lactamase, Biofilm-producing Uropathogenic Pathogens and Their Antibiotic Susceptibility Patterns from Urinary Tract Infection- An Overview

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Abstract: *Escherichia coli*, a member of the *Enterobacteriaceae* family, is a common flora of the human and animal guts. It is the most common cause of Gram-negative nosocomial and community-acquired infections. Uropathogenic *E. coli* (UPEC) are the predominant causative organisms of urinary tract infections (UTI) and one of the most frequently isolated organisms in neonate meningitis and nosocomial bacteremia. An acute UTI can lead to recurrent infection, which could be considered as re-infection. The possible relationship between bacteria persistence in the urinary tract and the presence of virulence factors (VFs) lead to biofilm formation. Biofilm is a group of microorganisms encased in an exopolymer coat. The less availability of new generation antibiotics necessitates looking for substances from alternative medicines with claimed antimicrobial activity. In order to avoid renal complicacy and achieve successful treatment of UTIs, updated information of antibiogram is essential. Extended-spectrum beta-lactamases (ESBLs) constitute a growing class of plasmid-mediated β-lactamases which confer resistance to broad spectrum beta-lactam antibiotics. They are commonly expressed by *Enterobacteriaceae* but the species of organisms producing these enzymes are increasing and this is a cause for great concern. This review provides an overview of UPEC, ESBL and biofilm and their antibiotic susceptibility pattern from UTI.

Key words: Biofilm Urinary Tract Infection · Uropathogenic *E. coli* (UPEC) · Extended-Spectrum Beta-Lactamase (ESBL) · Multi Drug Resistance

INTRODUCTION

Urinary Tract Infection (UTI) is defined as the presence of multiplying microorganisms (bugs) in the tract through which urine flows from the kidneys via the bladder to the outside world [1]. Infections of the urinary tract are the second most common type of infections in the human body. UTI poses a serious health threat because of the antibiotic resistance and high recurrence rates. *Escherichia coli* are the most frequently isolated microorganism in UTIs. Currently a history of UTI is accepted as an independent risk factor for developing bladder cancer [2].

Normally urine is sterile and usually free of bacteria, viruses and fungi but does contain fluids, salts and waste products. An infection occurs when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply. Most infections arise from one type of bacteria, *E. coli*, which normally lives in the colon. Any abnormality of the urinary tract that obstructs the flow of urine (a kidney stone, for example) sets the stage for an infection. An enlarged prostate gland can also slow the flow of urine, thus raising the risk of infection [1].

Most UTIs are caused by ascending colonization and/or infection by enteric bacteria of the perineum, the periurethral area, the urethra, the bladder and occasionally, the kidney. Infection results when the bacterial virulence factors overcome the numerous host defenses [3]. Generally predominant uropathogens acquired from any source are Gram- negative bacteria with *E. coli* accounting for the highest prevalence in most instances [4].

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The frequency of UTIs and the mounting pressure for cost containment in medical care emphasize the need to consider costs of evaluating and treating UTIs. If initial treatment is provided with a drug for which a pathogen is not sensitive, patients will be likely to continue to experience symptoms and return for re-evaluation, resulting in a more thorough evaluation and a second antibiotic, generally a more expensive fluoroquinolones, is prescribed. The most important predictor of high cost-effectiveness is high efficacy against *E. coli*. Increased follow-up care results in diminished cost-effectiveness. Antibiotic cost is a poor predictor of cost-effectiveness, which is illustrated by the finding that the most and least expensive drugs, ofloxacin and trimethoprim-sulfamethasoxazole, are approximately equally cost-effective. Both of these are more cost-effective than other drugs, nitrofuration and amoxicillin [5].

The pathogenicity and virulence of the infective microorganisms as well as the efficiency of local or systemic defence mechanisms determine the course and severity of the disease. Virulence properties (toxins, capsule and iron uptake) are encoded by genomic structures and the determination of virulence is influenced by the host situation. In renal insufficiency, a variety of quite different substances (uremic toxins, betaine, amino acids, creatinine, urea, glucose) influence the microbial environment. Defence factors (Tamm-Horsfall protein, defensin, phagocytic activity of granulocytes) and underlying anatomical lesions as well as pre-existing renal disease determine the severity of UTI and the prognosis of renal insufficiency [6].

Idowu and Odelola [7] studied the bacterial isolates of different genera collected from suspected cases of UTI in Ibadan and their prevalence. Sensitivity pattern of the organisms to quinolones antibacterial agent was also investigated by the antibiotic disc diffusion method using Kirby Bauer method. The study revealed the prevalence of uropathogenic organisms as follows: *E. coli* (46.2%), *Klebsiella spp.* (23.1%), *Staphylococcus aureus* (21.1%) and *Pseudomonas aeruginosa* (76.0%). The quinolones were found to be highly effective against all the organisms. The average percentage susceptibility of the organisms was as follows: *S. aureus* (92.7%), *E. coli* (81.7%), *P. aeruginosa* (76.0%) and *Klebsiella spp* (70.0%). As indicated by their high activity, quinolones are better alternatives to commonly prescribed antibiotics in UTI therapy although caution must be exercised in their prescription as the emerging low level of resistance may pose a great danger for their future use.

Urinary Tract Infection in Children: UTI is one of the most common medical conditions requiring treatment, affecting millions of children every year [8]. A study of 3581 infants found 3.7% of boys and 2% of girls to have urine cultures positive for bacteria in the first year of life [9]. During the preschool and school years (1 to 11 years of age), the incidence of screening for bacteriuria is 9 to 10 times higher in girls [10] because they have short urethras. The cumulative incidence of symptomatic UTI in children younger than 6 years of age is 6.6% for girls and 1.8% for boys [11].

Scholen *et al*. [12] have addressed several major issues concerning UTIs in uncircumcised male infants. They studied in large, relatively captive patient population and were able to access inpatient diagnoses of UTI during the first year of life. They corroborated the association between foreskin presence and an increased incidence of UTIs. Additionally, they have reported a relatively higher frequency of such infections than that which is generally recognized. Finally, Scholen *et al*. [12] have noted the greater economic burden of UTIs in this population, primarily because of their greater incidence during early infancy.

Urinary Tract Infection in Adults: UTIs are among the most common bacterial infections in women, *E. coli* being the most common pathogen [13, 14]. UTIs engender substantial morbidity as well as some mortality, exacting enormous healthcare costs. It is estimated that about 35% of healthy women suffer symptoms of UTI at some stages in their life. About 5% of women each year suffer with the problem of painful urination (dysuria) and frequency [15]. The incidence of UTI is greater in women as compared to men either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors [16]. Pregnant women see more prone to UTIs than other women. It is thought that, about 2-4% of pregnant women develop a urinary infection. The hormonal changes and shifts in the position of the urinary tract during pregnancy make it easier for bacteria to travel up the urethras to the kidneys [17].

Uropathogenic *Escherichia coli* (UPEC): E. coli is responsible for 54.7% of UTIs and the isolation of *E. coli* is decreasing in comparison to previous reports, especially in males and in patients with indwelling bladder catheters who instead show higher *Pseudomonas* spp and *Enterococcus* spp. Multivariate analysis of multi-resistant uropathogens showed a positive significant correlation with indewelling bladder catheter and age. An upward

trend in the resistance of *E. coli* to cotrimoxazole, ampicillin and fluroquinolones were observed from 1996 to 1999 and also more than 50% of *Pseudomonas* spp. strains were resistant to fluoroquinolones and gentamicin [18].

Uropathogenic *E coli* possess fimbriae (fingerlike projections, also called pili) that bind to glycoproteins on uroepithelial cells through sites called adhesins. This attachment allows uropathogenic *E coli* to withstand being flushed by urine from the system [20]. These fimbriae are seldom identified in asymptomatic bacteriuria [21]. After adherence, many pathogenic *E. coli* secrete toxins to mediate further transmigration, including α-hemolysin (cytotoxic-necrotizing factor) and secrete auto transporter toxin, leading to cellular apoptosis or cell lysis. The hemolysin toxin is present in 50% of isolates responsible for pyelonephritis [21].

The gold standard for identification of Enteroaggregative *E. coli* (EAEC) remains the HEp-2 cell adherence test, which is time-consuming and requires specialized facilities [19]. Strains of *E coli* that have a predilection for the urinary system are known as uropathogenic *E coli*. They have unique virulence factors that contribute to their ability to cause UTIs, including adhesion-promoting structures (types 1 and P fimbriae) and toxins such as cytotoxic necrotizing factor and its polysaccharide coating. These virulence factors allow the organism to attach, invade, find nutrients and evade the immune system.

Biofilm: Many bacteria are able to form biofilms, which are defined as matrix-enclosed microbial population adherent to each other and to surfaces or interfaces [22]. The formation of biofilms on surfaces can be regarded as a universal bacterial strategy for survival and for optimum positioning to effectively use available nutrients. The gel-like state, predominantly consisting of polysaccharides, prevents the access of antibacterial agents, such as antibodies, white blood cells and antibiotics, so that sessile bacterial cells in biofilms can withstand host immune responses and are much less susceptible to antibiotics than in their non-attached individual planktonic state [23, 24]. The phenotypic changes observed in microorganisms as they attach to surfaces are due to the differential expression of genes within biofilms [25]. Genetic analyses have revealed the diversity of genetic factors participating in biofilm formation and there are no doubt multiple pathways to build a biofilm [26]. These factors, especially when they are involved in the early stages of biofilm formation, can be functionally replaced or overridden by others, depending on the media and growth conditions [27]. Therefore, although the study of initial attachment probably still holds some surprises, the quest for an essential adhesion step might be in vain. Recently, there has been a change of focus from the simple hunt for genes involved in the initial step of adhesion toward the identification, through global analysis, of late biofilm functions. The biofilm producing *E. coli* strains were resistant to at least six antimicrobial agents which call for an urgent need to regulate the overuse of antibiotics. This would limit the spread of resistant microorganisms in the community as well as in hospital settings [28].

Bacteria within the biofilm differ both in behavior and in phenotypic from the planktonic, free-floating bacteria. Conventional clinical microbiology can detect only the planktonic, free-floating bacteria, which are absolutely different from bacteria enclosed in the biofilm [29-31]. The microbes have evolved other mechanisms to evade antimicrobial therapy and probably the most important among them is the ability to either form or live within a biofilm [32]. A single bacterial species can form a biofilm, but in natural environment often biofilms are formed from various species of bacteria, fungi, algae, protozoa, debris along with corrosion products. Adhesion to surfaces provide considerable advantage for the biofilm forming bacteria, such as protection from antimicrobial agents, exchange of nutrient metabolites or genetic material from close proximity to microorganisms. Biofilms can vary in thickness from a monocell layer to 6 to 8 cm thick, but mostly on an average of about 100μm [33].

The difficulty in eradicating a chronic infection associated with micro colony and biofilm formation lies in the fact that biofilm bacteria are able to resist higher antibiotic concentration than bacteria in suspension. They are being implicated in the pathogenesis and also clinical manifestations of several infections. They cause a variety of persistent infections, including chronic middle ear infections, heart valve infections, infections realted to implanted medical devices and lung infections [32].

Mechanism of Biofilm: The formation of biofilm generally consists of two main steps: (i) the deposition of the microorganisms and (ii) the attachment by microbial adhesion and anchorage to the surface. After the process, multiplication and dissemination can be observed [29, 30, 34-38]. The initial event in this process is bacterial adhesion and the deposition of host urinary components on the surface of the biomaterial leading to the biofilm and consists of proteins, electrolytes and some

unidentified molecules [29, 35]. The types of components that form the conditioning biofilm depend on several characteristics such as chemistry and hydrophobicity. Many of the protein molecules in the conditioning biofilm play an active role in the bacterial adhesion process. The conditioning biofilm does not cover the whole implant surface completely, but rare forms a "mesh-like" covering [39]. Several factors are thought to influence bacterial adhesion to outer body surfaces, such as biomaterial and characteristics, bacterial surface features and the behavior of microorganisms and the presenting clinical condition [30, 35].

The biofilm is commonly built up of three layers. The linking biofilm is attached to the tissue or biomaterial, the biofilm base consists of microorganisms and the surface film acts as an outer layer where planktonic organisms can be released free-floating and spread to the surrounding every parts [29, 30, 36, 38].

Biofilm consists of multilayered cell and embedded in a matrix of extracellular matrix of extracellular polysaccharide matrix biofilm, which facilitates the adherence of these microorganisms to biomedical surface and protects them from host immune system and antimicrobial therapy [40]. Biofilm formation is regulated by expression of polysaccharide intracellular adhesion (PIA), which mediates a cell to cell adhesion and is the gene product of *iac ADBC* [41]. It is well documented that biofilms are notoriously difficult to eradicate and are often resistant to antibiotic therapy and removal of infected device becomes necessary [35].To control a chronic infection, antibiotics are chosen on the basis of conventional *in vitro* diffusion and dilution evaluation methods are not involving in biofilm formation [42].

Antimicrobial Resistance of Biofilms: Microbial biofilms have been associated with a lot of persistent infections which respond poorly to conventional antibiotic therapy. This also helps in the spread of antibiotic resistant traits in nosocomial infection by increasing mutation rates and by the exchange of gene to gene which are responsible for antibiotic resistance. Antibiotic therapy against device associated with biofilm organisms often fails without the removal of the infected implant. An elevated expression of the efflux pump is another mechanism for the development of antibiotic resistance in biofilm pathogen. The specific up regulation of genes which encode antibiotic transporters, has been seen in biofilms which are formed by *P. aeruginosa*, *E. coli* and *Candida albicans*. Physiological heterogeneity is another important characteristic which is observed in biofilm bacteria. This phenomenon affects the rate of growth and metabolism of the bacteria and is reflected by inter bacterial quorum signals, the accumulation of toxic products and the change in the local environment. These so called persister cells are not resistant to antibiotics per use, but become resistant when associated with the biofilm [43].

The Overall Biofilm Process

Trapping of Antibiotics: The exopolysaccharide slime causes a diffusion barrier by restricting the rate of molecule transport to the interior of the biofilm, or chemically reacting with the molecules themselves. The exopolysaccharide is negatively charged and restricts the penetration of the positively charged molecules of antibiotics by chemical interactions or molecular binding. This also dilutes the concentration of the antibiotics before they reach to the single bacterial cells in the biofilm [25, 44].

Bacteria Escape the Host Immune System: Biofilm producing bacteria escape the damaging effect of the antibody which is produced by the host immune system in response to infections [45].

Metabolism and Decrease of the Growth Rate of Bacterial Biofilms: A cell to cell communication in bacterial biofilms is established through chemical signaling. Small, compound molecules of class of N-acylated homoserine lactones (AHLs) are liberated by biofilm bacteria into their surrounding local environment and these AHLs are associated with DNA binding proteins. As the amount of AHLs reaches a threshold level, it induces the transcription of specific genes throughout the population. The regulation process is known as quorum sensing. The cells lying deep within the biofilm have low metabolic activity and low growth rates. This makes the biofilm microorganisms inherently low susceptible to antibiotics. Due to the consumption of oxygen and glucose, a relative anaerobiasis is created at the deeper layers of the bacterial biofilm, where in order to survive, the microorganisms transform into slow growers or non growers. Older biofilms are relatively more resistant than newer biofilms [46].

After the attachment to a biotic or an abiotic surface, the bacteria undergo further adaptation, increased synthesis of exopolysaccharide and increased antibiotic resistance. They also develop an increased resistance to UV light, increased genetic exchange, altered metabolism and increased secondary metabolic production [25, 44].

Prophylaxis of Biofilm: This includes systemic perioperative and local antibiotic prophylaxis. The aim of the local antibiotic prophylaxis is to inhibit the colonization of microorganisms on devices and the contamination of the tissues. Antimicrobials can be applied in various steps such as:

Coating of Device: Device coatings are of two types - passive and active. Passive coating such as ethylene glycol, poly ethylene oxide and hydrophilic poly urethane can be used. The effectiveness of passive coating is limited. In active coating, the release of anti microbial agents in high fluxes occurs to inhibit the initial adhesion of bacteria [43, 46 - 48].

Immersion and Surgical Irrigation: The dipping of the device in antimicrobial solution, e.g. rifampicin dipped vascular graft. Also, skin antisepsis and the antimicrobial irrigation of the surgical field [48].

Antibiotic Loaded: The use of antibiotic loaded (usually in joint arthroplasties) provides the local delivery of antibiotics, the stabilization of soft tissues, scope for an easier re implantation and better clinical outcome [49].

Antibiotic Therapy: This method is done to prevent the bacterial colonization by catheter [43].

Antimicrobial Carrier: Antimicrobials can be added onto a carrier either preoperatively or during surgery. Biodegradable and non biodegradable polymers which are impregnated with antimicrobials are used in orthopaedic. The resulting effects of the antimicrobials persist for weeks to months [48].

Treatment: The common treatment against persistent infections which are produced by bacterial biofilm producers is the removal of the infected Indwelling Medical Devices (IMD), combined with antibiotic/antifungal therapy. In case of IMD in non surgical patients, long-term antibiotic therapy is required [47, 50, 51].

Experimental Therapy: The in vitro use of ultrasound electric fields and penetration of antibiotics through microbial biofilms: The device emits low energy surface acoustic waves, electric currents, or pulsed ultrasounds that reduce the colonization of the devices and enhances the release of locally applied antibiotics [43, 46].

The Disruption of Signaling Molecules: These are involved in the biofilm architecture and detachment, e.g. penicillin acid [52].

Inhibition of Biofilm: Designing small molecules which can prevent biofilm formation at some target point, e.g. amino imidazole. The treatment inhibits the transcription of the biofilm regulatory genes and might be able to completely inhibit biofilms [46, 53].

In the Future: Identifying the virulent factor and genes which cause biofilm formation, can help in preventing the colonization of the microorganisms [25, 43].

Use Sensors: Sensors which can detect biofilm formation as early as possible are a great help for treating clinicians. Research is underway on to two types of sensors for biofilm monitoring: bacterial touch sensors and electro chemical sensors (non bacterial sensors). Eg: *Vibrio cholerae* (bacterial sensor) [54].

Prevention of Fungal Biofilm: A polymer which is isolated from the crustacean exoskeleton inhibits candidal biofilm formation *in vivo*. It damages the fungal cells, therefore, it can be considered for the prevention of fungal biofilms of the central venous catheters and other medical devices [55].

Methods of Biofilm Production in *In vitro*: Poovendran *et al*. [28] studied 100 (60.2%) *E. coli* strains and found that 72 strains displayed a biofilm positive phenotype under optimized conditions in the Tube Method (Figure 2) and the strains were further classified as highly positive 17 (17 %), moderate positive 19 (19 %) and weakly positive 36 (36 %). Screening on CRA biofilm positive phenotype under optimized conditions in the CRA method (Figure 3), the strains were further classified as highly positive 23 (23 %), moderate positive 37 (37 %), weakly positive 40 (40 %) and TCP biofilm positive phenotype under the optimized conditions in the TCP method (Figure 1). The strains were further classified as highly positive 6 (6 %), moderate positive 80 (80 %) and weakly positive 14 (14 %) by TCP method. which do not correlate well with the tube method for detecting biofilm formation in UPEC.

Similarly, in the 96 (71%) *E. coli* strains, studied by Murugan *et al*. [56], 81(84.37%) strains displayed a biofilm-positive phenotype under optimized conditions in the Tube Method and the strains were further classified as strong positive 9 (9.4 %), moderate positive 33 (34.4 %),

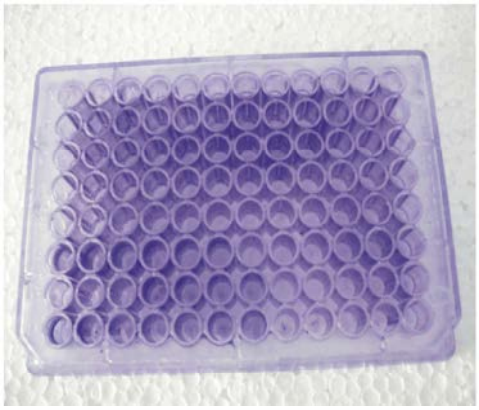


Fig. 1: Tissue culture plate (TCP) method [28]

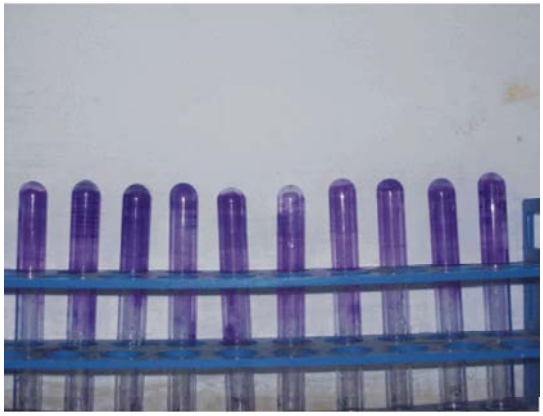
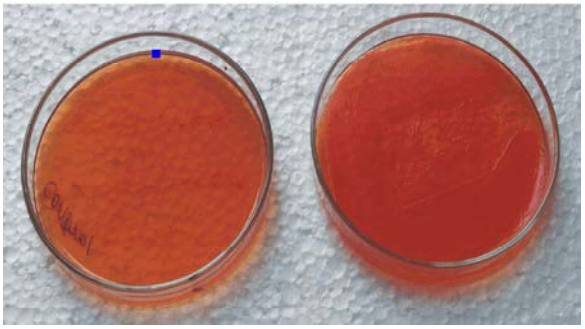


Fig. 2: Tube method [28]



A: Control B: Uropathogenic *E.coli*

Fig. 3: Congo red agar method (CRA) [28]

weakly positive 39 (40.6 %) and negative 15(15.6%). Screening on CRA biofilm positive phenotype under the optimized CRA method, the strains were further classified as highly positive 33 (34.4 %), moderate positive 24 (25.0 %) and weakly positive 39 (40.6 %), respectively.

Murugan *et al*. [56] have reported that the correlation between biofilm and multiple drug resistance towards UPEC. Similarly, Poovendran *et al*. [57] studied the correlation between biofilm and ESBL producing UPEC. According to Hanna *et al*. [58], biofilm formation protects bacteria from hydrodynamic flow conditions, for example in the urinary tract and against phagocytosis and host defence mechanisms, as well as antibiotics. Costerton *et al*. [59] have reported that more than 50% of all bacterial infections involve biofilm formation. Similarly Matija *et al*. [60] have reported 56% positive for biofilm production. Another study by Soto *et al*. [61] evaluated the prevalence of biofilm production in different clinical samples.

Pruss *et al*. [62] reported that the haemolysin and type 1 fimbriae expression are significantly associated with biofilm production. Type 1 fimbriae which promote adhesion to host epithelial cells, have been found to be important in the initial steps of biofilm formation. Bacterial biofilm has long been considered as a virulence factor contributing to infection associated with various medical devices and causing nosocomial infection [63, 64].

Tube method and Congo red agar method described here are based on the enhancement of exopolysaccharide production by using enriched media, TSB in the Christensen method [63]. Rakhshanda *et al*. [65] reported that the biofilm production by uropathogenic bacteria like *S. aureus* is (75 %) *E. faecalis* (75%) and *E. coli* is (40%). Soto *et al*. [61] have indicated that the *E. coli* as the most frequent cause of UTI and biofilm formation allows the strains to persist for a long time in the genitourinary tract and interfere with bacterial eradication. Although hemolysin is the main virulence factor by which *E coli* causes acute prostatic infection, the association between hemolysin and biofilm formation may result in increased ability of *E.coli* strains to persist in the prostate. Tenke *et al*. [66] have addressed easier methods for diagnosing and quantifying biofilm associated infection and development of more specific antimicrobial agents and ideal device surfaces would surely help in the fight against biofilm formation.

Extended Spectrum β-Lactamases (ESBLs) - History: Emergence of resistance to β-lactam antibiotics began even before the first β-lactam penicillin was developed. The first β-lactamase was identified in *E. coli* prior to the release of penicillin for use in medical practice [67]. All the early work on β-lactamases was concerned with those produced by Gram-positive organisms, e.g. *S. aureus* and the *Bacillus* spp. With the advent of new penicillin's, eg: ampicillin, carbenicillin and cephalosporins, attention changed from the Gram-positive species to the lactamases to Gram-negative organisms. Many genera of Gram-negative bacteria possess a naturally occurring,

chromosomally mediated β-lactamase. These enzymes are thought to have evolved from penicillin-binding proteins, with which they show some sequence homology. This development was likely due to selective pressure extended by β-lactamase producing soil organisms found in the environment [68]. The first plasmid mediated β-lactamase in Gram negative, TEM-l, was described in the early 1960s [69]. The TEM-l enzyme was originally found in a single strain of *E.coli* isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM [70]. Plasmid and transposon mediated has facilitated the spread of TEM-l to other species of bacteria. Within a few years after its first isolation, the TEM-l β-lactamase have spread worldwide and is now found in many different species of members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa, Haemophilus influenza* and *Neisseria gonorrhoea.* Another common plasmid mediated beta lactamase found in *Klebsiella pneumoniae* and *E. coli* is SHV-I (for sulphydryl variable). The SHV-l β lactamase is chromosomally encoded in the majority of isolates of *K.pneumoniae* but is usually plasmid mediated in *E. coli* [71].

Jabeen *et al*. [72] reported that the ESBL production in *Enterobacteriaceae* and the percentage of isolates which are falsely reported as sensitive in absence of ESBL detection, in a clinical microbiology laboratory of a tertiary care hospital in Karachi, Pakistan between September-October 2002 was determined. Selected isolates were identified according to standard biochemical tests and disc susceptibility tests were performed according to NCCLS. ESBL detection by combined disc (cefotaxime (30 µg) versus cefotaxime plus clavulanate (30/10 µg) was compared with detection using double discs (amoxi-clavulanic acid (20/10 µg) and aztreonam (30 µg) applied 10 mm apart.

Arora *et al*. [73] have undertaken a study on the prevalence of ESBL producers in major hospitals of Kolkata. 284 non-repeat clinical isolates were taken from five major hospitals of Kolkata and screened for ESBL production by Disk Agar Diffusion (DAD) using third generation cephalosporins (GC) and Double Disk Synergy Test (DDST) with and without clavulanic acid (CA), as per National Committee for Clinical Laboratory Standards (NCCLS). 87 (30.6%) strains were resistant to at least two 3GC out of which 46 (16.2%) were found to be ESBL-producers and confirmed phenotypically by DDST. They found ESBL production in 26 (56.5%) of *E. coli*, 12 (26.1%) of 14 (8.6%) of *Klebsiella Spp.* (4.3%) of *Pseudomonas aeruginosa,* 2 (4.3%) of *Proteus vulgaris*. Some of the representative isolates were screened for the presence of plasmid DNA. Both large and small plasmids were found in these strains; 16.2% of ESBL producing clinical strains were from Kolkata.

Extended Spectrum β-Lactamase (ESBL): In Gram negative pathogens, β-lactamase production remains the most important contributing factor to beta-lactam resistance [70]. The four major groups of β-lactams; penicillin, cephalosporins, monobactams and carbapenems have a beta-lactam ring which can be hydrolyzed by beta- lactamases resulting in microbiologically ineffective compounds [74]. The persistent exposure of bacterial strains to a multitude of beta-lactams has led to overproduction and mutation of beta-lactamases. These β-lactamases are now capable of hydrolyzing penicillin, broad-spectrum cephalosporins and monobactams. Thus these are new β-lactamases and are called as Extended Spectrum Beta Lactamases (ESBLs) [75]. In Gram negative bacteria these enzymes remains in the periplasmic space, where they attack the antibiotic before it can reach its receptor site [76]. The first plasmid mediated beta-lactamase was described in early 1960 [69]. ESBLs have been isolated from a wide variety of *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Capnocytophaga ochracea* [71, 77].

On the basis of mechanism of action, most common β -lactamases are divided into three major classes (A, C & D) depending on amino acid sequences. These enzymes act on many penicillin, cephalosporins and monobactam. Class B beta-lactamases called as Metallo Beta Lactamases (MBLs), act on penicillin, cephalosporin and carbapenems but not on monobactams [78]. MBLs differ from other beta-lactamases in using metal ion zinc, linked to a histidine or cysteine residue to react with the carbonyl group of the amide bond of most penicillin, cephalosporins and carbapenems [79]. Another class, Amp C- beta-lactamases is also clinically significant, since it confer resistance to cephalosporins in the oxyimino group, 7α-methoxy cephalosporins and is not affected by available β - lactamase inhibitors [80]. Amp C β-lactamases have been reported in *E. coli, Klebsiella pneumoniae, Salmonella* spp. *Citrobacter freundii, Enterobacter aerogenes* and *Proteus mirabilis* [81, 82].

Extended-Spectrum β-lactamase (ESBL) producing organisms are a major problem in the area of infectious disease after their discovery in 1983 [83]. ESBL productivity strains of *Enterobacteriaceae* have emerged as a major challenge in hospitalized as well as community based patients. Infections due to ESBL producers range

from uncomplicated UTI to life threatening sepsis [84]. The most common ESBL-producing organisms are *Klebsiella* species and *E. coli.* These organisms confer resistance to all β-lactam antibiotics except cephamycins and carbapenems [85]. In addition, ESBL-producing organisms frequently show cross-resistance to many other classes of antibiotics; including amino glycosides and fluoroquinolones, thus treatment of these infections are often a therapeutic challenge [83]. Detection of ESBL is challenging for the clinical microbiology laboratory. Its presence in the bacterial cell does not always produce a resistant phenotype; some ESBL isolates may appear susceptible to third-generation cephalosporins *in vitro* failure [86]. Resistant bacteria are emerging worldwide as a threat to the favorable outcome of common infections in community and hospital setting. A β -lactam is a lactam with a hetero automatic ring structure, consisting of 3 carbon atoms and a nitrogen atom. It is a part of several antibiotics [87].

β - lactams are globular proteins that posses II *alpha* helices and five beta-pleated sheets [88]. β- lactamases are heterogeneous bacterial enzymes that cleave the β- lactam ring of penicillin and cephalosporins to inactivate the antibiotic have TEM and SHV beta- lactamases conferring resistance to various antibiotics. A point mutation which alters the configuration around the active site of the TEM and SHV type enzymes has led to β- lactamases that are known as extended spectrum β-lactamase (ESBLs) can hydrolyze cefotaxime, ceftazidime, aztreonam and other expanded spectrum cephalosporin to varying degrees [89].

Beta-lactamase inhibits enzymes involved in the synthesis of bacterial cell wall endangering their survival. A common mechanism of bacterial resistance to beta-lactam antibiotics is the production of beta-lactamase enzymes that cleave the structural beta-lactam ring of penicillin group of drugs. More than 60 different types of beta-lactamase have been described from Gram-negative and Gram-positive organisms [73].

ESBLs have become a challenge both from the diagnostic as well as on the management point of view. β -lactam antibiotics are the most common treatment for bacterial infections. Concurrently the β - lactamase are the major defense of Gram negative bacteria against beta lactam antibiotics. These enzymes cleave the amide bond in the beta lactam ring, rendering beta lactam antibiotics harmless to bacteria [90]. The number of these enzymes now is more than 150 which were initially limited to *E. coli* and *Klebsiella.* ESBL phenotypes and detection have become more complex due to the diversity of the enzymes produced, emergence of inhibitor resistant ESBL variants plasmid borne resistance genes, concurrent Amp-C production enzyme hyper production and porin loss. During the last decade, a number of ESBL phenotype has been reported [91].

ESBL producers are associated with increased morbidity and mortality, especially amongst patients on intensive care and high-dependency units. Accurate laboratory detection is important to avoid clinical failure due to inappropriate antimicrobial therapy [92]. ESBL producing *E.coli* in Europe, North, Latin America and Western Pacific was reported at 1-8% [93]. Mathur *et al.* reported 68% ESBL positivity rate in their *Enterobacteriaceae* isolates from India [94].

β-Lactam Antimicrobials: The discovery of antibiotic drugs to treat infections caused by bacteria has been an important development of modern medicine. The bacterial cell wall is the obvious target for antibiotics. The two important classes of antibiotics that inhibit bacterial cell wall synthesis are β-lactams and glycopeptides. β-lactam antimicrobial agents are used commonly as first line therapy for the treatment of serious infections. The β-lactam family of antibiotics includes many of the most heavily used antibacterials in clinical medicine. They are important, both historically and currently, because of their effectiveness and generally low toxicity. The majority of clinically useful β-lactamase belongs to either the penicillin or cephalosporin group. The β-lactam also includes the carbapenems the monobactams, e.g. aztreonam and the β-lactamase inhibitors (eg. Clavulanic acid). β-lactam antibiotics are useful and frequently prescribed antibiotics. The orally active β-lactams are used frequently to treat community-acquired infections and the parenteral forms of penicillin (with or without β-lactamase inhibitors) and third generation cephalosporins are usually reserved for the treatment of nosocomial infections. The increasing and wide spread use of these classes of drugs exert a selective pressure that act as driving force in the development of antibiotic resistance. β-lactams are prescribed more often than any other antibiotics, this heavy usage has selected pressure that act as driving force in the development of antibiotic resistance [95].

Action of β-1actam Antibiotic of Bacteria: β-lactam antibiotics act on bacterial cell and can kill susceptible bacteria by interfering with bacterial cell wall synthesis. Bacteria have a cytoplasmic membrane much like that of eukaryotes. Surrounding this membrane is a periplasmic

space, which in turn, enclosed by a peptidoglycan layer and finally the outer membrane. The peptidoglycan layer is a cross linked polymer that forms a net-like structure that helps to provide structural rigidity to the organism and allows it to survive in the medium to which it may be strongly hypertonic. Without cell wall and its net-like peptidoglycan layer, the bacterial protoplast (cytoplasm plus cell membrane) would swell and burst. The composition of cell wall varies with species. The peptidoglycan layer is composed of repeating units of N- acetyl glutamic acid (NAG) and N-acetyl muramic acid (NAM) acid. The cross linking of peptidoglycan reaction is catalyzed by a peptidoglycan transpeptidase (penicillin binding protein) located in the cell membrane. β-lactam antibiotic can serve as the substrate for the transpeptidase. Once they have combined with the transpeptidase enzyme, they remain bound regardless of drug concentration in the media and inhibit bacterial cell wall synthesis and growth. Inhibition of cell wall construction ultimately leads to cell lysis and death [96].

Resistance two β-1actam antibiotics (β-1actamase action): Bacteria develop resistance to β-lactam antibiotics by a variety of mechanisms. Most common is the destruction of the drug by β-lactamases. These enzymes have a higher affinity for the antibiotics than the antibiotic has for its target. Commercially available β-lactam antibiotics fall into two groups the penicillin and cephalosporins; these compounds are susceptible to enzymatic modification and degradation. The most important of the degradation enzymes are the β-lactamases. Penicillin and cephalosporins are distinguished from other antibiotics by their possession of a β-lactam ring in the nucleus of the antibiotic molecule and the integrity of this structure is essential for the anti bacterial activity of the compounds. β-lactamases attack the amide bond in the β-lactam ring of penicillins and cephalosporins causing disruption of the molecule with subsequent production of penicilloic acid and cephalosporic acid respectively and ultimately rendering the compounds antibacterially inactive. Thus the enzymes play an important role in the resistance of many bacteria to penicillin and cephalosporins. Penicillin destroying enzymes have been known almost as long as penicillin has been available for therapy [97]. Genes encoding β-lactamases have been found in both chromosomes and extra chromosomal locations and in both Gram positive to Gram-negative bacteria; these genes are often on mobile genetic elements called plasmids.

ESBL Detection Methods: The increased prevalence of *Enterobacteriaceae* producing ESBLs creates a great need for laboratory testing methods that will accurately identify the presence of these enzymes in clinical isolates. Although most ESBLs confer resistance to one or more of the oxyimino-β-lactam antibiotics, the β-lactamase does not always increase the MICs high enough levels to be called resistant by the National Committee for Clinical Laboratory Standards (NCCLS) interpretive guidelines. The sensitivity and specificity of a susceptibility test to detect ESBLs vary with the cephalosporin tested. The NCCLS is currently reevaluating the testing procedures and interpretive criteria that should be used for the detection of ESBLs. The failure of either MIC or disk tests alone to accurately detect the presence of an ESBL in all strains of *E. coli* and *K. pneumoniae* has been well documented. It also appears that there is a difference in the ability of various susceptibility-testing methods used for detecting cephalosporin resistance in an ESBL producing strain. Steward *et al.* reported lack of sensitivity and specificity in traditional susceptibility tests to detect ESBLs. In the years since ESBLs were first described, a number of different testing methods have been suggested [98].

Agrawal *et al*. [99]; Basavaraj *et al*. [100]; Naik and Desai.[101] reported the prevalence of ESBL producer to be 22, 32.1 and 66%, respectively. Other studies from India have reported the ESBL production varying from 6 to 87% [94,102-104]. In recent years increase in ESBL production was reported from several countries such as USA, Canada, China and Italy [105-108]. Similarly, in a large survey of 1610 *E. coli* isolates from 31 centers, 10 European countries found that the prevalence of ESBL in these organism range from as low as 1.5% in Germany to high as 39-47% Russia, Poland and Turkey [109]. In the Arabian Gulf region, high ESBL production is 31.7% in Kuwait and 41% in the United Arab Emirates [110,111]. Similarly, Husam *et al*. [112] have reported that prevalence the ESBL production is 60% in Saudi Arabia. Babypadmini *et al*. [113]; Poovendran *et al*. [57], reported that in Coimbatore (South India) ESBL production is 41 and 34% in *E. coli*.

Antibiotics

Classes of Antibiotics: Some antibiotics can be used to treat a wide range of infections and are known as 'broad-spectrum' antibiotics. Others are only effective against a few types of bacteria and are called 'narrow-spectrum' antibiotics [114]. There are different kinds of antibiotics. The main classes of antibiotics are:

- Aminoglycosides
- Cephalosporins
- Fluoroquinolones

Aminoglycosides are used to treat infections caused by Gram-negative bacteria. Aminoglycosides may be used along with penicillins or cephalosporins to give a two-pronged attack on the bacteria. They work quite well, but bacteria can become resistant to them. The aminoglycosides are drugs which stop bacteria from making proteins and the effect is bactericidal [115]. The most commonly-prescribed aminoglycosides are amikacin, gentamicin, kanamycin, neomycin, streptomycin and tobramycin.

Cephalosporins are grouped into "generations" by their antimicrobial properties. Each newer generation of cephalosporins has greater Gram- negative antimicrobial properties than the preceding generation. The later-generation cephalosporins have greater effect against resistant bacteria. Cephalosporins are closely related to penicillins. Cephalosporins have a bacteriocidal effect by inhibiting the synthesis of the bacteria cell wall [115]. The most commonly-prescribed cephalosporins are cefotaxime, cefixime, cefpodoxime, cefpodoxime, ceftazidime, cefepime and cefpirome.

Fluoroquinolones are known as broad-spectrum antibiotics because they are effective against many bacteria. Fluoroquinolones are used to treat most common urinary tract infections, skin infections and respiratory infections. Fluoroquinolones inhibit bacteria by interfering with their ability to make DNA. Thus, making the bacteria difficult to multiply and the effect is bacteriocidal [115]. The most commonly-prescribed fluoroquinolones are ciprofloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, norfloxacin, ofloxacin and trovafloxacin.

Antibiotic Resistant Pattern of Uropathogenic E. Coli: Antibiotic resistance is the ability of an organism to withstand the effects of an antibiotic. It is a specific type of drug resistance. Antibiotic resistance involves naturally via natural selection through random mutation, but it could also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called multi resistant or, informally a superbug [116].

The luster of antimicrobial era soon began to show evidence of tarnish, however, at first bacteria, then fungi and then virus began to develop resistance to chemotherapeutic agents directed against them. This is especially of bacteria that have modified their DNA by chromosomal mutation and by acquiring resistance genes via conjugation, transformation and even transduction. Most bacteria have multiple routes of resistance to any drug; can rapidly give rise to vast number of resistance progeny. Antimicrobial resistance has been fueled by the inappropriate use of antibiotics by the physician and the public. Antibiotic resistance is a serious global problem, which results in morbidity, mortality and increased health care costs. Widespread antibiotics usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistances. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infection as well as for community-acquired infections [117].

Antimicrobial resistance is complex and dynamic. Although the major genetic and biochemical mechanisms have been recognized, new factors continue to be discovered, including integrons, multidrug efflux, hypermutability and plasmid addiction. Natural selection favors mechanism that confers resistance. Selection may also favor determinants that are least burdened by their resistance. Selection may also favor determinants that prevent their own counter selection and resistant strains with enhanced survival ability or virulence. The major mechanism used by bacteria to resist the action of antimicrobial agents are inactivation of the compound, alteration of the antibacterial target, decreased permeability of the cell envelope to the agent and active elimination of the compound from the interior of the cell [118]. Several bacteria, including *E. coli*, construct a multiple-antibiotic-resistance (MAR) efflux pump that provides the bacterium with resistance to multiple types of antibiotics, including erythromycin, tetracycline, ampicillin and nalidixic acid. This pump expels the antibiotic from the cell's cytoplasm, helping to maintain the intracellular levels below a lethal concentration [119,120]. The MAR pump is composed of the proteins MarA and MarB, whose synthesis is inhibited by the regulatory protein, MarR [121] mutations that reduce or eliminate the repression control of MarR resulting in over production of the MarAB efflux pump, which enables the cell to expel higher concentrations of antibiotics or other antimicrobial agents [117,122].

Antibiotic resistance of urinary tract pathogens has been known to increase worldwide, especially to commonly used antimicrobials. The antibiotic sensitivity patterns of either one or more of the commonly used antimicrobial drug in UTI cases [123 - 126].

The 20th century saw a series of remarkable discoveries that changed the face of medical practice. Among the most important was the discovery of antimicrobial agents, beginning with the synthesis of arsphenamine by Paul Ehrlich as the century dawned. With this discovery, the dreaded scourage of syphilis was brought under control, although not eradicated. However, the toxicity of the drug made it less than ideal as an antimicrobial agent. Shortly thereafter, optochin (ethyl cupreine) was tried for therapy of *Pneumococcal pneumonia*, but it was too toxic and was not effective enough to be successful. Moreover, pneumococci with resistance to this drug were isolated from patients who failed to respond to treatment- one of the first observations of antimicrobial resistance. The middle of the century saw an even more remarkable set of discoveries, the development of the first true antibiotics, beginning with the sulfonamides and penicillin and progressing through a whole series of effective antimicrobials that attached the bacterial cell at numerous vulnerable points. The discovery of effective anti tuberculosis agent and antifungal agents soon followed [90].

Resistance is an ability of an organism to grow in the presence of an elevated level of an antimicrobial agent. In short, a strain for which the Minimum Inhibitory Concentration increased is resistant. By this conventional criterion, biofilm cells do not necessarily show increased resistance. With some exceptions, biofilm cells do not grow better than planktonic cells in the presence of a broad range of antimicrobials [127].

Bacterial UTIs are frequent infections in the outpatient as well as in the nosocomial setting. The stratification into uncomplicated and complicated UTIs has proven to be clinically useful. Bacterial virulence factors on the one side and the integrity of the host defense mechanisms on the other side determine the course of the infection. In uncomplicated UTIs *Escherichia coli* is the leading organism, whereas in complicated UTIs the bacterial spectrum is much broader including Gram-negative and Gram-positive and often multi resistant organisms. The therapy of uncomplicated UTIs is almost exclusively antibacterial, whereas in complicated UTIs the complicating factors have to be treated as well. There are two predominant aims in the antimicrobial treatment of both uncomplicated and complicated UTIs: (i) rapid and effective response to therapy and prevention of recurrence of the individual patient treated; (ii) prevention of emergence of resistance to antimicrobial chemotherapy in the microbial environment. The main drawbacks of current antibiotic therapies are the emergence and rapid increase of antibiotic resistance. To combat this development several strategies can be followed. Decrease the amount of antibiotics administered, optimal dosing, prevention of infection and development of new antibiotic substances [128].

Antimicrobial activity of imipenem was measured using 4725 strains isolated from patients with complicated UTIs (CUTIs) between 1988 and 2000. Imipenem was inactive against methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Enterococcus faecium* and some non-fermenting Gram-negative rods. The prevalence of imipenem-resistant strains of *S. aureus, S. epidermidis* and *P. aeruginosa* was sporadically high in some years; no steady increase was seen over the period. Resistant strains were rare in other major uropathogenic species [129].

Bacterial infection of the urinary tract is a common health problem in young women but also the most common nosocomial infection (33%) contributing to the mortality of patients and increasing the duration and cost of hospitalization. *E. coli* are the most predominant organism and its prevalence varies in different studies. The high consumption of inappropriately prescribed antibiotics, combined with multiple pathology and frequent use of invasive devices, is a major factor contributing to high levels of resistance. There is a serious decrease in susceptibility of *E. coli* strains to amoxicillin, due to the presence of R-TEM enzymes, to cotrimoxazole and trimethoprim. Nitrofuration and fosfomycin-trometamol remain highly active against urinary *Enterobacteriaceae*, with over 90% of *E. coli* being susceptible [130].

The selective pressure of use and overuse of new antibiotics in the treatment of patients has resulted in the new variants of β-lactamases. One of the new classes was the oxyimino- cephalosporins, which became widely used for the treatment of serious infections due to Gram-negative bacteria in the 1980s [81].

Supriya *et al*. [131], have stated that multidrug resistance is expected to be more common in ESBL producing organisms. In their study, 38 (90.5%) ESBL

producing isolates were found to be resistant to three or more drugs whereas multidrug resistance in non ESBL producers was seen in only 31 (68.9%) isolates. The difference was statistically significant (P<0.05).

According to Neelam *et al*. [132], resistance to antimicrobial agents has become common among all pathogens. Fifty one isolates (42.9%) were multi-drug resistant (resistant to 3 or more commonly used antibiotics). Resistance to amoxycillin, nalidixic acid, cotrimoxazole, cefotaxime, chloramphenicol and ciprofloxacin was 62.5, 66.7, 34.6, 48.1, 37 and 18.5 %, respectively.

Suranjana *et al*. [73], observed that out of a total of 13,091 Gram-negative bacteria isolated, 9004 (68.78%) were found to be ESBL producers. Overall, piperacillin/ tazobactam exhibited the best activity (81.37% organisms susceptible) followed by ceftazidime/ sublactam (76.06% organisms susceptible). Ticarcillin/ clavulanic acid (45.48% organisms susceptible) was found to have a poor activity against all the organisms.

Manchanda *et al*. [102] described that multidrug resistance (three or more drugs) was observed in 90% (n=46) of the isolates. Resistance to aminoglycosides was high, with as many as 72% (n=37) of the isolates showing resistance to gentamicin and 69% (n=35) to amikacin. Decreased susceptibilities to cefotetan and cefoxitin were observed among 51% (n=26) and 43% (n=22) of the isolates, respectively.

Poovendran *et al*. [133] found the antibiotic resistance were 90, 89, 88, 86, 73, 71 and 58% for amikacin, piperacillin/tazobactam, cotrimoxazole, amoxyclv, norfloxacin, ampicillin and tobramycin, respectively. The susceptibility was found to be 97 and 100% for chloramphenicol and imipenem.

Subramanian *et al*. [134] observed that all the isolates are resistant to ceftazidime with 70% of the isolates displaying high level of resistance. However, the analysis of the 336 confirmed ESBL isolates revealed that ESBLs are predominantly present among *E. coli* (63.7%) compared to *K. pneumoniae* (14%) and other *Enterobacteriaceae* spp. that exhibit resistance to any one of the third generation cephalosporins must be reported as resistant to all third generation cephalosporins.

CONCLUSION

Biofilm can be composed of a single or multiple organisms on various biotic and abiotic surfaces. Hence, in UTI caused by biofilm producing *E. coli*, may promote the colonization and lead to increased rate of UTIs. The differentiation with respect to its biofilm phenotype might help to modify the antibiotic therapy and to prevent infection related to biomedical devices. A suitable and reproducible method is necessary for screening biofilm producers in any healthcare setup in adult women. This causes a number of persistent infections, which respond poorly to conventional antibiotic therapy. The overall healthcare costs which are attributed to the treatment of biofilm associated infections are much higher due to their persistence. Besides, a longer hospital stay is another factor for higher costs. Early detection of biofilm associated infections and newer treatment options for the management of the same are needed. The emerging threat of ESBL pathogens in our setting with the occurrence of these strains as etiological agents of infection in the hospital and community was cleared. While the findings shed light on *E. coli*, which are the predominant ESBL producers, we recommend further work on evaluating the ESBL types in these isolates as well as the prevalence of other ESBL-producing Gram negative bacteria which are emerging as pathogens of concern in the clinical setting. In conclusion of this study uropathogenic *E. coli* was higher in ability to form significant biofilm and ESBL production. It has been proposed that a number of *E. coli* gene re-arrangement occurs upon acquisition of the ESBL plasmid. Based on the findings the acute uncomplicated UTI affects a large proportion of the population. The review confirmed *E. coli* to be a major uropathogens. These indicate a need for continued surveillance of antimicrobial resistance among uropathogens causing UTI, so as to increase positive outcomes of clinical interventions.

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