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Original scientific article

MOLECULAR BASED IDENTIFICATION OF *Tem ß-lactamase* AND *Tet A* RESISTANCE GENE IN *E. coli*

Madiha AMJAD[1], Qaisar AKRAM[1,2], Humara KAUSER[1], Azam ALI[1*], Qurban ALI[1*]

[1]Institute of Molecular Biology and Biotechnology, the University of Lahore, Lahore Pakistan
[2]Department of Microbiology, University of veterinary and Animal Sciences, Lahore, Pakistan

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Escherichia coli is a universal bacterium causing infections in humans and animal and serves as a major pathogen of urinary tract infections (UTI) and Extraintestinal infection. The present study was conducted for current antibiotic resistance pattern of *E. coli* and molecular detection of resistance related gene in clinical isolates of *E. coli*. The study was a hospital based, prospective study which was done for a period of twelve months. This study was done by using the standard culture techniques for urine, pus, semen and sputum samples, Maximum number was from samples of urine 73 followed by pus 23 semen 2 and sputum 2. Hundred pathogenic *E. coli* isolates was further identified by standard microbiology techniques such as colony morphology, Gram staining and biochemical testing methods. Drug resistance was evaluated by disc diffusion method and relevant drug resistance gene detection done by Multiplex PCR. Out of 130 clinical samples total (n=100) isolates were identified as *E. coli* and their susceptibility patterns for different antibiotics were determined. Results showed that Gentamicin among aminoglycosides and Colistin sulfate among polymyxin were showed relatively less resistance in *E. coli*. Bacitracin, Ampicillin, trimethoprim, Erythromycin, Tetracycline, Ciprofloxacin, Amoxicillin and Piperacillin were found more resistant. Imepenum, Meropenum among β lactam were most effective drug. PCR was employed to identify resistance causing gene. Among 100 pathogenic *E. coli* isolates 87% shown ampicillin resistance encoded by Tem B lactamase gene and 86 % shown tetracycline resistance causing by Tet A gene. Highest level of drug-resistance was observed against ampicillin and tetracycline (AMP-TET) among clinical isolates of pathogenic *E. coli* collected from hospitalized patients.

Key words: Escherichia coli, antibiotics, pathogen, drugs, molecular detection

Corresponding author: Azam Ali, Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan. E-mails: azam.skyblue@gmail.com, saim1692@gmail.com

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INTRODUCTION

Human beings are susceptible to different contagious diseases caused by microorganism such as bacteria, viruses, fungi and parasites. The pathogenic variants caused infection in urinary tract and upper respiratory tract like meningitis, septicemia gastroenteritis, and peritonitis. Gram negative bacteria can cause many types of infections in human body including wound or surgical site infections, pneumonia, meningitis infections and blood stream infections. Gram negative organisms such as *Klebsiella pneumonia* and *Escherichia coli* has emerged as a developing serious health problem occurring in patients surviving in the community as well as in those who have a recent hospital contact (LESKI *et al.*, 2013). Among these bacteria *Escherichia* **coli** is main causative agent of life threatening diseases and found to be associated with different systemic diseases such as colorectal cancer and inflammatory bowel syndrome, UTI and also responsible for endogenous infections in pregnant women (UTI's) intra-abdominal infections, and meningitis in neonates, women surgical site infections and sepsis in all age groups (PATHAK *et al.*, 2013). *E. coli* affects as much as 50% nosocomial UTI patients. *E. coli* is one of the significant causes of acute diarrhoea in Kenya and other under developed countries and may account up to 80% enteritis in children. Now days, there is major threat caused by the attainment of antibiotic resistance by pathogenic bacteria. Rapid occurrence of multiple drug resistant strains is becoming serious health hazard. In the last 50 years bacteria have developed Resistance to almost every antibiotics like resistance to trimethoprim is mostly linked to resistance to other antimicrobial agents like ampicillin. *E. coli* is a major cause of both community infections ad nosocomial infections in humans (DUREJA *et al.*, 2014; KHALID *et al.*, 2021; ARIF *et al.*, 2021).

Incorrect use of antibiotic is the largest selection pressure for antibiotic resistance and supports horizontal transfer of bacterial resistance by mobile genetic elements including transposons, plasmids and gene cassettes are important factors that can play an important role in increase in Multi resistant bacteria**.** Ampicillin antibiotic are among β lactam class of drugs, which are most extensively used in the clinics. They act on penicillin binding proteins (PBPs), which are involved in cell wall synthesis. Beta lactamases are enzymes which are produced by some bacteria and responsible for their resistance to beta lactam antibiotics like Ampicillin, Carbapenem and Penicillin's, used in veterinary and human medicine to treat human and animals infections (MA *et al.*, 2014). Which hydrolyse the five membered beta lactam rings and thus inactivate the antibiotic .Resistance to ampicillin is generally the result of one or more of the three following mechanisms; 1) Resistance through alteration in target site, 2) resistance by alteration in access to the target site and 3) resistance by production of β lactamases. About 200 Beta lactamases have been categorised into eight subgroups and four main groups according to their functional and structural characteristics. TEM-1, TEM-2 and TEM β lactamase is predominant plasmid mediated B lactamases of gram negative rods (YUSUF *et al.*, 2014).

In opportunistic and pathogenic bacteria tetracycline resistance has been emerged.Tetracycline resistance usually results from the acquisition of genes that are involved mainly in three processes: antibiotic efflux through energy dependent membrane associated proteins, ribosomal protection, and enzymatic inactivation of tetracycline. Many bacterial pathogens have developed or acquired resistance to Tetracycline. Tetracycline inhibits the binding of aminoacyl-tRNA's to the A site of the 30S ribosomal subunit, which inhibits protein synthesis. To date, about sixty one tetracycline resistance genes have been sequenced More than

40 different classes of tetracycline resistance genes have been identified. *Tet*(A) and *Tet*(B) most frequently detected *tet*(A),tet(b),tet(C),*tet*(D),*tet*(E) and tet(G) commonly involved in tetracycline resistance These resistance genes are passed as plasmids, integrons, and transposons (GEYER *et al.,* 2013). The present study was conducted to investigate the genetic background for Tem ß--lactamase resistance genes that encode ampicillin resistant and *Tet (A*)genes that encode tetracycline resistance. Report what we believe to be the first identification of full-length *Tet* (A) gene and Tem ß-lactamase Among 100 *Escherichia coli* clinical strains isolated from human samples (KARHAR *et al.,* 2014).

MATERIAL AND METHODS

Collection, identification and storage of E. coli strains

This study was carried out from March 2015 to March 2016 in the Department of Microbiology and Biotechnology University of Lahore, Pakistan. In this study 130 *Escherichia coli* isolates were collected from (males/females with different ages) who attended hospital for UTI's infection Collection were made from the city lab near Jinnah hospital Lahore. Maximum number was isolated from samples of urine 73 followed by pus 23 semen 2 and sputum 2.In order to isolate *E. coli*, samples were directly inoculated on MacCankoy agar (Merck-Germany) Plates. A calibrated wire loop (0.001ml) was used to inoculate each sample After overnight incubation at 37°C, lactose fermenting colonies were streaked on EMB agar (Merck-Germany)(MOMTAZ *et al.,* 2012). It slightly inhibits the growth of gram positive bacteria and provide color indicator distinguishing between lactose fermenter (*E. coli*) from non-lactose fermenter (Salmonella and Shigella), lactose fermenters (*E. coli*) appeared as green sheen on EMB (DERAKHSHANDEH *et al.,* 2013). Typical *(E. coli)* were tested for oxidase presence, citrate utilization, Indole production, Triple sugar iron and hydrogen sulfide production. Isolated strains of *E.coli* were grown on lactose broth and kept as a stock in 25 % glycerol solution and stored at -70°C for long term use.

Antimicrobial Susceptibility testing

This study was done by using the standard culture techniques for clinical samples .Antibiotic susceptibility testing was done on Muller Hinton Agar and the Kirby Bauer disk diffusion method was used to confirm the ESBL production by the clinical isolates of *E. coli* in urine and other samples. Susceptibility tests were performed by disc diffusion method for the following antimicrobial agents. The antibiotic disc and concentration used included Cefaclor (30mcg), imipenem (10mcg) tetracycline (30mcg), meropenem (10mcg) erythromycin (15mcg), piperacillin (100mcg), bacitracin (10mcg), colistinsulphate (10mcg), ampicilin (10mcg), streptomycin (10mcg), gentamycin (10mcg), ciprofloxacin (5mcg), amoxicillin(30mcg) Trimethoprim,(1.25mcg) (MA *et al.,* 2-013).

DNA extraction

For DNA extraction, boiling methods was performed for the preparation of DNA templates. Its methodology is as following, firstly, in 100 µl sterile distilled water few colonies was re-suspended and mixed properly. For 10 minutes at 100˚C cells was lysed by heating. After heating, they were immediately put on ice for 5 min. In order to harvest supernatant

centrifugation was done at 14000 rpm, for 5 minutes. The supernatant were separated by using micropipette and stored at -20°C. The supernatant was used as source of template for PCR amplification. DNA extraction was confirmed by directly visualizing on Agarose gel. PCR were performed for the precise identification of bacterial isolates (ABDULLAH *et al.,* 2011).

Detection of blaTEM –β lactamase and Tet A resistant genes

All 100 Ampicillin-Tetracycline resistant isolates were tested for the presence of TEM β lactamase using the primers Am1-F and Am2-R for amplification on the *bla*TEM gene and Tet A-F and Tet A-R for amplification of *Tet A* genes by using PCR and Among 100 pathogenic *E.coli* isolates 87 shown ampicillin resistance encoded by Tem B lactamase gene and 86 shown tetracycline resistance causing by Tet A gene. The PCR reaction was performed in a DNA thermocycler under the following thermal cycler, conditions: Initial denaturation for 5 min at 94°C followed by 30 cycles of denaturation each of 5 sec at 94°C, 30 sec at 54°C for annealing and elongation for 30 sec at 72°C, and a final extension step of 7 minutes at 72°C, followed by a hold at 4°C (CLERMONT *et al.,* 2000). The PCR products were visualized by running them on 2% Agarose gel electrophoresis and photographed by using smart phone**.**

RESULTS

During the study period total 130 samples were collected from patient with suspected UTIs, among which 100 were confirmed to be an *E. coli.* Then green sheen appearance on EMB Eosin Methylene Blue agar was further purified as *E. coli.* (Figure 1). Rose pink color colonies 2 to 3 mm in diameter and non Mucoid colonies on MacConkey agar were further identified as *E. coli* (Figure 2). 66% isolates were obtained from female and 44% were obtained from male. The percentage of males and female was shown in (Table 1). Antibiotic susceptibility was performed by using different classes of antibiotics which included cefaclor (30mcg), imipenem (10mcg), tetracycline (30mcg), meropenem (10mcg), erythromycin (15mcg), piperacillin (100mcg), bacitracin(10mcg), colistinsulphate (10mcg), ampicilin (10mcg), streptomycin (10mcg), gentamycin (10mcg), ciprofloxacin (5mcg), amoxicillin (30mcg), Trimethoprim (125mcg) (1st, 2nd, 3rd and 4th generation). The zone size around each antimicrobial disk was interpreted as sensitive, intermediate or resistant shown in (Table 2).

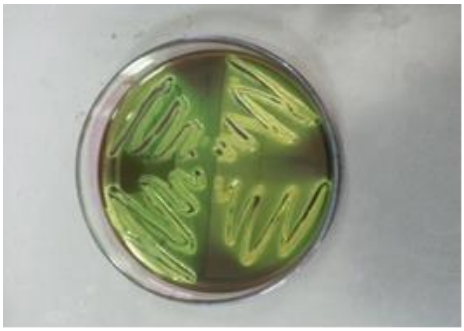

Fig 1. Isolation and purification of *E. coli* on Eosin Methylene Blue agar showing Metallic green Sheen

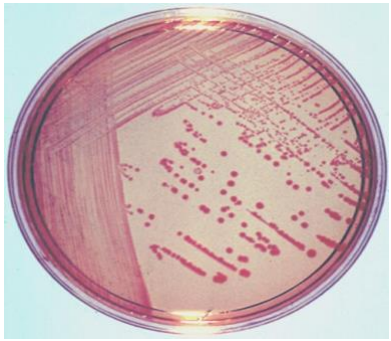

Fig 2. Isolation and purification of *E. coli* on MacCankoy agar showing typical rose pink colonies

These isolates were highly resistant to Bacitracin (100%) and ampicillin (87%). However, some other β-lactam drugs such as Meropenen (97%) and imepenem (99%) were more effective. Among aminoglycosides, although streptomycin (68%) was very ineffective, gentamicin was relatively better (90% susceptibilty).

Table 1. Percentage of male and female positive samples from their source for infected individuals

Total number of positive samples	Gender	No	Percentage	Source of samples	Percentage
N= 100	Male	36	36%	Urine	73%
				Pus	23%
	Female	64	64%	Semen	2%
				Sputum	2%

Table 2. Different antibiotics and their zone of inhibition for E. coli

Sr. No	Antibiotic	Abbreviation	Drug Content	Resistance	Intermediate	Sensitivity
1	Cefaclor	CEC	30 mcg	14	15-17	18
2	Imipenem	IPM	10 mcg	19	20-22	23
3	Tetracycline	TE	30 mcg	11	12-14	15
4	Trimethoprim	W	1.25mcg	10	11-15	16
5	Meropenem	MEM	10 mcg	19	20-22	23
6	Erythromycin	E	15 mcg	13	14-22	23
7	Pipercyline	PRL	100 mcg	17	18-20	21
8	Bacitracin	B	100 mcg	13	14-16	17
9	Colistin sulphate	CT	10 mcg	10	11	12
10	Ampicillin	AMP	10 mcg	13	14-16	17
11	Streptomycin	S	10 mcg	11	12-14	15
12	Amoxicillin	AML	30 mcg	13	14-17	18
13	Gentamycin	CN	10 mcg	12	13-14	15
14	Ciprofloxacin	CIP	5 mcg	15	16-20	21

Table 3. Relevance of drug resistance by disc diffusion method

Groups	Drugs	No of resistant isolates (n:100)	No of intermediate isolates (n:100)	No of sensitive isolates (n:100)	% resistant	% intermediate	% susceptibility
B Lactams	Ampicillin	87	4	9	87	4	9
	Amoxicillin	60	4	26	60	4	26
	Piperacillin	68	8	24	68	8	24
	Imipenem	1	0	99	1	0	99
Carbapenem	Meropenem	2	1	97	2	1	97
Aminoglycosides	Gentamicin	5	5	90	5	5	90
	Streptomycin	68	1	31	68	1	31
Fluoroquinolones	Ciprofloxacin	60	4	26	60	4	26
Polymyxin	Colistin sulfate	9	0	91	9	0	91
Macrolide Antibiotic	Erythromycin	82	18	0	82	18	0
Tetracycline	Tetracycline	86	1	13	86	1	13
	Bacitracin	100	0	0	100	0	0
Dihydrofolate reductase inhibitors	Trimethoprim	89	1	10	89	1	10
Cephalosporin	Cefaclor	72	2	26	72	2	26

Trimethoprim and tetracycline were largely ineffective (89%, and 86% resistance respectively). Cefaclor was relatively ineffective (72% resistance) but as expected fluoroquinolone, ciprofloxacin had better results (60% resistance). However, surprisingly, colistin sulfate had very good results as only 9% isolates showed resistance against this drug. All 100 isolates were multiple drug resistant (MDR). Prevalence of MDR strains of pathogenic *E. coli* was investigated and it appeared that all the screened isolates were resistant to three or more than three of the tested antibiotics 3 (3%) isolates showed resistance to 11 drugs belonging to 9 major groups of antimicrobial agents. Out of total strains 23% shown resistance to 10 different antibiotics. And 33% isolates were resistant to 9 different drugs. And 18% of isolates shown resistance to 8 drugs. While 3% isolates showed resistance to 7 drugs. Out of total stains 6% isolates showed resistance to 5 drugs as shown in (Table 3).

It was noteworthy that the resistance rate of the isolates to tetracycline and Ampicillin was strikingly high, which may in part be due to the frequent, heavy and long-term use of this antibiotic group for the control of bacterial infections in human. In this study, interpretation of drug sensitivity results was done by disc diffusion method; efforts were made to detect most common relevant resistance causing genes: Gene encoding resistance to ampicillin (TEM β Lactamase gene), and one gene for resistance to tetracycline (Tet A). Among 100 (87%) ampicillin resistant isolates, and (86%) tetracycline resistant (Table 3) We have therefore reported the highest prevalence, to the best of our knowledge, of *tet*(A) among tetracycline-resistant *Escherichia coli* and demonstrated that the *tet*(A) gene is highly endemic in Pakistan.

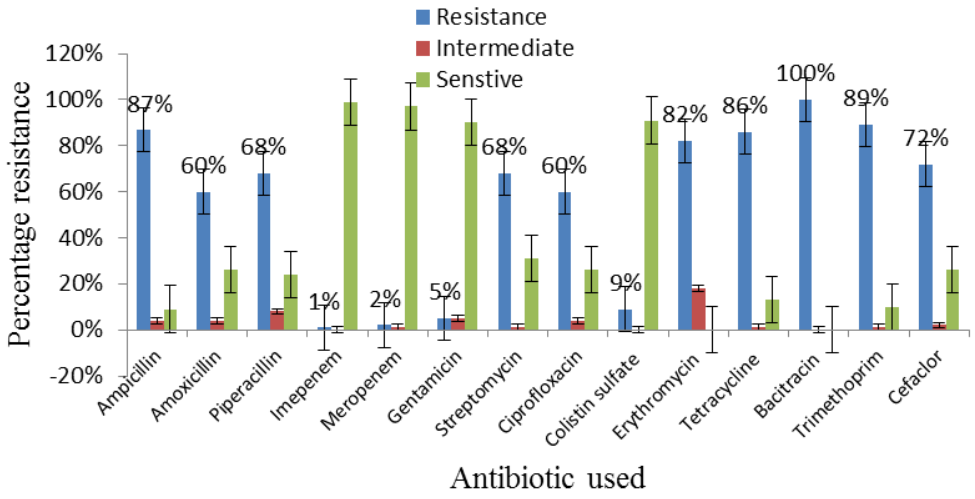

Fig 3. Graphic representation of drug resistance in *E.coli* isolates

The emergence of drug resistance patterns showed high variability among local *E. coli* isolates. All the isolates showed very high level of multiple drug resistance. Similar studies indicating relative efficacy of streptomycin and gentamicin have been reported from Pakistan (FAROOQI *et al.*, 2000). However it has also been reported that there is an increase in resistance against these drugs in urinary *E. coli*. Penicillin's are the major β lactam drugs. We found high resistance to ampicillin (87%). Similar observations have been reported recently Among Tetracycline Bacitracin 100% was totally ineffective, whereas piperacillin (68%) and amoxicillin (60%) colistin sulfate(9%) gave much better results with disc diffusion method. ciprofloxacin (60%) had intermediate activity. Majority of resistant isolates belonged to beta lactam and tetracycline groups (BRANGER *et al.*, 2005). Tetracycline resistant isolates of *E.Coli* was found to be dominant as far as resistance to other antibiotics is concerned as shown in Figure 3.

Each isolate was investigated by molecular methods for the presence of relevant drug resistance genes. In local isolates, resistance to β lactams was due to the presence of *Tem β lactamase* Among 49 ampicillin resistant isolates, 34 isolates were positive for tem β lactamase (Amplification product of 876bp) (Fig 5/Table 4) (CHIU *et al.*, 2002).

Ampicillin resistance related β lactamase gene (TEM β lactamase gene)

Primer	Primer sequence	Amplicon size	Targeted gene	Reference
Am1-F	ATGAGTATTCAACATTTCCGTGT	876	Tem Beta	Chu *et al.*
Am2-R	TTACCAATGCTTAATCAGTGAGG		lactamase	(2002)

Tetracycline resistance related genes (Tet A gene)

Primer	Primer sequence	Amplicon size	Targeted gene	Reference
Tet A-F	GGTTCACTCGAACGACGTCA	577	TET A	MUMTAZ *et al.*
Tet A-R	CTGTCCGACAAGTTGCATGA			(2012)

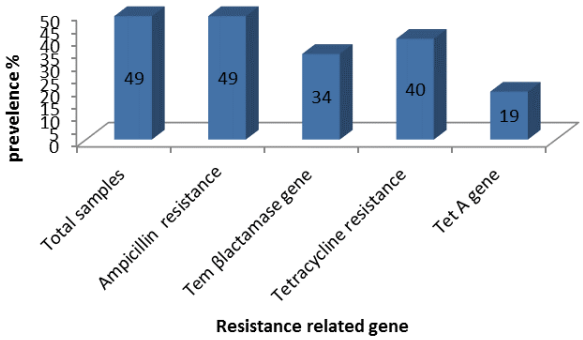

Figure 4. Graphic representation of Ampicillin and Tetracycline resistance related gene in *E.coli* isolates

Table 4. Drug resistance gene profile of each isolates

Sr.no	Tem B lactamase	Sr.no	Tem B lactamase	Sr.no	Tet A	Sr.no	Tet A
1	+	26	-	1	+	24	-
2	-	27	+	2	-	25	+
3	+	28	+	3	+	26	-
4	+	29	-	4	+	27	-
5	+	30	-	5	-	28	-
6	+	31	+	6	+	29	+
7	+	32	+	7	+	30	-
8	-	33	+	8	-	31	+
9	+	34	-	9	-	32	+
10	-	35	+	10	-	33	-
11	+	36	+	11	+	34	+
12	+	37	-	12	+	35	-
13	+	38	+	13	-	36	+
14	+	39	-	14	+	37	+
15	-	40	+	15	-	38	-
16	+	41	+	16	+	39	+
17	+	42	+	17	-	40	-
18	+	43	+	18	-		
19	+	44	+	19	-		
20	+	45	-	20	-		
21	-	46	+	21	-		
22	+	47	+	22	+		
23	+	48	-	23	+		
24	-	49	-				
25	+						

Similarly, for Tetracycline resistance one tetracycline resistance gene Tet A gene were targeted. 19 isolate out of 40 was positive for Tet A gene amplification product of 577 bp (Fig 6 and Table 4) (MOMTAZ *et al.*, 2012).

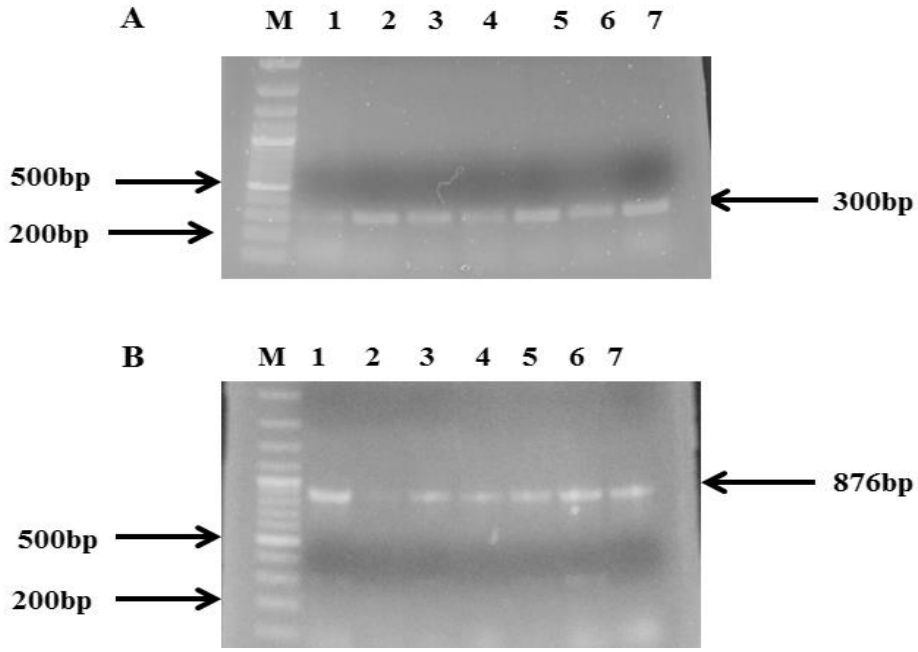

Figure 5. PCR for identification of ampicillin resistance gene (*tem β lactamase* gene)

(A) Represent the GAPDH gene amplification product of *E.coli* as an internal control
Lane M: Gene Ruler (Fermentas) showing bands of 100,200, 300, 400, 500, 600, 700, 800, 900 and 1000 bps.
Lane 1-7: *E. coli* isolates showing amplification product of 300bp

(B) PCR for identification of ampicillin resistance gene (*tem β lactamase* gene)
Lane M: Gene Ruler (Fermentas) showing bands of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100
Lane 1-7:*E. coli* isolates showing amplification product of *tem β lactamase* gene (876 bp)

In the present study 89 % resistance for trimethoprim was observed which showed that it has become relatively ineffective for *E. coli*. This level of resistance is much higher than that reported elsewhere**.**

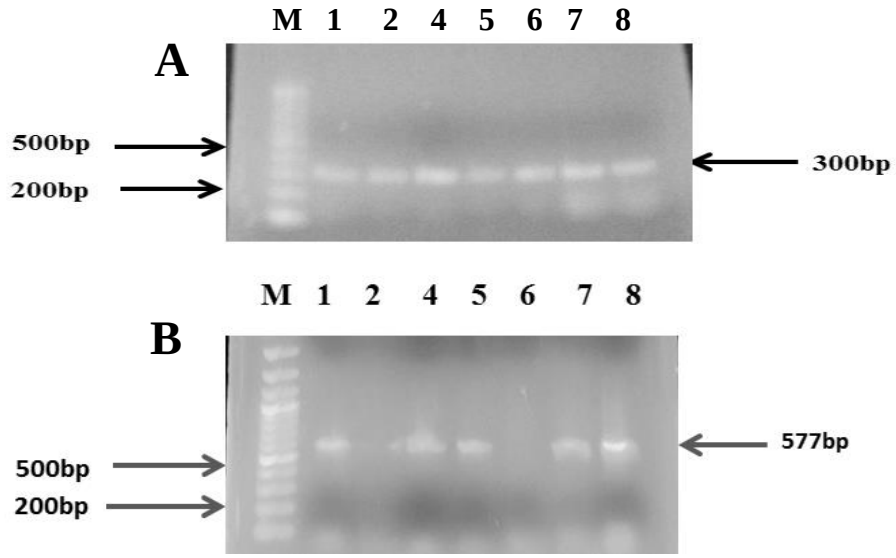

Figure 6. PCR for identification of tetracycline resistance gene (Tet A)

(A) Represent the GAPDH gene amplification product of *E.coli* as an internal control

Lane M: Gene Ruler (Fermentas) showing bands of 100,200, 300, 400, 500, 600, 700, 800, 900 and 1000 bps.

Lane 1-8: *E. coli* isolates showing amplification product of 300bp

(B) PCR for identification of Tetracycline resistance gene (*Tet A* gene)

Lane M: Gene Ruler (Fermentas) showing bands of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100

Lane 1-8:*E. coli* isolates showing amplification product of *Tet A* gene (577 bp)

DISCUSSION

In the present study we found that 87% of our isolates were resistant to ampicillin belonged to beta lactam group, where as 86% resistant to tetracycline were belonged to tetracycline group. Our findings are in line with other studies where it was found that major strains of *E. coli* mainly resistant to tetracycline and ampicillin. In the present study the drug resistance in *E. coli* was studied by using standard disc diffusion method. The isolates showed different multiple drug resistance patterns. Fourteen drugs encompassing all major groups and their respective generations were used in this study. All the 100 isolates studied showed resistance to at least 10 drugs by disc diffusion methods. So, all the isolates can be labeled as MDR.

In local isolates, resistance to β lactams was due to the presence of, *tem β lactamase* (TIMOFTE *et al.*, 2014). Found *TEM* type of β lactamase genes responsible for drug resistance. Point mutations in the *TEM* type of beta lactamase genes were highly responsible for resistance. Form the past two decades; trimethoprim alone has been used widely as empirical therapy against *E. coli.*

In this current research very high level of tetracycline resistance (86%) was observed by disc diffusion method (MOMTAZ *et al.,* 2012) also reported an increase in the tetracycline resistance in human isolates and considered it as unexpected due to the fact that in humans tetracycline use is less than in animals. Among 40 tetracycline resistance isolates, 19 *Tet A* genes were detected. This finding is in accordance with some previous reports which showed that *Tet A* gene is frequently responsible for resistance to tetracycline in the clinical isolates. Among quinolones and fluoroquinolones, resistance to ciprofloxacin was also found in 60% isolates respectively by disc diffusion method. This high occurrence of resistance is in sharp contrast to some other studies (DAREHABI'S *et al.,* 2013). Reported only one ciprofloxacin resistant strain of *E. coli*. However, SHARIF *et al.,* (2013) has reported an emergence of fluoroquinolone resistant *E. coli* responsible for UTI.

Our finding was that gentamicin show 5% resistance only. No resistance to imipenem was observed in isolates studied. Same results have been obtained by other studies; they found no imipenem resistance in *E. coli* isolates. High sensitivity of *E. coli* strain to imipenem has been reported earlier. It seems that this drug can be used as drug of choice for treatment of UTIs caused by *E. coli*. However it should be noted that non limited administration of antibiotics drugs can gradually lead to antibiotic resistance. Out of 50 isolates, 35 showed correlation between drug resistance gene and drug susceptibility findings (Table 4), where as in some isolates gene was absent but showed resistance by disc diffusion method. This may be because of inactivated genes in this isolate (OHEIKU *et al.,* 2013; ALI *et al.,* 2014; DANISH *et al.,* 2020; ALI *et al.,* 2020; KHALIL *et al.,* 2020ab). The geographical patterns and social practices may play a significant role in determining resistance patterns. Our results show that disc diffusion method and molecular detection of relevant genes were not always comparable. The reason for failure in molecular detection may be that a variety of mechanisms are responsible for acquired bacterial resistance to various antibiotics and these resistance mechanisms are encoded by different genes and it is not possible to check for all those genes. So it is a common practice that only the most common genes are selected for investigation (ALI *et al.,* 2014; TAHIR *et al.,* 2020; BASHIR *et al.,* 2020; MUSTAQ *et al.,* 2020). On the other hand absence of phenotypic drug resistance in presence of relevant genes may be due to inactivation of these genes due to some mutation. Our study showed that, isolates were positive for tem Beta lactamase gene. Among 40 (19) isolates were positive for Tet A gene. The difference in findings among distinct populations in different studies may be due to geographical climatic conditions, health status of the host, use of antibiotics, dietary factors, or host genetic factors, additionally to the differences arising from different sampling areas (Fig 4).

CONCLUSION

In summary, we have reported the extensive study of the prevalence and distribution of tetracycline resistance and ampicillin resistance genes in *Escherichia coli* isolates from clinical samples. From Our data it can be concluded that all local pathogenic *E.coli* are multiple drug resistant (MDR) and have a battery of virulence factors which makes them a serious and challenging health problem. In literature we found that among local isolates, beta lactam group have harbored multiple drug resistance and related genes to a greater extent and are responsible for high pathogenicity. Out of 49 ampicillin resistance isolates 34 were positive for *tem β lactamase* gene and 19 samples carrying *Tet (A)* gene were identified from 40 tetracycline

resistance isolates. Twelve isolates were detected carrying both *Tem B lactamase* and *Tet A* resistance gene. It was also found that most effective drugs were gentamicin among aminoglycosides; imipenem and meropenem among β lactams and colistan sulfate. Streptomycin, trimethoprim, tetracycline and ampicillin were relatively ineffective. In summary this study reveals the effective and non-effective drug to be used against most infectious bacteria *E. coli*. Clinically, antibiotics should be used according to the results of the susceptibility tests to reduce the prevalence of drug resistant strains, which will allow us to control the increase and spread of bacterial resistance. Further study would be done to solve universal problem of antibiotic resistance. This study will contribute significantly in collecting national data regarding tem β lactamase gene and Tet A gene distribution in Pakistan.

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MOLEKULARNO ZASNOVANA IDENTIFIKACIJA *Tem ß-lactamase* I *Tet A* REZISTENTNIH GENA KOD *E. coli*

Madiha AMJAD[1], Qaisar AKRAM[1,2], Humara KAUSER[1], Azam ALI[1*] and Qurban ALI[1]

[1]Institut za molekularnu biologiju i biotehnologiju, Univerzitet u Lahoru, Lahor, Pakistan
[2]Departman za mikrobiologiju, Univerzitet za veterinu i animalne nauke, Lahor, Pakistan

Izvod

Escherichia coli je univerzalna bakterija koja izaziva infekcije kod ljudi i životinja i služi kao glavni patogen urinarnih infekcija (UTI) i ekstraintestinalnih infekcija. Ova studija je sprovedena za trenutni obrazac rezistencije *E. coli* na antibiotike i molekularnu detekciju gena povezanog sa rezistencijom u kliničkim izolatima *E. coli*. Studija je sprovedena u bolnici I rađena je u periodu od dvanaest meseci. Ova studija je urađena primenom standardnih tehnika culture urina, gnoja, sperme i ispljuvka. Maksimalan broj je bio iz uzoraka urina 73, zatim gnoja 23, sperme 2 i ispljuvka 2. Standardnom mikrobiološkom tehnikom dalje je identifikovano stotinu patogenih izolata *E. coli* kao što su morfologija kolonija, bojenje po Gramu i biohemijske metode ispitivanja. Otpornost na lekove je procenjivana metodom disfuzije diska, a odgovarajuća detekcija rezistencije gena na lekove izvršena je Multiplex PCR-om. Od 130 kliničkih uzoraka, ukupni (n = 100) izolati su identifikovani kao *E. coli* i utvrđeni su njihovi obrasci osetljivosti na različite antibiotike. Rezultati su pokazali da su gentamicin među aminoglikozidima i kolistin sulfat među polimiksinima pokazali relativno manju rezistenciju *E. coli*. Bacitracin, ampicilin, trimetoprim, eritromicin, tetraciklin, ciprofloksacin, amoksicilin i piperacilin su bili otporniji. Imepenum, meropenum među β laktamima bili su najefikasniji lek. PCR je korišćen za identifikaciju gena koji izaziva rezistenciju. Među 100 patogenih izolata *E. coli* 87 % je pokazalo rezistenciju na ampicilin kodiran genom Tem B laktamaza, a 86 % pokazalo rezistenciju na tetraciklin uzrokovanu genom Tet A. Najviši nivo rezistencije na lekove primećen je kod ampicilina i tetraciklina (AMP-TET) među kliničkim izolatima patogene *E. coli* prikupljenim od hospitalizovanih pacijenata.

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