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Antibiotic Susceptibility Profile of *E. coli* Isolated from urine pus of Leady reading hospital Peshawar Pakistan

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Abstract
The bacterial resistance to antibiotics is one of the main problem worldwide. This study investigated the antibiotics sensitivity and resistance pattern of isolated bacteria from UTIs patients, thus big challenge for antibiotics era. Total 30 urine pus samples isolates during 1 month's period to study and check antibiotic pattern of various organisms. Organisms were identified as per standard operative procedure and drug sensitivity was done. Commonly founded organism was E.coli but some mixed growth also found such as S.aureus and pseudomonas. It was showing sensitivity towards vancomycin, clindamycin and cefoxitin whereas gram negative organisms were showing sensitivity towards penicillin, gentamycin and eztromycine. The study observes that the prevalence of urine pus is high among females (70%) then males (30%). Most of the females of the age between 18-49 years constituted 60.37% of the total patient with urine pus. It has been reported that the adult woman have a higher prevalence number of pus infection then male.

Principally due to the reason sexually or menstrual cycle or physical factors in females. Among male increase prevalence of pus infection was recorded in young age in 20-35 year group.

The study demonstrate that E.coli remain and causing agent organism which cause uropathogen being responsible for 60% of puss infection both male and female. This is inconsistence with finding of other studies in which E.coli was the most frequently reported isolated from patient with pus culture. Following over study show S.aureus spp 27%, and pseudomonas 27% , and E.coli is 40% are the other common uropathogens. Antimicrobial resistance pattern of several antibiotics against E.coli as below ciprofloxacin is 55%, gentamycin 30%, ampicillin 45%, penicillin 25%, tetracycline 24%, erythromycin 15%. Its showed that ciprofloxacin and ampicillin is the best choice toward E.coli spp. Antimicrobial therapy allow to stop microbes growth and mutate into new forms that help them to survive antibiotic treatment and common pathogens such as E.coli other microbes and their resistance pattern can best idea for clinician to choose appropriate antibiotic for experimental treatment of patients.

Keywords: *E.coli*, Antibiotic, susceptibility, pus, culture, urine

Introduction

E. coli is a gram-negative rod-shaped bacteria which belong the genus *Escherichia spp* that is commonly found in the esophagus and fundus region of the stomach lower intestine of human [1]. Most *E. coli* strains are beneficial and help out in digestion and produce metabolites and help in enzymatic activity but some strain of *E.coli* serotypes can cause serious food poisoning in their hosts [2]. *E. coli* found 0.1% of gut flora in human intestine, and spread through fecal oral route which cause disease such urine trace infection, digestive problem and some strain typhoid fever. *E.coli* are able to survive outside the body for short time, *E.coli* is responsible for causing UTI urine trace infection and other skin infection [3].

The virulence strain which cause diarrhea worldwide [4]. The *E. coli* globally, is becoming resistant to antimicrobial agents and has been rising day by day. These strain is particular combination of virulence genes include enterotoxins, endotoxin, capsulated, and lipopolysaccharide. *E. coli* differentiated by serotyping, based. *E.coli* cause certain disease Urinary tract infection (UTI) 70%, Septic infections of wound 56% , Diarrhea 89% ,Dysentery70% ,Septicemia 40% ,Pneumonia 15% ,Neonatal meningitis80% ,Abscess in various organs 23% [5,6].

Diagnosis of *E.coli* Phenotypic/conventional Method, Culturing, *E.coli* can be detected by its colony morphology, physical appearance (Colour, shape, size, margins etc), Microscopy. Microscopy technique can be applied to visualize the cells morphology. The following techniques can also be utilized to detect the *E.coli*, Biochemical techniques, Serological techniques, Molecular techniques [7,8].

Antibiotic therapy and resistance

Antibiotics are usually uses to treat bacterial infection. *E. coli* are showed resistant to many antibiotics that are effective against gram-positive organisms.[9] Antibiotics which may be used to treat *E. coli* infection include tetracycline , clavulanic acid , gentamycin , as well as other generation such penicillin's, cephalosporin's, carbapenems, cefixime , trimethoprim-sulfamethoxazole, ciprofloxacin, aminoglycosides and nitrofurantoin. Broad spectrum Cephalosporin, Aminoglycoside and Fluoroquinolones are the drugs of choice against *E.coli*. Antibiotic resistance is a particular problem in Gram-negative *bacilli*. Some strains are showing high level resistance to aminoglycosides, β-lactam, and quinolones [9,10,11,12].

Antibiotic resistance is a major problem now a days due to misuses of antibiotics. Research study published in the journal of life Science in August 2018 found that the rate of mutations in *E. coli* is much higher than other organism "on the order of 10^{-5} per genome per generation to generation. Which may have significance for the study and management of bacterial antibiotic resistance [13, 14, 15].

E.coli may pass the gene to other species to showed antibiotic resistance this process is called horizontal transfer gene mechanism. *E. coli* bacteria carry multiple drug resistance genes, and under stress situation transfer those plasmids genes to other species is called conjugation process [16].

Beta-lactamase strains

Infections with MDR multi drug resistance *E.coli* increasing with resistance by extended spectrum b-lactamase (ESBL) production. These isolates from hospitalized patients and as well increased cause of community acquired infection too. The risk factors for infection include UTI, ESBLs are able to hydrolyze of the b-lactam antibiotics, including second and third generation such cephalosporin, gentamycin. In addition showed co-resistance to trimethoprim and aminoglycosides. Generally carbapenems considered the drug of choices for the treatment of ESBL-E.coli infections. Extended Spectrum Beta-Lactamases (ESBLs) are the enzymes that persuade resistance to several beta-lactam antibiotics like monobactam, cephalosporin and penicillin. The beta-lactamases production by the bacteria is the main protection tool of *Enterobacteriaceae* against β-lactam antibiotics. These microorganisms are proficient of dropping the efficacy of updated extended spectrum cephalosporin excluding carbapenems and cephamycins through the production of plasmid mediated ESBLs [17,18,19].

Material and methods

This study was carried out in Microbiology Research Laboratory, Abasyn University Peshawar.

Collection of samples

In the proposed study, about 100 samples collected from indoor and outdoor patients and medical equipment from Lady Reading Hospital, Peshawar by using sterile swab, and further processing will be conducted in microbiology research laboratory of Abasyn University Peshawar.

Isolation of *E.coli*

Samples from the clinical patients and medical equipment (BP apparatus, catheters and respiratory equipment, etc) will be inoculated on nutrient agar, Blood agar and MacConkey agar. On nutrient agar *E.coli* appear as pink colour.. MacConkey agar was used as a selective media for the growth of gram negative bacteria and *E.coli* was identified as lactose fermenter on MacConkey. Samples was streaked on media and the plates was at incubated at 37°C for 24hours.

Identification and characterization of *E.coli* isolates

The following tests and procedures were carried out for the identification and characterization of *E.coli* in collected samples.

Morphological and Cultural Characterization

Cultural characteristic is useful for identification of target microbes. Nutrient agar was used for the growth and colonies selected on the bases of size, shape and pigmentation.

Microscopy and Gram staining

Through Gram staining method the bacterial species identified and classified into gram positive and gram negative.

Biochemical Characterization

Clinical samples will be biochemically characterize according to Bergey's Manual of Determinative Bacteriology (9th edition) and for characterization different biochemical tests were be performed (22,).

For characterization and identification of *E.coli* following tests were performed:

- Triple Sugar Iron test (TSI): glucose and lactose fermentation.
- Indole test
- Citrate Utilization test
- Catalase test
- Oxidase test
- Urease test

Synergy disc diffusion method

Synergy Disc Diffusion method will be performed for the detection of ESBL producers. The antibiotic discs of Ceftriaxone (CRO), Cefotaxime (CTX), Ceftazidime (CAZ) and (AZM) will placed at 25 to 30 mm from Clavulanic acid-Amoxicillin. Clavulanic acid + Amoxicillin (AMC =20+10g) placed in the center of inoculated Muller Hinton agar (MHA) plates according to the recommendation of Clinical and Laboratory Standards Institute (CLSI). After overnight incubation, zones of inhibition around the Aztreonam and third-generation Cephalosporin discs will be observed. If the zones of inhibition around the 3G Cephalosporin disc and Aztreonam will close or extended sides to AMC, the isolate bacterial specie will be ESBL producer [20].

Antibiotics sensitivity testing

Disk diffusion method used to determine the susceptibility of the *E.coli* isolates to antimicrobial agents according to Clinical and Laboratory Standards Institute (CLSI, 2006; 2007) guidelines. The antibiotic disc to be used placed on the media plates for 24hours at 37 °C. Zones of inhibition measured for each antibiotic [21].

Following antibiotics were used.

1. Ttracyline
2. Erthromycine
3. Gentmycine
4. Ciprofloxacin
5. Ampecillin
6. Pincilline

Results

Clinical specimens and processing for bacterial isolation

In the current research study among total 30 samples 24 samples were positive for bacterial growth while 6 samples had no growth on culture plates were collected from pathology section and surgical B ward Lady Reading hospital Peshawar Pakistan. Total 24 samples were positive on culture plates for bacterial growth including Gram negative and Gram positive bacteria. Identified bacterial species were *Staphylococcus aureus*, *P. aeruginosa*, *E. coli*, and. These clinical samples through pure culture techniques including various types of media i.e Nutrient agar (N.A). Blood agar, Mannitol salt agar (MSA), Eosin Methylene blue (EMB), and MacConkey agar. While characterization of these collected isolates were screened out by performing microscopy and different biochemical tests, which showed in percentage in graph. In gram staining *Staphylococcus aureus,* was Gram positive and remaining isolates were Gram negative. It was observed that *E.coli* showed gram negative and rod shape bacilli and acidic with no colour change in TSI test but showed negative response toward oxidase, urease, citrate and coagulase test. *E.coli* also showed indole positive response.

Table. 1. Isolated bacterial species from the deep wounds surgical infection.

S.NO	Identified species	No. of isolated species (n)	Percentage
1	*Staphylococcus aureus*	6	27 %
2	*Pseudomonas aeruginosa*	7	27 %
4	*Escherichia coli*	11	46 %

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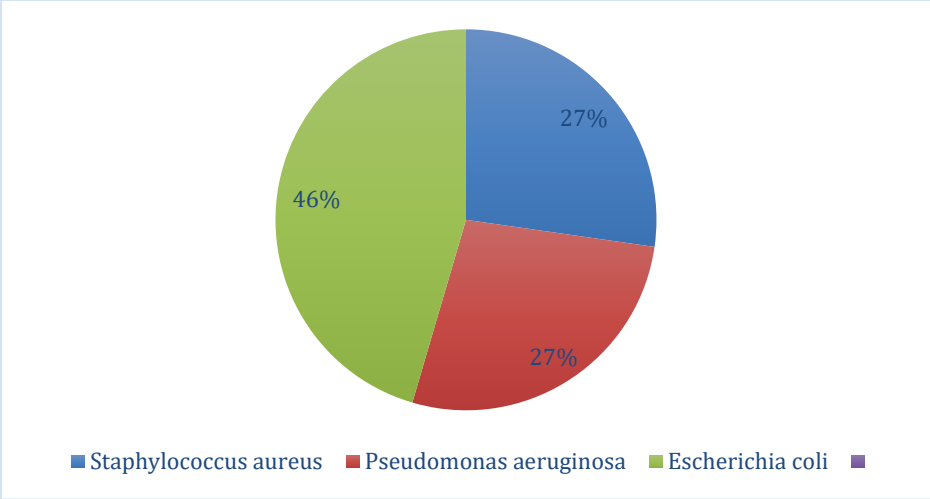

Figure 1.

Identification of collected bacterial isolate

Table 2. Detailed description of microscopy and biochemical test response of test bacterial organisms.

Bacterial species identified	Gram staining	Biochemical tests								
		Lactose	**Dextrose**	**Sucrose**	**H_2S Test**	**Indole test**	**Citrate test**	**Urease test**	**Catalase test**	**TSI test**
E. coli	-	AG	AG	-	-	+	-	-	+	A/NC
Pseudomonas sp.,	-	-	-	-	-	-	+	+	+	+
S. aureus	+	-	-	-	-	+	+	-	+	N/A

Key: + = Positive reaction, - = Negative reaction, d = Variable reaction, Cat = Catalase, Oxi = Oxidase, Cou = Cougulase, Cit = Citrate utilization, Ind = Indole production, Urea = Urease production, TSI = Triple sugar iron KK =no fermenter, K = Red, A = Yellow, K = Alkaline, A= Acidic, NA= Not applicable

Multi drug resistant bacterial isolates

E. coli

Total 24 multi drug resistant bacterial species were screened out and total 6 Antibiotic were used against *E.coli*. The multi-drug resistant bacteria *E. coli* isolate 1 to 7 revealed resistance to Penicillin, Tetracycline, and Ampicillin, Erythromycin and Gentamycin and ciproflxine. Multi drug resistant *E. coli* isolate 1 to 7 showed 18, 16, 14, 15, 18, 17, 14mm inhibition zone respectively when Ciprofloxacin was used.

Table 3.

Sample	P	TET	AMP	CIP	E	GN
***E. coli* 001**	R	12mm	R	17mm	R	12mm
***E .coli* 002**	R	8mm	R	14mm	8mm	18mm
***E. coli* 003**	9mm	R	10mm	13mm	R	R
***E. coli* 004**	R	R	13mm	15mm	R	R

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***E.coli* 005**	8mm	R	9mm	18mm	R	9mm
***E. coli* 006**	11mm	R	9mm	17mm	R	R
***E. coli* 007**	R	9mm	R	14mm	16mm	R

Key: R= Resistant, P= Penicillin, TET=Tetracycline, AMP= Ampicillin, CIP= Ciprofloxacin, E= Erythromycin and GN= Gentamycin.

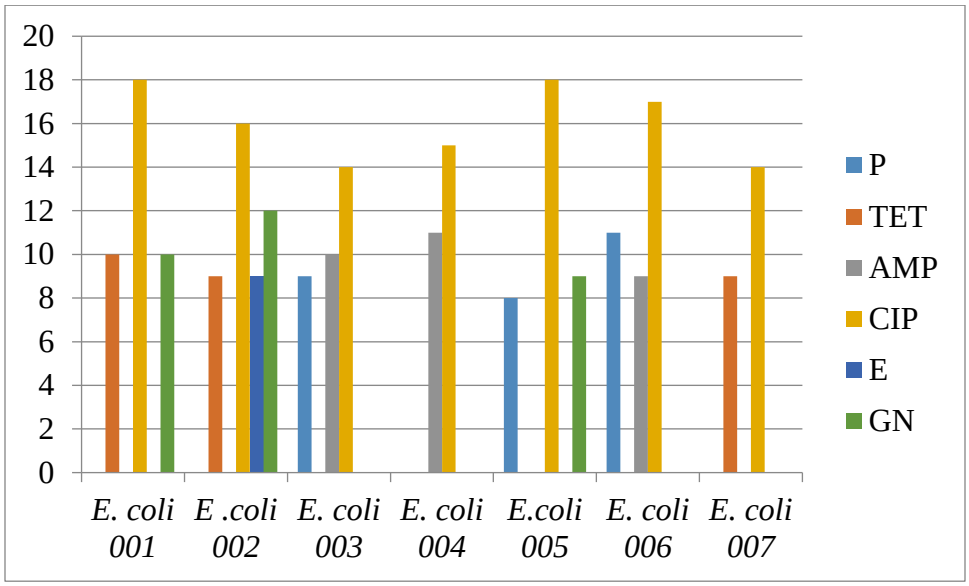
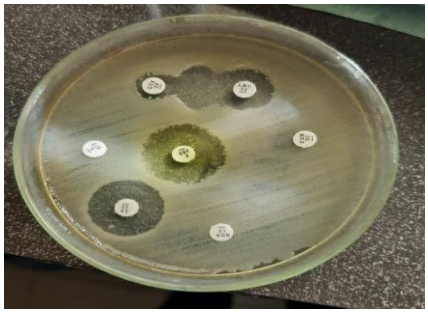
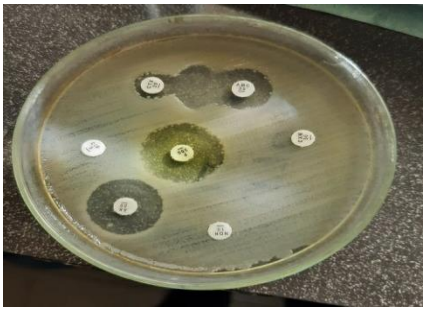


Figure 2.



(a)



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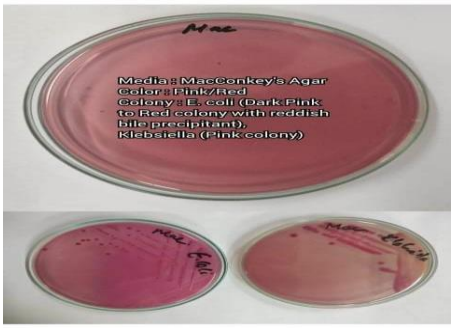

(c)

Fig 3 *E.coli* drug resistivity and culture shown in figure

Discussion

Antibiotic-resistant is a global issue worldwide, but under developing countries are more in threat because of less hygienic conditions and poor clinical infrastructure. In the present study, urine pus infection was among the highest reported clinical diagnosis. In bacterial pathogen, E. coli was reported in the maximum number of studies[22]. UTI are the great issue needs hospitalization among patients. It is also usually the great cause of non-traumatic lower extremity amputations. The Physicians play an important role in the safety measurement, early investigations and management of UTI problems. Management however involves an extensive knowledge of the major risk factors. In the present research study bacterial species were isolated from pus sample. Which were S. aureus, P.aeruginosa, E. coli species from urine pus samples The multi drug resistant bacteria E.coli isolate 1 to 8 revealed resistance to different sort of antibiotics like Penicillin, Tetracycline, Ampicillin, Erythromycin and Gentamycin. Multi drug resistant bacteria E.coli isolate 2, 3, 6, 7 and 8 showed to Ciprofloxacin while isolate 1 and 5 showed susceptibility towards Ciprofloxacin making inhibition zone like 16 and 15mm respectively. Ciprofloxacin is least potent antibiotics towards E.coli. This result which ciprofloxacin was most effective antibiotic. Penicillin, Tetracycline, Ampicillin and Erythromycin were the most ineffective antibiotics against all isolated E.coli spp Gentamycin, Erythromycin and antibiotics were ineffective against E. coli in our study. Ciprofloxacin and ampicillin is the most effective antibiotics against all type of E.coli

Conclusion

The present study is design to identify resistance antibiotics against ***E.coli*** and determine prevalence of in different clinical samples.

1. Collect urine samples from male & female patients having urine pus problem.
2. Identify bacterial pathogens by growth on selective media and different biochemical tests.
3. Check the antibiotic susceptibility of ESBL& MBL production of the isolates towards commercially available antibiotic.

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