INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 6, p. 240-246, 2019

RESEARCH PAPER OPEN ACCESS

# Biofilm formation and antimicrobial susceptibility patterns of *Escherichia coli* isolates from urine samples of "urinary tract infections (UTIs)" patients in "District Kohat, Pakistan"

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**Key words:** Urinary tract infection, Antimicrobial susceptibility patterns, *E. coli,* Biofilm.

http://dx.doi.org/10.12692/ijb/15.6.240-246 Article published on December 29, 2019

## Abstract

Urinary tract infections are the most frequent bacterial infections both in nosocomial and community settings. *E.coli* is the prime causative agent of UTIs. Worldwide resistance in *E.coli*against commonly used antibiotics is a major threat to public health. So, there is a need to determine biofilm formation and antimicrobial susceptibility patterns of *Escherichia coli* isolates from urinary tract infection patients in district Kohat. Total 120 Positive mid-stream "urine samples" having "pus cells >10" were collected from patients in sterile urine containers. *E.coli* isolates were identified and confirmed by morphological and biochemical tests. Congo-red agar and Standard Kirby-Bauer methods were performed to find biofilm formation and antimicrobial susceptibility patterns of all isolates respectively. Of 120 positive urine samples, "50 were detected as*E.coli* and out of which, 34 (68%) isolates formed biofilm. All isolates were 100% resistant to ampicillin, 68% to cefixime, 64% to ticarcilin, 48% to gentamicin, 8% to amikacin, piperacillin+tazobactum and meropenem, followed by 4% toimipenem and nitrofruantoin". Fosfomycin showed 100% activity against all *E.coli* isolates. Fosfomycin was the most effective drug against all isolates, so this can be the optimal drug for treatments of UTIs in district Kohat. Moreover, proper susceptibility patterns tests should be performed before prescribing any antibiotics and defined approaches should be adopted to overcome biofilm-based antibiotics resistance to avoid treatment failure.

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## Introduction

Urinary tract infections arethe utmost common bacterial infections worldwide (Stefaniuk *et al.*, 2016). Each year around "150 million people are diagnosed with UTIs", costing above 6 billion US dollars to the world economy"(Akram *et al.*, 2007)".Naturally, urinary tract is sterile. UTIs occur when bacteria from perianal region invade one or more parts of the urinary system and grow into a significant number there. In general population, UTIs are the most common infections with global "occurrence rate of 18 per 1000 person per year(Puca, 2014)". Bacterial access into the blood stream may cause terrible problems, comprising septicemia, shock and hardly death (Al-Badr *et al.*, 2013). Infections in lower urinary tract is known as cystitis which can be recovered by healthy individuals without any serious consequences while infections in the upper urinary tract is known as pyelonephritis which may cause serious morbidity and in some circumstances mortality too (Marrs *et al.*, 2005). Etiologic agents causing UTIs originate from skin or nearby opening of urethra (Kalsoom *et al.*, 2012).

It is the most common infectionnext to upper respiratory infections (Jafri *et al.*, 2014). UTIs are 10-20% and 20-40% of entire infections attended in primary care and hospitals respectively. Due to several causative agents, high rate of occurrence, reappearance, severe consequences and an increase in antibiotics resistance, it is a major concern for medical affiliated professionals (Stefaniuk *et al.*, 2016).The incidence rate is high in patients from lower socio-economical class (Islam *et al.*, 2016). It infects mainly sexually active women, aged people, neonatal, pre-school girls and immunocompromised patients(Puca, 2014; Toval *et al.*, 2014).

It is believed that more than '65% of nosocomial and 80% of total microbial infections"occurs due to biofilms. "Biofilm is defined as immobile microbial community embedded in extracellular polymeric substances (EPS)" that they have made and are irreversibly attached to an interface or substratum or each other.Various studies reported that majority of the isolates from patients with relapse infections were *in vitro* biofilm producers so showing strong relationship of it with persistence and relapse. In addition, the most valuable benefit of the biofilm to bacteria is the increase in antibiotics resistance as it can be able to resist antimicrobials 1000-fold more than planktonic cells due to several mechanisms (Soto, 2014).

*E.coli* is the major causative agent among the gram-negative bacilli as it causing more than 70-90% of UTIs (Abuhandan *et al.*, 2013). For the prescriptions of most sensitive antibiotic, the general practitioners should determine the microbiological profile and antibiotics susceptibility patterns of *E.coli"*(Pradhan *et al.*, 2017)".

Antibiotic discovery was one of the significant achievements as it changed the course of modern medicine. Worldwide antimicrobial resistance is an alarming situation which occurred due to misuse and overuse of antibiotics. According to the 2014 World Health Organization report, the antibiotics resistance is gradually increasing and becoming a major threat for public health (Prestinaci *et al.*, 2015).

This study was performed to determine biofilm formation and antibiotics susceptibility patterns of *E.coli*isolatesin District Kohat so that clinicians can prescribe the most effective drug for empirical treatment of UTIs.

## Material and methods

*Sample collection*

This study was performed in the "Department of Microbiology, Kohat University of science and technology from February to June 2017". Total 120 positive urine samples having >10 pus cells were collected from both indoor and outdoor patients in sterile urine containers from "District Head Quarter Hospital (DHQ) and Liaqat Memorial Hospital (LMH) Kohat". Later on, samples were transferred and instantly processed in "Microbiology research laboratory" of the department under sterile conditions.

*Isolation and identification*

Urine samples were inoculated on "Cysteine Lactose Electrolyte Deficient (CLED) agar" at 37Ċ for 24 hours. To obtain pure growth, colonies expected to be *E.coli* were further sub cultured on MacConkey agar. Confirmation of all isolates was done through morphological and "standard biochemical tests (oxidase, citrate, indole, gas production and sugar fermentation)."

*Antimicrobial susceptibility testing*

"Antibiotic sensitivity testing was performed according to the standard Kirby-Bauer disk diffusion method on Mueller Hinton Agar by using commercially available discs (OXOIDS)" (Kariuki *et al.*, 2007; Shahid *et al.*, 2008). Following ten commonly used antibiotics were used against all isolates. "Ampicillin (10), fosfomycin (50), imipenem (10), amikacin (30), ticarcillin (75), piperacillin-tazobactum (110), gentamycin (10), meropenem (10), cefixime (5) and nitrofurantoin (300)."As per Clinical and laboratory Standard Institute (CLSI) guidelines, listed in Table 1, zones of inhibitions were measured with matric ruler and were interpreted accordingly (Shahid *et al.*, 2008).

*Biofilm production*

Biofilm formation was determined by "Congo-red agar (CRA) method". Congo-red was made as aqueous solution then autoclaved, added to agar and cooled to 55. After inoculation, "plates were incubated for 24 hours at 37°C"(Tajbakhsh *et al.*, 2016).

**Results**

*Urine culture*

A total of 120 positive mid-stream urine samples of both male and female and of all ages were collected in sterile urine containers and 50*E.coli* were identified through morphological, microscopic and "standard biochemical tests including oxidase, citrate, urease, indole, gas production and sugar fermentation".

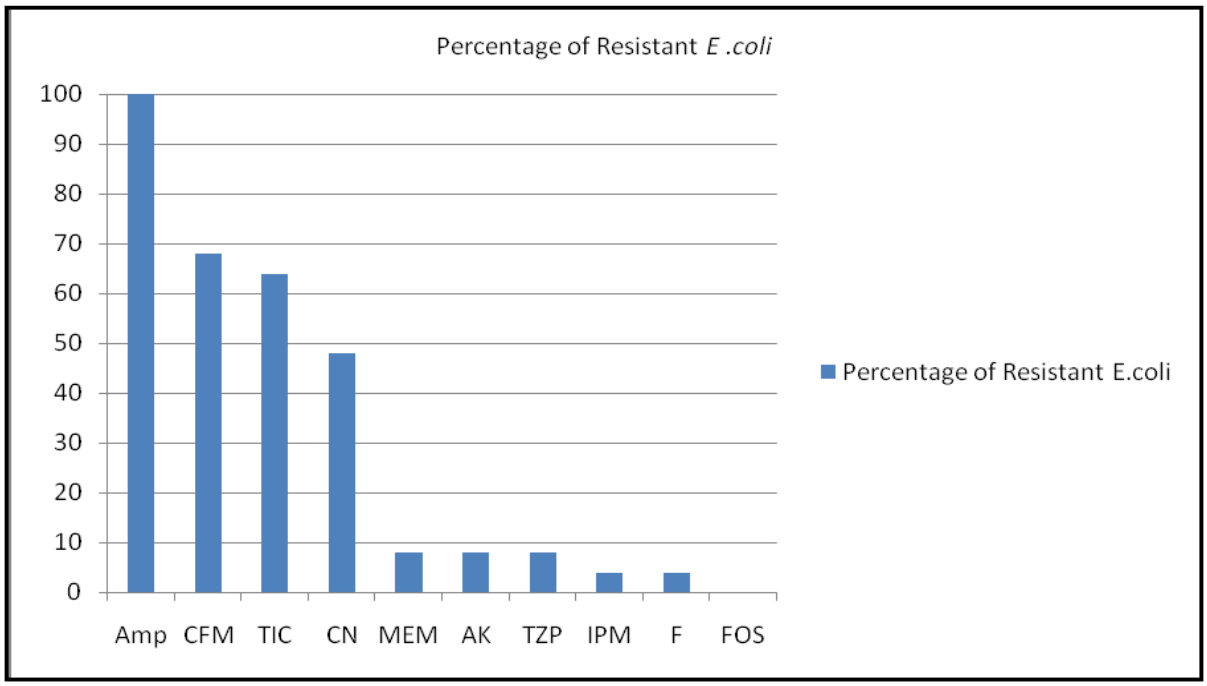
**Table 1.** Standard Kirby-Bauer chart for *E.coli*.

| Antibiotics | Sensitive (mm) | Intermediate (mm) | Resistance (mm) |
|---|---|---|---|
| Ampicillin 10 | 17 or more | 14-16 | 8 or less |
| Fosfomucin 50 | 16 or more | 13-15 | 12 or less |
| Imipenem 10 | 23 or more | 20-22 | 19 or less |
| Amikacin 30 | 17 or more | 15-16 | 14 or less |
| Ticarcillin 75 | 24 or more | 16-23 | 15 or less |
| Pieracillin+Tazobactum110 | 21 or more | 18-20 | 17 or less |
| Gentamicin 10 | 15 ore more | 13-14 | 12 or less |
| Meropenem 10 | 23 or more | 20-22 | 19 or less |
| Cefexime 5 | 19 or more | 16-18 | 15 or less |
| Nitrofurantoin 300 | 17 or more | 15-16 | 14 or less |

*Antimicrobial susceptibility patterns of E.coli isolates*

The antimicrobial susceptibility pattern was done through standard Kierby-Bauer disk diffusion method on Muller Hington Agar (MHA) for all isolates against ten commonly used antibiotics as shown in figure2 and the results are reported as resistant (R) and susceptible (S). As β-lactam antibiotics are frequently used in UTIs treatment however in our study, we have noticed a modification in trends of susceptibility to it.

"All isolates were 100% resistant to ampicillin, 68% to cefixime, 64% to ticarcillin, 48% to gentamicin, 8% to amikacin, piperacillin+tazobactum and meropenem, 4% to imipenem and nitrofurantoin as shown in figure 1. Fosfomycin was the only antibiotic which showed 100% activity and remained effective against all isolates."




AMP= Ampicillin, CFM=Cefixime, TIC= Ticarcillin, CN=Gentamicin, MEM=Meropenem, AK=Amikacin, TZP=Piperacillin+Tazobactum,IPM=Imipenem, F=Ntrofurantoin, FOS=Fosfomycin.

**Fig. 1.** Antibiotics resistance Percentage rate of *E.coli*.

*Detection of biofilm*

In this study, all "*E.coli*"isolates were examined for biofilm production. Among 50*E.coli* isolates, 34(68%) were biofilm producers as they produced dry, black crystalline colonies while 16(32%) are non-biofilm producers showing light pink colonies on Congo-red agar plates.

**Discussion**

This study indicates antibiotics sensitivity testing of *E.coli* isolates from UTIs patients in district Kohat. Resistance in *E.coli* is increases globally, making UTIs treatment and management difficult "(Kariuki *et al*., 2007)".(Kariuki, Revathi *et al*., 2007). In previous study, it is reported that *E. coli* is the major etiologic agent causing 73% of UTIs (Noor *et al*., 2004). "*E.coli* is a Gram-negative bacillus", normal flora of gastrointestinal tract and one of the significant pathogen in humans (Vila *et al*., 2016).

In present study all *E.coli* isolates showed 100% resistance against ampicillin indicating that this antibiotic cannot be used for empirical treatment of UTIs in this region. Earlier studies conducted by "Sabir *et al*., 2014 and Ahmad *et al*., 2015" reported 100% and 92% resistance in *E.coli* against ampicillin which is similar to our results (Ahmad *et al*., 2015). In our study, isolates showed 68% and 64% resistance against cefixime and ticarcillin respectively. Comparable results are reported by(Rahman *et al*., 2009). We observed 48% resistance in *E.coli* isolates against gentamicin. High rate of resistance indicates that this antibiotic should not be used for preliminary therapy of UTIs in this region.Prior studies done by Sumera *et al*., 2014 and Saghir *et al*., 2014, stated 59.8% and 67% resistance of *E.coli* against gentamicin. Gales *et al*., 2001 suggest that rates of resistance in *E.coli* vary between regions and from year to year (Abuhandan *et al*., 2013; Sabir*et al*., 2014).

"To meropenem, piperacillin+tazobactum and amikacin 8% of isolates were resistant in current study. Yavuz *et al*., 2014 reported 0% resistance of *E.coli* to meropenem". Similarly, Min Eui Kim *et al*., 2008 described high activity of amikacin and piperacillin+tazobactum against *E.coli*. This shows that these antibiotics were effective against *E.coli* once but due to self-medication and overuse of antibiotics which is a quite common in this region,

the isolates acquiring new resistance mechanisms. "Another study from Pakistan, performed by Bano *et al.* 2012 reported different resistance rates of amikacin. However, Abuhandan*et al.* 2013 stated 8.5% resistance rate of *E.coli* against amkacin which is very close to our results".

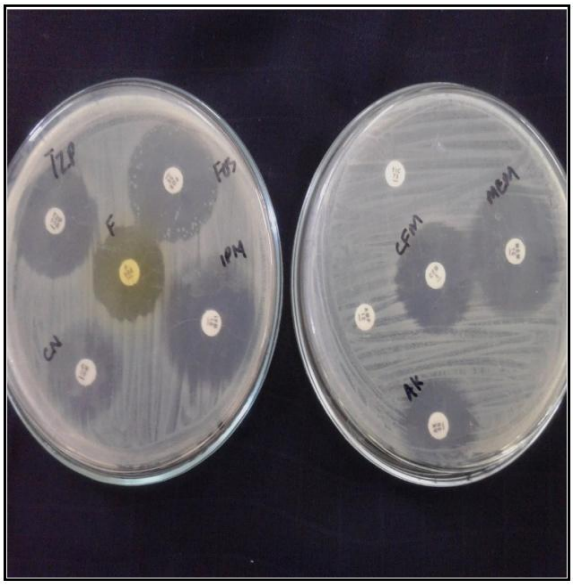



**Fig. 2.** Standard Kirby Bauer method. Circles denote zones of inhibition of different antibiotics discs.

Susceptibility testing of imipenem and nitrofurantoin showed 4% resistance rate of *E.coli* isolates in our study. This indicates that these antibiotics can be used in empirical therapy for UTIs as the isolates demonstrated maximum degree of susceptibility.

These findings were very similar to the results published by Sohail M *et al* 2015 and Abuhandan *et al.* 2013 as they reported 3% and 3.5% of *E.coli* resistance against imipenem respectively. Similarly, Bano *et al.* 2012 stated 5% resistance in *E.coli* against nitrofurantoin.

Fosfomycin showed 100% activity against all isolates in current study so this may be the optimal drug for empirical treatment of UTIs in Kohat region. These findings were similar to previous reports published by Nickel *et al*2007 and Bano *et al* 2012 as their studies demonstrated 98.4% and 96.43% fosfomycin activity against *E.coli* respectively (Nickel, 2007). Another significant aspect of our study was biofilm formation rate in *E.coli* isolates. It is believed that recurrence and persistence of UTIs by uropathogenic*E.coli* (UPEC) is linked with biofilm production (Tapiainen *et al.*, 2014). UPEC can easily form biofilms at bladder wall, surface of catheter material, and in bladder epithelial cells that can defend enclosed bacteria both from antibiotics and host immune system so obstructs UTIs treatment. In addition, bacteria within the biofilms live in close contact so genetic material including antibiotics resistance plasmids and transposons can be easily exchanged(Eberly *et al.*, 2017). As per literature, microorganisms in biofilms can resist antibiotics because of various reasons including low diffusion of antibiotics within the biofilm due to extracellular matrix, less sensitive to growth dependent killing of antibiotics as bacteria in biofilms enter to a non-growing state because of oxygen and nutrients depletion(Patel, 2005). In our study, we found that isolates forming biofilms were less susceptible to antibiotics as compare to non-biofilm producers.

In the present study, it was observed that most of the commonly used antibiotics were ineffective and lost their efficacy against *E.coli*isolates. Increase rate in antimicrobials resistance may be because of inappropriate use, transmission of resistant isolates among people, self-medication, consumption of food from animals that have taken antimicrobials, sales of inferior and unauthorized drugs (Ayukekbong *et al.*, 2017). Various strategies and policies need to be implemented to control an increase rate in antibiotics resistance which includes hygiene and sanitation measures, determination of antimicrobial susceptibility patterns before prescription, strict measures regarding antibiotics prescription and various defined methods adoptation to overcome biofilm based antibiotics resistance(Bano *et al.*, 2014).

**Conclusion**

In conclusion, we found that majority of the isolates were multidrug resistant. Globally, antibiotics resistance is believed as a major risk to public health. Based on our results, we recommend fosfomycin as the drug of choice for treatment of UTIs in Kohat

region. Before prescription, urine culture, antimicrobial susceptibility patterns and biofilms formation should be checked so that chances of treatment failure can be avoided. “Limitations of our study are less sample size due to financial and time constraints. Additional studies with larger sample size will be effective to explain the situation effectively.

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