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Prevalence of Antibiotic Resistance in Clinical Strains of *E. Coli* and Their Control

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Abstract : *The present study was to evaluate the isolates of antibiotic resistant E. coli in clinical samples and their control. A total of 50 E. coli strains were isolated from samples such as ENT, Pus and UTI. 5 different antibiotics were tested. 20% of the strains showed resistance to single antibiotic (AMP, AMX), whereas rest of the strains to 2-5 antibiotics (i.e.) AMP-AMX (6%); AMP-AMX-CXM (16%), AMP-AMX-CN (20%), AMP-AMX-CN-CPD (20%), and AMP-AMX-CXM-CN-CPN (18%). MAR index for the isolates was found to be from 0.4-1.0. 4% of the strains showed a MAR index of 0.4 and 20% strains showed a MAR index of 1.0. The presence of ESBL production in various E.coli strains which was indicative through exhibition of resistance to first, second as well as third generation cephalosporin. Among 50 strains, 30 strains (60%) were observed to possess CTX type of β-lactamases. 62% of clinical strains were positive for haemolysis. Among them 20 (87%) of them were from UTI samples, 5 (29.41%) of them were from ENT samples and 6 (60%) of them were from Pus samples. Plasmid profile of the E.coli isolates was found to be >23kb. All the resistant strains tested against β-lactam antibiotics, lost resistance during plasmid curing.*

Keywords: *E.coli, Haemolytic, β-lactam, Antibiotic resistance*

I. Introduction

The increase in fecal pollution in source water is a menace not only in developing countries but also in developed countries. Water borne bacterial pathogens viz., *E.coli*, *Salmonella*, *Shigella* and *V.cholerae* can lead to outbreak of intestinal diseases and result in serious health implications as well as economic loss. Improper management of sewage as well as industrial wastes and their entry in to the water ways finally pollute the coastal waters.

Most populated cities as well as most of the industrial units are located either on the banks of the rivers (or) nearer to the coastal cities and this situation holds good worldwide. Few studies demonstrated statistically significant correlation between industrial pollution and the spatial distribution of antibiotic resistance (Goni-urriza *et al.,* 2000 and McArthur and Tuck field, 2000). Heavy use of antibiotics for medical and veterinary purposes (White *et al.,* 2000 and Balague and Vescovi, 2001) as well as the domestic and agricultural use of pesticides and related compounds (Balague and Garcia Vescovi, 2001) caused significant antibiotic contamination of the natural environment and consequent development of resistantance in communities.

The resistance developing in one part of the country, or indeed in the world, can be disseminated readily (Greenwood, 1998). The problem of microbial drug resistance is a major public health concern due to its global dimension and alarming magnitude. The major resistance issues overall are, those which are related to the methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRF), extended-spectrum β-lactamase producing *Enterobacteriaceae*, and the multidrug-resistant *P.aeruginosa* and *Acinetobacter baumannii* (Rossolini and Mantengoli, 2008). In the present investigation *E. coli*, an emerging pathogen was dealt with regarding its antibiotic resistance in the clinical resistance environment.

Resistance mechanisms can either be an intrinsic property of a bacterial species or an acquired trait. Acquired resistance occurs as a result of chromosomal mutations or by the acquisition of genetic elements. Intrinsic resistance covers a whole bacterial species and provides resistance without the addition of genetic elements or mutations.

The β-lactamases execute their effect in hindering the work of β-lactams by hydrolyzing the β-lactam ring structure (Goering, 2008 and Bush, 2010). These can be encoded on both plasmids and chromosomes (Poole, 2004; Goering *et al.,* 2008 and Bush, 2010). The plasmid-encoded β-lactamases are related to the chromosomally-encoded enzymes (Jacoby, 2009). B-lactamases can be transferred within different bacteria (Poole, 2004 and Bonnet, 2004). The β-lactamases are mostly extracellular in Gram-positive bacteria, while periplasmic in Gram-negative species (Goering *et al.,* 2008 and Bush, 2010). *Enterobacteriaceae* are the significant causes of serious infection, and many of the most important members of this family are becoming increasingly resistant to currently available antimicrobials. Two organisms of concern are *E. coli* and *Klebsiella*

pneumoniae, opportunistic pathogens of humans and animals are responsible for a wide range of infections, such as urinary tract infections, pneumonia, wound infections and septicemia (Slama *et al.,* 2010). The present study was to evaluate the isolates of antibiotic resistant *E. coli* in clinical samples and their control.

II. Materials And Methods

Collection of clinical samples

Clinical samples (25 nos. were collected from hospitals located in Chidambaram (Cuddalore district) area using sterile containers and were brought to the laboratory and stored at 4°C. All the samples were brought to the laboratory immediately and analyses were made within two hours of collection.

Antimicrobial susceptibility testing

Antibiotic susceptibilities of the isolates were determined by both well diffusion using Muller Hinton agar and 5 antibiotics namely Ampicillin (AMP) - 25µg, Cefuroxime (CXM) - 30µg, Amoxcillin (AMC) - 30µg, Cefpodoxime (CPD) - 10µg, Cephalexin (CN) - 30µg.

Multiple antibiotic resistances (MAR) index

The multiple antibiotic resistance (MAR) index of each strain was calculated according to the method described by Krumperman (1983) using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested.

Disc susceptibility test to screen for ESBL

All clinical isolates were screened for ESBL production using three indicator cephalosporins, namely ceftazidime (30µg), cefotaxime (30µg) and cefpodoxime (30µg). The isolates were considered to be resistant if the diameter of the inhibition zone for ceftazidime, cefotaxime or cefpodoxime was ≤22 mm, ≤27 mm or ≤17 mm, respectively. The strains that showed resistance to at least one of the three cephalosporins were tested further using phenotypic confirmation methods as per Srisangkaew and Vorachit (2004).

Double disc synergy test (DDST)

E. coli isolates showing resistance to any of the three indicator cephalosporin's were tested for ESBL production by the DDST. Cefotaxime (30µg), cefuroxime (30µg), cefpodoxime (30µg) and amoxicillin/clavulanic acid (Amoxicillin 20µg + clavulanic acid 10µg) (Hi-Media Laboratories Ltd., Mumbai, India) were used for ESBL detection (Datta *et al.,* 2004 and Duttaroy and Mehta, 2005). Amoxicillin/clavulanic acid and third generation cephalosporin discs were placed at a distance of 20 mm from center to center on lawn cultures on Muller-Hinton agar plates. The plates were incubated at 37^{o}C overnight. Any enhancement in zone of inhibition of cephalosporin towards the amoxicillin/clavulanic acid disc was considered a positive result for an ESBL.

Haemolytic activity

For detection of hemolytic activity, the *E. coli* isolates were grown on tryptose soy agar with 5 % defibrinated sheep blood or 5 % sheep erythrocytes washed in saline (0.85 %) with or without 10 mM $CaCl_2$ or 10mM EDTA added. The blood agar plates were incubated at 37^{o}C for 18-24hrs and then examined for the presence of haemolysis zones around bacterial colonies (Beutin *et al.,* 1989).

Plasmid isolation

Plasmid DNA profiling of the strains was done by the alkaline lysis method (Sambrook *et al.,* 1989). All bacterial strains were incubated overnight in 5 mL Luria Bertani broth at 37 °C. The bacterial cells were collected by centrifugation at 10,000 rpm for 5min. in a 1.5 mL micro centrifuge. To the pellet 100 µL of glucose-Tris-EDTA buffer was added and completely re-suspended. To that 200 µL of lysis buffer solution was added and mixed gently for 5 min. The tube was spun at 10,000 rpm for 5 min. and supernatant was transferred to a sterile eppendorf tube. Equal volume of phenol: chloroform was added vortexes, centrifuged at 10,000 rpm for 5 min and supernatant was transferred to a clean eppendorf tube. Plasmid DNA that extracted was precipitated and concentrated by using ice cold isopropanol. The resolution of the isolated plasmid DNA was checked on 0.8% agarose gel. 1kb DNA ladder and Lambda DNA Hind III digest marker DNA were used as size standards (Fermentas).

Plasmid curing

The role of plasmids in the antibiotic resistance was confirmed by curing the plasmid with acre dines orange at a concentration of 500µg/ml added to the nutrient broth. The culture was incubated at 37°C for 12 hrs (Fugii *et al.,* 1997). Antibiotic resistance patterns of the strains before and after curing of plasmids were compared. The curing was confirmed by loss of plasmid and antibiotic susceptibility testing using antibiotics to which organisms were resistant.

III. Results

Antibiotic Resistance of Clinical Strains

In the present study 5 different antibiotics were tested to study the antimicrobial susceptibility of the *E.coli* isolates. Among the tested antibiotics Ampicillin and Amoxicillin were belong to β-lactams, whereas, cephotaxime, cephalexin, cefpodoxime respectively belong to first, second and third generation cephalosporines which are also considered group of β-lactams. A total of 50 *E. coli* strains were used for testing their antimicrobial susceptibility. Regarding susceptibility of *E. coli*, the resistant and sensitive patterns were tested to 5 antibiotics at a concentration of Ampicillin (AMP) - 25µg, Cefuroxime (CXM) - 30µg, Amoxicillin (AMC) - 30µg, Cefpodoxime (CPD) - 10µg and Cephalexin (CN) - 30µg.

In the present study Ampicillin showed 100% resistance followed by Amoxicillin (82%), Cephalexin (58%), Cefuroxime (54%) and Cefpodoxime (38%) (Table-1).

In the present study 20% of the strains showed resistance to single antibiotic (AMP, AMX), whereas rest of the strains to 2-5 antibiotics (i.e.) AMP-AMX (6%); AMP-AMX-CXM (16%), AMP-AMX-CN (20%), AMP-AMX-CN-CPD (20%), and AMP-AMX-CXM-CN-CPN (18%) (Table-2).

In this study, MAR index for the isolates was calculated and the range was observed from 0.4-1.0. Among the tested isolates for multiple antibiotic resistance (MAR), 4% of the strains showed a MAR index of 0.4 and 20% strains showed a MAR index of 1.0 . The present study indicated the presence ESBL production in various *E.coli* strains which was indicative through exhibited resistance to first, second as well as third generation cephalosporins. Among 50 strains, 30 strains (60%) were observed to possess CTX type of β-lactamases (Table 3). The other strains may possess other type of β-lactamases (or) any possess other mechanism to attain resistance status against these antibiotics.

Haemolytic activity

Regarding haemolytic activity 62% of clinical strains were positive for haemolysis. Among them 20 (87%) of them were from UTI samples, 5 (29.41%) of them were from ENT samples and 6 (60%) of them were from Pus samples, (Table -4).

Plasmid

Regarding the plasmid profile of the *E.coli* isolates used in this study plasmids of >23kb were found. In this work plasmids of different types (i.e.) <10kb and >23kb were found in *E. coli* strains analyzed. 10 strains covering all patterns of antibiogram were selected for plasmid study. Their resistance patterns are given in Table -2. One strain which was resistant AMP alone did not possess any plasmid whereas another similar strain possessed 1 plasmid. The resistant patterns of varying nature (resistance up to 4 antibiotics) also possessed only one plasmid. However 2 strains which possessed 4 and 5 plasmids were resistant to all the 5 antibiotics tested and were isolated from UTI patients (Fig. 1 and Table 5).

Plasmid isolation and curing

Plasmid curing showed interesting results. Towards antibiotics like Ampicillin, Amoxicillin and Cephalosporin's, strains lost their resistance status after plasmid curing. When plasmid curing was done all the resistant strains tested against β-lactam antibiotics tested in the present investigation lost resistance.

Table 1: Antibiotic resistance percentage

Antibiotic	No. of strains resistant for the tested total of 50 isolates	% of resistance
AMP(Ampicillin)	50	100%
AMX(Amoxicillin)	41	82%
CXM(Cefuroxime)	27	54%
CN(Cephalexin)	29	58%
CPD(Cefpodoxime)	19	38%

Table 2: Antibiotic resistance pattern and MAR index

Sample sources of the isolates	Total No. of isolates	Source wise total isolate %	Antibiotic pattern	Antibiotic pattern %	MAR index
ENT	5	ENT – 30%	AMP	10	-
ENT	5		AMX	10	-
Pus	3	UTI – 46%	AMP-AMX	6	0.4
UTI-4, ENT-2, Pus-2	8		AMP-AMX-CXM	16	0.6
UTI-5, ENT-2, Pus-3	10	Pus – 10%	AMP-AMX-CN	20	0.6
UTI-5, ENT-3, Pus-2	10		AMP-CXM-CN-CPD	20	0.8
UTI	9		AMP-AMX-CXM-CN-CPD	18	1.0

Table 3: Extended spectrum of Beta lactamase (ESBL)

Strain	ESBL %	No. of strains tested
E.coli from clinical samples	60%	30/50 strains

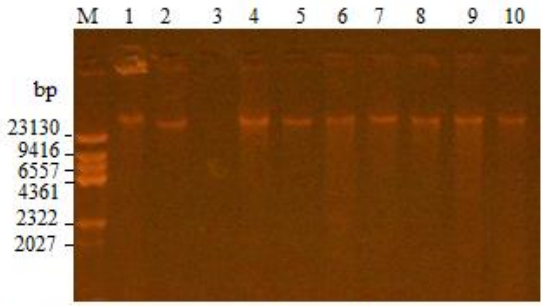
Table 4: Hemolytic activity

Sample source	No. of isolates	No. of haemolytic strains	Haemolytic activity %	Overall Haemolytic activity %
UTI	23	20	87	62
ENT	17	5	29.41	
Pus	10	6	60	

Table 5: Plasmid profile of *E. coli* isolates from clinical samples

Strain/Isolate	Plasmid size profile (in kb)			Antibiotic resistant pattern
	<10kb	>23kb	Total No. of plasmids	
1	--	1	1	AMP
2	--	1	1	AMX
3	--	--	--	AMP
4	--	1	1	AMP-AMX
5	--	1	1	AMP-AMX-CXM
6	--	1	1	AMP-AMX-CXM
7	--	1	1	AMP-AMX-CXM
8	2(9.5,8 kb)	2	4	AMP-AMX-CXM-CN-CPD
9	3(8,5.5,3 kb)	2	5	AMP-AMX-CXM-CN-CPD
10	--	1	1	AMP-CXM-CN-CPD

Fig. 1: Plasmid profile of multidrug resistant *E.coli* isolates isolated from clinical samples



Lane 1-10 : Plasmid profile of *E.coli* isolates isolated from clinical samples
Lane M : Lambda DNA Hind III digest - DNA marker

IV. Discussion

A total of 50 *E. coli* strains were used for testing their antimicrobial susceptibility. Regarding susceptibility of *E. coli*, the resistant and sensitive patterns were tested to 5 antibiotics at a concentration of Ampicillin (AMP) - 25µg, Cefuroxime (CXM) - 30µg, Amoxicillin (AMC) - 30µg, Cefpodoxime (CPD) - 10µg and Cephalexin (CN) - 30µg. Jeyasekharan *et al.,* 2015 in a long surveillance of *E. coli* in UTI, found in *E. coli,* an increasing trend of resistance to gentamycin, cephalosporin and flouroquinolones which were reflected in the results of the present study also.

Sohail *et al.,* 2015 collected pathogenic isolates from patients with UTI in Punjab, in which they observed *E. coli* was highly resistant to antimicrobial drugs, viz. cephalexin (95%), cephradine (95%), pipemidic acid (92%), amikacin (91%), and nalidixic acid (91%). Most of the routine β-lactam antibiotics like amoxicillin/clavulanic acid, ampicillin, and aztreonam were also ineffective against *E. coli*, with resistance rates of 84%, 84%, and 72%, correspondingly.

The present study indicated the presence ESBL production in various *E.coli* strains which was indicative through exhibited resistance to first, second as well as third generation cephalosporin. Among 50 strains, 30 strains (60%) were observed to possess CTX type of β-lactamases. The other strains may possess other type of β-lactamases (or) any possess other mechanism to attain resistance status against these antibiotics.
According to a study previously performed in Beirut (Daoud and Afif, 2011), *E. coli* was the most frequent isolate (60.64% of the total isolates) and an increase in the production of ESBL was observed between the years 2000 and 2009 (2.3–16.8%). Unfortunately, very limited data concerning UTIs is available from other regions of the country. In Indian context also studies are limited.

The highest MAR index of 1.0 in 18% of strains in the present investigation emphasizes the public health risk under study. Boon and Catlanach (1999) observed the antibiotic resistance of THB which was greater than that of *E.coli* in river samples of Victoria region of Australia.

In the present investigation the strains were resistance to the β-lactam antibiotics including cephalosporin. Agwu and Oluwagunke (2014) also recorded resistance to β-lactam antibiotics such as ampicillin and amoxicillin in surface water *E.coli* isolates were high (i.e.) respectively 96 and 88%.

Only few studies reported this much higher resistance (Lobova *et al.,* 2008 and Al-Hussaini *et al.,* 2012). However in general among β-lactam antibiotic ampicillin and amoxicillin showed higher resistance compared to other group of antibiotics (Sivri *et al.,* 2012; Mehta *et al.,* 2012 and Rehman *et al.,* 2013).

Plasmid curing was done all the resistant strains tested against β-lactam antibiotics tested in the present investigation lost resistance. Plasmid is one of the important mediators for fast spreading of antibiotic resistance in bacteria (Datta and Park, 2004).

V. Conclusion

The results obtained in this study clearly showed that the 5 different antibiotics (Ampicillin, Amoxicillin, Cefuroxime, Cephalexin, and Cefpodoxime) were tested to study the antimicrobial susceptibility of the *E.coli* isolates. In this research work not to all the β-lactam antibiotics, the *E.coli* strains were equally resistant. Though 100% resistance was observed against ampicillin, only 50% resistance was observed regarding amoxicillin. When plasmid curing was done all the resistant strains tested against β-lactam antibiotics tested in the present investigation lost resistance. By performing this study we can extend further research to manage common and lethal bacterial infections, critically compromised by the appearance and the rapid spread of antibiotic resistant bacteria and to begin the process of developing actionable policy recommendation relevant to low and middle income countries.

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