

# ANTIMICROBIAL RESISTANCE; COMPARISON OF ESCHERICHIA COLI IN DIFFERENT AREAS OF LAHORE

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**ABSTRACT... Introduction:** Since the development of antibiotics there is a growing concern about the increasing incidence of antibiotic resistance. As a result the therapeutic value of originally effective antibiotics become significantly reduced overtimes. Extensive data is available on antibiotic susceptibilities of hospital isolates but very little information is available about the susceptibilities of community strains. **Design:** Descriptive. **Period:** July 2004 to June 2005. **Setting:** Department of Microbiology, Shaikh Zayed Hospital Lahore. Hence the present study was design to assess the environmental load of the antibiotic resistance using fecal flora as an indicator of overall problem. It will also provide guidance in antibiotic protocol for antibiotic policy. **Objective:** Objective of the present study was to determine the developing resistance to  $\beta$  – Lactam Antibiotics which is the commensal microbe of enteric tract. **Materials & Methods:** One hundred samples were collected from ten different areas of Lahore city (10 samples from each area) and were inoculated on Mac Conkey's agar. Five morphologically distinct lactose fermenting colonies were selected & identified using standard laboratory methods. Five hundred different colonies of E.coli were identified and analyzed for their susceptibility to b-lactam antibiotic. **Results:** Out of 500 isolates, the resistant isolates with ampicillin (48%), co-amoxiclav (40%) and cephradine (37%) were detected, with cheaper oral agents high prevalence of resistance was detected. **Conclusions:** Ampicillin, co-amoxiclav and cephradine are not much useful for the treatment of urinary tract infection and septicemia caused by E-coli & other Members of fecal flora.

**Key words:**E-coli, Resistance, b-lactam.

## INTRODUCTION

Pathogenic bacteria which cause waterborne infectious diseases are released into the water system via feces of warm-blooded animals mainly human beings. To estimate sanitary condition in the water system, fecal coliforms have been commonly used as an indicator of fecal pollution<sup>1</sup>.

E.Coli is the most common cause of urinary tract infection. It is the most abundant facultative anaerobe in the colon and faeces. E-coli causes a variety of diseases both within & outside the intestinal tract. It is the most frequent cause of nosocomial urinary tract infection in both men and women. It may produce cystitis and pyelonephritis. It may also produce neonatal meningitis and diarrhoea<sup>2</sup>.

Betalactam antibiotics are penicillins and cephalosporins. The penicillins share features of

chemistry, mechanism of action pharmacology and immunologic characteristics with cephalosporins, monobactam, carbapenems and  $\beta$ -Lactamase inhibitors. All are  $\beta$ -Lactam compound, so named because of their unique four membered lactone ring<sup>3</sup>.

## Aim & objective

Objective of the present study was to determine the developing resistance to  $\beta$  – Lactam Antibiotics which is the commensal microbe of enteric tract.

## MATERIALS & METHODS

1. Sterile swabs to get sewage samples.
2. MacConkey's media (differential media) for inoculation of the specimen and isolation of Escherichia coli (lactose fermenting colonies).
3. Biochemical media including.
  - a) Triple Sugar Iron Agar
  - b) Sulphide Indole & Motility Media

- c) Kligler Iron Agar
4. Muller Hinton agar for sensitivity testing.
5. Antibiotic discs.

### Reagents

- Staining Material for Gram Staining.
- Kovacs Reagent for Indole Test.

### Method

#### Sample Collection and Transportation to the Laboratory

Source of bacterial isolate in this study is Sewage. Specimens were collected from Sewage in different areas of Lahore. One hundred samples collected from ten different areas of Lahore city (10 Samples from each area). Samples were collected with the help of sterile swabs and were transferred to the Microbiology Laboratory, Shaikh Zayed Hospital Lahore. (N-500)

### CULTURING THE SAMPLES

MacConkey's agar and selenite-F broth were inoculated by the swabs. Both plates and broths were incubated aerobically at 37°C for 18-24 hours. Selenite-F broth was subcultured on XLD agar.

### IDENTIFICATION OF ESCHERICHIA COLI

From MacConkey's agar, five morphologically distinct lactose fermenting colonies were selected and identified using standard laboratory methods which included:

#### Gram Staining<sup>4</sup>

#### Triple sugar iron medium<sup>5</sup>

#### Indole & motility tests<sup>6</sup>

### Results

Rapidly emerging nosocomial pathogens and the problem of multi-drug resistance necessitates periodic review of isolation patterns and sensitivity in surgical practice<sup>7</sup>.

According to the present study susceptibility of E.Coli strains obtained were determined for b-lactam antibiotics the study shows that out of 500, total resistant to ampicillin were 48%, whereas the prevalence of

resistance to amoxiclav was 40%, resistance to cephradine was 37% very few isolates showed resistance to cefurixome 12%. No resistance was observed against fourth generation cephalosporins (Table I & II). Prevalence of resistance to both ampicillin and co-amoxiclav was moderately high 48% and 40% respectively. Resistance to ampicillin was highest in Shadbagh Area 70% whereas; it was lowest in Cantt. Area 20%, in other eight areas resistance to ampicillin ranged from 30-50%. Prevalence of co-amoxiclav resistant isolates was also lowest in Cantt. Area 18% and was highest in Madina Colony and Chung 50%. In other area, its ranged from 30-40. No resistance to piperacillin was observed (Table-I).

### First generation cephalosporins

Overall resistance to cephradine was recorded 37%, resistance was found about 50% highest in Madina Colony, Wahdat Colony and Chung while it was lowest in Cantt. Area 16% in other six area resistance ranged from 30-40%.

### Second generation cephalosporins

Overall resistance to cefuroxime was 12%. The isolates from Cantt. Area showed no resistance to cefuroxime while the isolates from Chung and Allama Iqbal Town showed a resistance of 20% highest, In other area its ranged from 5-16%.

3rd generation cephalosporins over all resistance to cefatoxime was 3.6 %. Isolates from Chung area showed 20% (highest) resistance while the isolates from muslim town, wahdat colony, jail road, cantt madina colony, allama iqbal town & Shadbag showed no resistance. In other area it ranged from 6 to 10% non of the isolates showed resistance to cefatazidime and ceftriaxone.

### 4th generation cephalosporins

No resistance was observed against 4th generation cephalosporins (cefpirome & cefiopime). (Table-II).

### DISCUSSION

Antibiotic resistance is a matter of great concern since the introduction of antibiotic have changed the conditions but resistance to newly introduced antibiotics is also developing. There is a good data for antibiotics

Table-I. Resistance to penicillins in different geographical areas

Area	Ampicilin		Co-amoxiclav		Pipracilin	
	No.	%	No.	%	No.	%
New Muslim Town (n=50)	25	50.0	15	30.0	-	-
Cantt (n=50)	10	20.0	09	18.0	-	-
Jail Road (n=50)	27	54.0	16	32.0	-	-
Shadman (n=50)	25	50.0	25	50.0	-	-
Madina Colony (n=50)	27	54.0	25	50.0	-	-
Garden Town (n=50)	15	30.0	15	30.0	-	-
Wahdat Colony (n=50)	25	50.0	25	50.0	-	-
Chung (n=50)	30	60.0	25	50.0	-	-
Allama Iqbal Town (n=50)	22	44.0	20	40.0	-	-
Shadbag (n=50)	35	70.0	20	40.0	-	-
Total (n=500)	241	48	199	40	-	-

Table-II. Resistance to cephalosporins in different geographical areas

Area	1 <sup>st</sup> Generation		2 <sup>nd</sup> Generation		3 <sup>rd</sup> Generation				4 <sup>th</sup> Generation				
	Cephadrine	%	Cefuroxime	%	Cefotaxime	Ceftazidime	Ceftriaxone		Cefpirome	Cefipime			
New Muslim Town (n=50)	15	30.0%	02	4.0 %	-	-	-	-	-	-	-	-	-
Cantt (n=50)	08	16.0%	-	-	-	-	-	-	-	-	-	-	-
Jail Road (n=50)	15	30.0%	05	10.0%	-	-	-	-	-	-	-	-	-
Shadman (n=50)	22	44.0%	03	6.0 %	03	6.0%	-	-	-	-	-	-	-
Madina Colony (n=50)	25	50.0%	05	10.0%	-	-	-	-	-	-	-	-	-
Garden Town (n=50)	15	30.0%	05	10.0%	05	10.0%	-	-	-	-	-	-	-
Wahdat Colony (n=50)	35	70.0%	05	10.0%	-	-	-	-	-	-	-	-	-
Chung (n=50)	25	50.0%	10	20.0%	10	20.0%	-	-	-	-	-	-	-
Allama Iqbal Town (n=50)	20	40.0%	08	16.0%	-	-	-	-	-	-	-	-	-
Shadbag (n=50)	15	30.0%	05	10.0%	-	-	-	-	-	-	-	-	-
Total (n=500)	185	37%	58	12 %	18	3.6%	-	-	-	-	-	-	-

resistance in hospitalized patients; but very little information is available about the prevalence of antibiotic resistance in the community<sup>8,9</sup>. These data are mainly from the developed countries<sup>10</sup>. In Pakistan, there is no data available for the prevalence of antibiotic resistance in the community. Furthermore, the previous studies regarding antibiotic resistance were mainly performed on the healthy volunteers. In this study, *Escherichia coli* isolated from the sewage was used as an indicator of resistance in the community. *E.Coli* is a member of normal fecal flora and is capable of transferring resistance to the other fecal flora via plasmid mediated mechanism. Because it is the largest reservoir of antibiotic resistant determinants<sup>11</sup>. Advantage of using sewage sample is that not only it represents the human fecal flora but also flora from the animal birds and poultry sources get represented.

In the present study, a moderately high prevalence of resistance in fecal *E.Coli* to ampicillin, co-amoxiclav and cephadrine were observed. A significant difference between Cantt. Area (Socio-economically Rich) and other area was found for ampicillin, co-amoxiclav and cephadrine, this resistance percentage for ampicillin was 20% as compared to the other areas where its was from 40-50%.

Similarly the prevalence of resistance to co-amoxiclav and cephadrine was 18% and 16% respectively in Cantt Area which was very low when compared to the resistance in other area which ranged from 30-50% in Madina Colony, Whadat Colony, chung and Shadbag the thickly populated areas mixed middle and lower class population. It is most likely due to lack of awareness about antibiotic use, low literacy rate, use of substandard antibiotics and rather misuse antibiotics. Studies performed in different parts of the world among them the study performed<sup>12</sup> provides the prevalence of antibiotic resistant fecal *E.Coli* in healthy children in Boston (USA).

Similar study performed on fecal samples taken from students in South East of Netherland<sup>13</sup>. Two years later Bonten et al detected the prevalence of antibiotic resistance *E.Coli* in stool samples of healthy volunteers from maastricht and Zwolle Netherland<sup>8</sup>. London also determined the prevalence of antibiotic resistant fecal

*Escherichia coli* by healthy volunteers from two cities Weert & Roermond Netherlands<sup>14</sup>.

Similar study was also performed on fecal samples of healthy volunteers of two different towns Gula and Marida of Venezuela<sup>10</sup>.

#### **Comparison of present study with previous studies**

High incidence of resistance to antimicrobial agents in the developing nations is mainly due to over crowding, poverty and irrational use of antibiotics and their availability without prescription<sup>10</sup>.

Resistance to ampicillin in the present study was almost comparable to prevalence of resistance to ampicillin (amoxicillin) in Merida (39%) Gula 46 % and Qinqu China 47%, whereas the resistance to ampicillin was very high in caracas (85%). Relatively lower resistance to ampicillin was detected in southeast of Netherland (28%), Boston 23%, Weert 8.4% and Roermond (12.4%).

In the present study, a high incidence of resistance to co-amoxiclav (augmentin) 37% was observed whereas no resistance to co-amoxiclav was observed in weert and roermond (Table I).

The above data from the previous studies revealed that resistance to ampicillin (amoxicillin) in the developing countries like Venezuela<sup>10</sup> Caracas and Qinqu<sup>12</sup> was higher or equivalent to that present study while it was low in Boston<sup>12</sup>, Weert and Roermond which are the areas of developed nation. Low prevalence of resistance to antibiotics in the developed countries is mainly due to rational use of antibiotics in these countries.

It has also been observed that there is high prevalence of resistance to co-amoxiclav and cephadrine in the present study which is higher than the other studies performed in developing countries, these differences can be justified because the samples were obtained in this study from the sewage which contained fecal flora of not only human but also animal. In contrast, the samples used in previous studies were fecal matter from healthy volunteers.

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