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Original Article

Identification of Biofilm and Multidrug Resistance Uti Isolates of *E.coli*

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Abstract

Totally 50 urine samples were collected and subjected to isolation of *E.coli*. Among them 27(54%) of *E.coli* were obtained. In this current study, highest occurrence observed from female (61.29%) and 42.10% from male. Among the 6 age groups 21-30 groups had highest prevalence and all isolates were carryout to following parameters such as slime, betalactamase and antibiotic susceptibility. Totally 92.59% of *E.coli* produces biofilm formation and 96.29% of isolates expressed betalactamase activity. In addition, 73.8% of antibiotic resistance was observed from our isolates. In this current goal was determining the relationship between above said parameter. Fortune biofilm produced isolates had betalactamase activity and higher drug resistant against commonly using antibiotics. The results of this study indicate *E. coli* is prevalent in women and it possesses virulence associated properties of biofilm. So, appropriate measures should be taken for detection and treatment of such cases.

Key words: *E.coli*, biofim,antibiotic resistance, Betalactamase, UTI

Introduction

Urinary tract infection (UTI) is defined as the colonization of microbes in any part of the urinary tract. Infection of the urinary tract is an extremely common clinical problem. It can be affected by variety of organisms, UTI which act as opportunists and by pathogenic species as well (Syeda *et al*., 2010). UTIs mostly caused by bacteria have been showing increasing trends in recent years. The etiology of UTI is not well understood, and though to be related to gender, mainly affected by female compared to male. The common uropathogens identified in adult patients with UTIs include enteric gram-negative bacteria, with *E. coli* being the most common. *E. coli* was most important pathogen associated with UTI in number of countries and documented by number of authors (Jency Roopa, 2010; Maya *et al*., 2011).

Multiple antimicrobial resistances among gram negative organisms have been a long term and well recognized problem with UTIs. Several studies conducted in India have reported a steady increase of the resistance rate of uropathogens to commonly prescribed antibiotics (Amoxicillin, ampicillin, Chloramphenical, Kanamycin, Nalidixic acid, norfloxacin). In some developing countries resistance to fluoroquinolones is also emerging (Nasreen *et al.*, 2009).

Escherichia coli produce many virulence factors contributes to its pathogenicity. Biofilm is defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. This was associated with production of exopolysaccharide matrix and biofilm leads to chronic or persistent infection (Costerton *et al*., 1999). 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).

In recent years, ESBL production in *Enterobacteriaceae*, particularly *Escherichia coli*, has significantly increased in several countries, including India (Maya *et al*., 2011). More than 100 different ESBL types have been described, with the most widespread type being the CTX-M beta lactamase enzymes encoded by the blaCTX-M gene. ESBLs of the CTX-M family display increased hydrolytic activities against ceftazidime (Jesus Oteo *et al*., 2006). Recently this type of antibiotic resistance isolates of *E. coli* was observed from clinical samples (Maya *et al*., 2011).The objectives of this retrospective study were to the prevalence of *E.coli* from urine samples and determine the relationship between biofilm and drug resistance.

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Materials and Methods

Samples collection

Fifty urine samples were collected from patients suspected to have urinary tract infection at the discretion of the provider at Namakkal, South India, surrounding hospitals and clinical laboratories.

Bacterial isolation

Culture plates of EMB (Eosin Methylene Blue) agar and MacConkey agar (HiMedia, Bombay, India) were used for the isolation. The primary identification of bacterial isolates was made based on the colonial appearance and pigmentation. Biochemical tests were performed to identify microbes (Cheesbrough, 2004).

Determination of biofilm production

All isolated *E.coli* were carried out to biofilm formation according to Mathur *et al.,* (2006). The isolates were inoculated in brain heart infusion agar (BHI) supplemented with 5% sucrose and Congo red. After 24hrs incubation period, observe the result. Black colony formation was considered as biofilm positive.

Beta lactamase Assay

Beta lactamase production was assayed using the method of Lateef, (2004). Broth culture of the test organism was spot inoculated on to Mueller-Hinton agar containing penicillin and 1% starch then incubated overnight at 37^0C. Phosphate buffered saline containing potassium iodide was added to plate. The presence of clear colourless zones around the bacterial growth is an indication of β lactamase production.

Antibiotic susceptibility tests

Antibiotic susceptibility tests were performed on Mueller-Hinton (MH) agar (Himedia India). Fifteen antibiotics were selected based on the UTI treatment. Antibiotic disks were obtained from Himedia, India. Agar plates were evaluated after 18 hours of incubation at 37 °C (Hosoglu *et al*., 2007).

Results

Totally 54% of *E.coli* isolates were observed from 50 urine samples. The highest occurrence was found in female sample (61.29%) followed by male (42.10%). The occurrence was highly observed from 21-30 age group peoples. Those *E. coli* isolates were subjected to biofilm, betalactamase and antibacterial resistance analysis (Table-1).

Table-1: Occurrence of *E.coli* from urine samples

Sex	Age Group						% of Occurence	Total% of occurrence
	0-10	11-20	21-30	31-40	41-60	+60		
Male	0/1	2/4	1/4	1/3	3/4	1/3	42.10%	54%
Female	1/2	3/6	11/15	3/4	1/2	0/2	61.29%	

In this study 92.59% of isolates were produced biofilm formation, among them 94.73% occurrence from female sample and 87.5% from male samples. The highest occurrence was observed from maximum all age group of peoples except above 60 age groups (Fig.1). All isolates were carrying out to the betalactamase test. Totally 96.29% of isolates were produced betalactamase enzymes. The highest prevalence was occurred in female (Fig. 2 & Plate.1a and b).

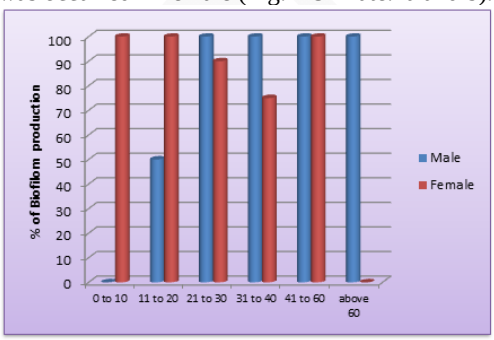

Fig- 1: Prevalence of biofilm producing *E. coli*

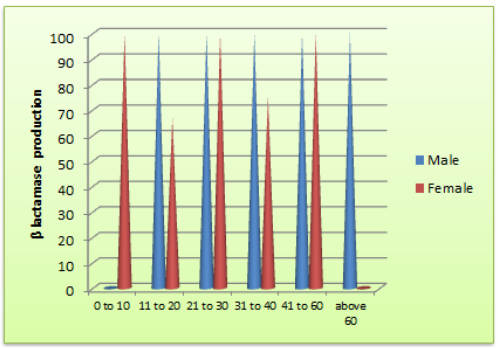

Fig- 2: Prevalence of beta lactamase producing *E. coli*

Antibiotic resistance isolates was observed by antibiotic disk diffusion method. Each isolates were resistance to at least single antibiotic. Among them single isolates was resistance to all 15 antibiotics and 2 isolates were resistance to 14 antibiotics. In the current reveals 14.8% of isolates were resistance to 8 antibiotics. Among the 15 antibiotics, 100% of isolates resistance against cefodoxime and second most ampicillin and novobiocin (96.29%) followed by

vancomycin (92.59%) tetracycline (81.48%), cephotaxime (77.77%), Gentamycin and co-trimoxazole (48.14%) had lowest resistance. Among the 6 types of age group people, 31-40 has highest antibiotic resistance. In this current goal most of the slime producing isolates had betalactamase and multidrug resistance character.

Plate -1a: Biofilm in Congo red plate

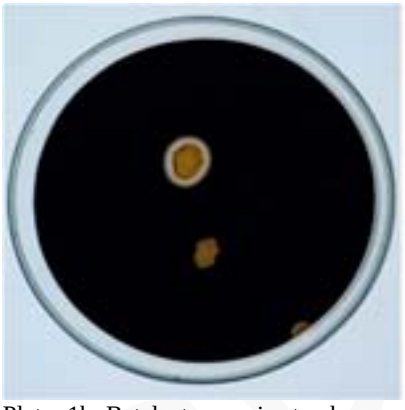

Plate -1b: Betalactamase in starch agar plate

Discussion

The prevalence of uropathogenic microbes has been commonly gram negative bacteria accounting for most infections. This record is in agreement with previous studies (Gupta *et al* 1999). According to a study conducted in Tamilnadu it was found that among the isolated organisms, *E.coli* was the predominant uropathogen (Maya *et al*., 2011). We investigated the possible relationships between *in vitro* biofilm formation, antibiotic resistance and betalactamase.

The present study conveys that, the highest percentage of *E.coli* was found in female samples. The total occurrence was found to be 54% an observation similar to other reports in India (Hasan *et al*., 2007). *Escherichia coli* UTIs were more prevalent in 21-30 of age group persons and specifically in women due to short urethra, controlling sense to urinate, non-hygienic practices, quick access of bacteria to bladder, urethral opening situated near the source of bacteria from anus and vagina which elevates the infections risk. Other studies like, pregnant women, hormonal changes and shifts in UT position during pregnancy makes it easier for bacteria to travel up urethras to the kidneys (Sharma *et al*., 2009). Previous foreign studies also demonstrated that *E.coli* was the most frequent agent causing UTIs (Bronsema *et al*., 1993, Weber *et al*., 1997).

In the present study, it is reveals that most of the isolates from patients of the age group 21-30 years showing high UTI. Our result was like to previous study of Jency roopa and Sudha (2010). Mostly adults are highly affected than other age groups especially women, because they are more likely to get an infection after sexual activity or when using a diaphragm for birth control. Menopause also increases the risk of UTI (http://www.umm.edu/ency/article/000521.htm). In this present study 33.3% and 50% of isolates observed between 0-10 and 11-20 age groups respectively. This could be because of wet bedding, unhygienic condition and toilet training problems. These UT infections result in significant financial and personal costs for both male and female patients and the health care system.

Biofilm formation allows the strains to persist for a long time in the genitourinary tract and interfere with bacterial eradication and initiate colonization and dispersion of pathogenic bacteria inside the host leading to bloodstream infection to UTI (Rajesh *et al*., 2009). It was found that 92.59% of *Escherichia coli* isolates in our study were found to be slime producers. This record was slightly high compared to the previous records (Suman, *et al*., 2005; Sharma *et al*., 2006).

All the isolates offered high degree of resistance against the antibiotics. Totally 73.8% of antibiotic resistance was observed from our isolates. In this study highest resistance of cefodoxime was found to be 100%, which is high as compare to other reports by Kausar *et al.,* 2009. The data reported here indicate that dissemination of high level cefotaxime resistant *E. coli* may be attributable to the rising

prevalence of the CTX-M family of ESBLs has been emerged as an important and rapidly developing worldwide problem. In recent years, ESBL production in *Enterobacteriaceae*, particularly *Escherichia coli*, has been significantly increased in several countries (Jesus Oteo *et al*., 2006).

This ascendant incidence may be due to the indiscriminate use of antibiotics in India. Now a day, ampicillin resistance is budding in clinical isolates of our community. According to an Indian report (Asad *et al*., 2006) the resistance was found to be 90%, while in our studies resistance was found to be 92.5%. In addition, the latter study showed that 91.5% of ampicillin resistance isolated from UTIs (Kausar *et al.,* 2009). In the current study, it reveals that 14.8% of isolates were resistance to 8 antibiotics. This is lowest percentage of antibiotic resistance in our study.

In this current investigation, most of the biofilm producing isolates had multidrug resistance. Retarded diffusion will descendent the concentration of antibiotic entering the biofilm helping an enzyme like betalactamase destroy the incoming antibiotic (Murugan *et al*., 2011). Among the 27 isolates, 96.2% produced betalactamase enzymes. Previous reports also describing the increasing prevalence of ESBL producing organisms among UTI isolates (Taneja *et al*., 2008 and Hassan *et al*., 2007). ESBL-producing *E. coli* showed a significant reduction in the susceptibility to most of the antimicrobials, it results in difficulty in detection and treatment, and increased mortality. At the presence, we found a relation between higher resistance, biofilm and betalactamase forming capabilities of isolates. In this investigation, most of drug resistance isolates produce betalactamase enzymes. 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.

The very difficulty in treatment of UTI associated with biofilm formation because antimicrobial agents are ineffective to penetrating the biofilm decreasing the concentration acting on the bacterial cells. In addition biofilm formation suppresses the phagocytic activity (Zubair *et al*., 2011). 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).

In conclusion this study shows larger number of *E.coli* recovered from UTI from this region produces ESBL. Thus, they are resistant to cefodoxime and Ampicillin, which are important drugs for treatment. The ascendant occurrence of Multi Drug Resistance (MDR) in this region is a cause for concern. Towards the search for newer antimicrobials, it is important to identify the specific mechanism and genes conferring antibiotic resistance in prevalent MDR strains. Further molecular studies may have to be conducted to establish the basis of MDR and government should make substantial effort to establish an antibiotic policy for the country.

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