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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESCHRICHIA COLI ISOLATES FROM CLINICAL SPECIMENS AT PIMS, ISLAMABAD

Jafar Khan[1,4], Irum Khalil[1], Naser M. AbdEl-Salam[2], Sehrish Liaqat[1], Naila Younas[1], Sameerah Mushtaq[1], Riaz Ullah[3]

[1]Department of Microbiology, Kohat University of Science and Technology, Kohat KPK Pakistan
[2]Riyadh Community College, King Saud University, Riyadh 11437, Saudi Arabia
[3]Department of Chemistry, Government College Ara Khel, FR Kohat, Khyber Pakhtunkhwa 26000, Pakistan
[4]Directorate of Research Innovation and Commercialization (DORIC) University of Swat KPK Pakistan

Abstract

Background: *E. coli* is the most common producer of extended spectrum beta lactamase enzyme (ESBL) which confers broad spectrum resistance to antibiotics like penicillin, cephalosporin and monobactum.
Methods and Materials: The present study was carried out at Pakistan Institute of Medical Sciences, Islamabad. The marked resistance was viewed against amoxicilline-clavulanic acid, ceftriaxone and ceftazidime. The most effective drugs established were sulbactum-cefoparazone, amikacin, pepircillin-tazobactum. A total of 220 samples of wide range were selected, i.e., blood, urine, pus, sputum, etc. and were analyzed using various techniques of Gram staining and biochemical identification.
Results: After performing antibiotic sensitivity tests, 83% samples came out to be ESBL positive and 17% were ESBL negative.
Conclusion: It was concluded that to ensure adequate treatment of infections arising especially from urinary pathogens and controlling spread of bacterial resistant strains, the continuous monitoring by bacterial susceptibility testing is essential.

Key words: Susceptibility pattern, *Eschrichia coli*, PIMS

Introduction

Antibacterial resistance has become an important problem worldwide. The development of antibiotics resistance in *E. coli* has important clinical implications. Antimicrobial agents are most important in treatment of bacterial infections (Dworkin and Falkow; 2006). Urinary tract infection (UTI) is a common community acquired bacterial infection which frequently affects female outpatient, children and is one of the main causes of nosocomial infections in humans. It has also been stated that these opportunistic pathogens are responsible for ulcerative colitis and hemolytic uremic syndrome (HUS) and a potentially fatal kidney disease (Kaper et al 2004, Rolhion and arfeuille; 2007). *E. coli* accounts for 75-90% of all UTI's in both the in patients as well as outpatients of the hospitals. Specific risk factors that lead to the spread of ESBL producing organism include prolonged hospitalization, severity of illness, incubation and urinary arterial catheterization, low body weight and previous exposure to broad spectrum of antibiotics (Lin et al; 2003, Tumbarello et al; 2007).

The first isolation of ESBL production by *E. coli* strain was done in 1987; a number of outbreaks caused by these organisms have been reported worldwide. Multiple drug resistance has significantly increased in recent years. The existences of extended-spectrum beta lactamase producing organisms are resistant to virtually all beta lactam antibiotics (Karlowsky et al., 2002). The increase in drug resistance in these organisms has made therapy of UTI very difficult and has consequently led to the greater use of expensive broad spectrum antibiotics with their third and fourth generations.

Materials and Methods

This study was carried out at Pakistan Institute of Medical Sciences (PIMS) Islamabad and a total of 220 *E. coli* isolates were collected from different wards of the hospital. Different specimen like blood, sputum, urine, pus, wound, etc. were screened for the infection. The specimen was inoculated into blood agar, MacCkonkey agar and CLED agar. The *E.coli* was also cultivated on glucose and other carbohydrates containing media to observe fermentation with indication of the production of acid and gas during the growth (Manges et al. 2001).

Gram Staining

Using sterile techniques, a smear of each isolate was prepared, dried and heat-fixed after which it was processed for Gram staining and microscopy.

Biochemical Identification

Indole test was carried out to check the presence of tryptophanase enzyme to which *E. coli* is positive. Organisms that can use citrate as their sole source of carbon can turn the color of Simon's Citrate agar from green to blue which is tested by Citrate utilization test. It differentiates between Enterobacteriacea from other Gram negative organisms. Motility test was done by stabbing deep a loop full of organism into the nutrient

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agar which differentiates the bacteria as motile or non-motile. Testing for urease enzyme activity was done to identify enterobacteria from other non-urease producers. It was done by inoculating the test organism into urease agar. TSI test was carried out to check the ability of the test organism to ferment different sugars. A wire loop loaded with test organism was inoculated onto the TSI agar slants and results were noted after 24 hours of incubation.

API 20E Analysis

The purpose of using the API 20E was for the micro standard system of specific identification of *E. coli* from the rest of the fastidious Gram negative rods. It contains a strip with 20 microtubules containing dehydrated substrates, inoculated with the bacterial suspension (Philipon et al. 1989). The previously isolated known colonies of *E. coli* were subjected to analysis as control. By using a sterile syringe, all wells of strip were filled according to the instructions of API20E manual. An anaerobic environment was created in ADH, LCD, ODH, H_2S, UREA tubules by putting a drop of mineral oil overlay. Incubation box was closed and placed in incubator at 37C for 24 hours.

Antibiotic Sensitivity Tests

Kirby-Bauer disk diffusion technique was used for antimicrobial susceptibility testing (Lin et al; 2003, Tumbarello et al; 2007). The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire surface in order to make lawns. Antibiotic disks were impregnated on to the streaked agar surface and incubated for 24 hours.

Results

The present study was conducted on various clinical isolates from indoor and outdoor patients to determine the prevalence of clinically significant *E coli* at Pakistan Institute of Medical Sciences (PIMS), Islamabad. Two hundred twenty samples of *E. coli* were isolated from blood, urine, sputum, wound pus etc. and were processed for the assessment of antibiotic susceptibility

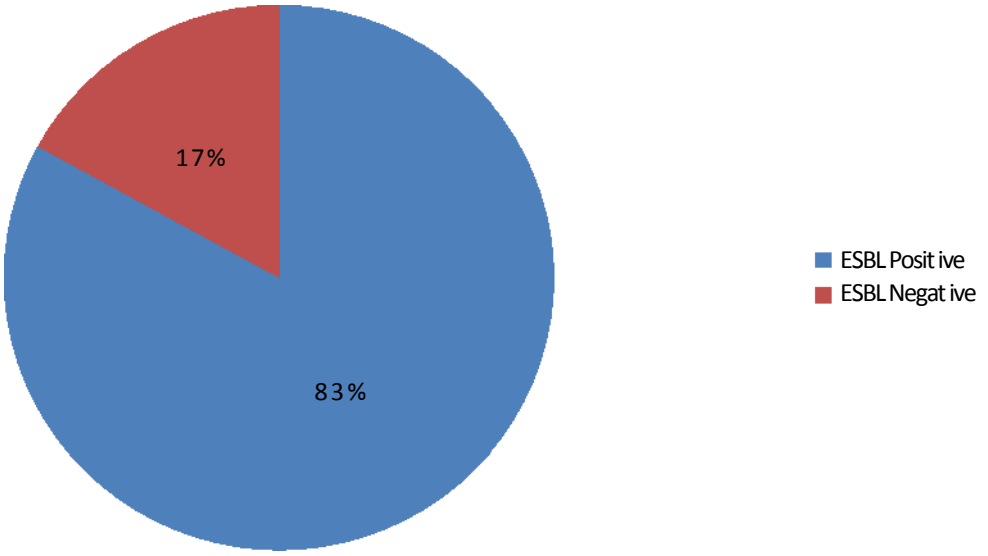

Figure 1: Distribution of Isolates on the basis of ESBL production.

Table 1: Age and gender wise distribution of isolates

Groups	Age	No. of Samples	No. of affected Females	% of affected Females	No. of affected Males	% of affected Males
A	1-10	18	03	10%	15	12.5%
B	11-20	30	09	10%	21	10.5%
C	21-30	54	18	9.5%	36	6%
D	31-40	42	19	6%	23	6.5%
E	41-50	25	06	4.5%	19	7%
F	51-60	25	15	2.5%	10	5.5%
G	61-70	14	05	3.5%	09	2.5%
H	71-80	12	05	2%	07	1.5%
Total		**220**	**80**	**48%**	**140**	**52%**

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Cultural Characteristics of Isolates

Isolates were cultivated on blood agar, MacCkonkey agar and CLED agar. Following were the results after 24 hours of incubation period.

Table 2: Culturing results of obtained isolates:

Media	Incubation time	Colony Color	Colony Shape
Blood agar	24 hours	Grey	Round and moist
MacCkonkey agar	24 hours	Dark Pink	Pinpointed
CLED agar	24 hours	Yellow	Round

Figure 2: E.coli culture on MacCkonkey, CLED and Blood agar.

Gram Reaction

After Gram staining *E. coli* appeared pink and rod shaped in microscopy and as Gram negative.

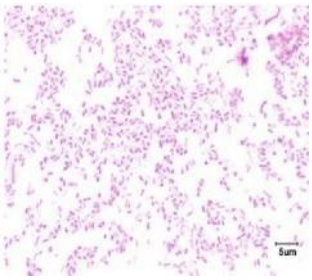

Figure 3: Microscopic appearance of E.coli rods

Biochemical Characteristics

For biochemical characterization, a series of biochemical analyses for *E. coli* were performed such as citrate utilization, TSI, VP, indole, oxidase, urease and motility tests.

Table 3: Biochemical test results for *E. coli*

Biochemical Test	Observation	Result
Indole	Red ring appeared	+
Voges-Poskeur	Yellowish brown color appeared	-
Citrate utilization	No color change of media	-
Oxidase	No color appearance	-
Motility	Streaks along the stab were seen	+
Urease	Orange color appeared	-
TSI	Yellow color of medium + gas production	Fermentation and gas production

Figure 4: Indole, VP, Citrate utilization and TSI tests

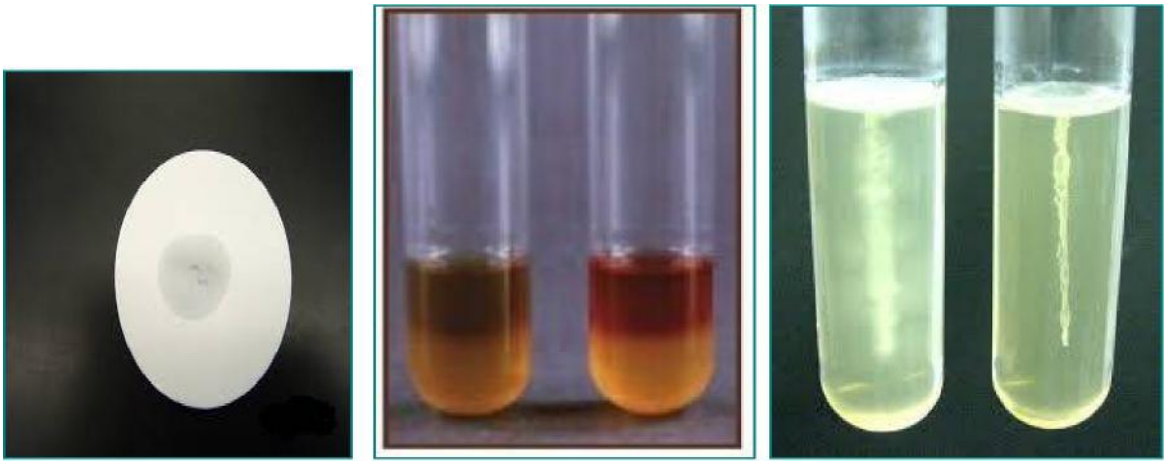

Figure 5: Oxidase, Urease and Motility tests

Results on API 20E System

API 20E is a micro standardized identification system for enterobacteriacea. After biochemical identification of E. coli they were confirmed with API 20E. The results were taken on analysis slips by comparing with the reading table. The generated number for E. coli was 8101, which was same as per API 20E manual. The results for *E. coli* on API 20 E system were in conformity with the manual biochemical method.

Figure 6: API20E strip results against *E.coli*

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Antibiotic Susceptibility Analysis

Antibiotic sensitivity was checked for all *E. coli* isolates and 83% were found to be ESBL positive. The department and ward wise distribution of antibiotic sensitivity pattern of the isolates was determined in percentage calculations in each case:

Table 4: Antibiotic sensitivity pattern of specimens referred from General Ward.

Antibiotics	Resistant Count(%age)	Sensitive Count (%age)	Intermediate Count(%age)	Total Count(%age)
Amoxicillin	47 (94%)	3(9%)	0 (0.0%)	50 (100%)
Ceftazidime	45 (90%)	6 (5%)	0 (0.0%)	50 (100%)
Ceftriaxone	44 (88%)	6 (12%)	0 (0.0%)	50 (100%)
Tazobactum	19 (38%)	29 (58%)	2 (4%)	50 (100%)
Sulbactum-cefparazone	8 (16%)	41 (82%)	1 (2%)	50 (100%)
Amikacin	14 (28%)	30 (60%)	1 (2%)	45 (90%)
Imipenem	13 (24%)	28 (56%)	0 (0.0%)	40 (80%)
Polymyxin B	14 (28%)	26 (52%)	0 (0.0%)	40 (80%)
Tobramycin	24 (48%)	20 (40%)	0 (0.0%)	44 (88%)
Piperacilline	15 (30%)	19 (38%)	1 (2%)	35 (70%)
Levofolxacin	20 (40%)	19 (38%)	5 (10%)	44 (88%)
Norfloxacin	5 (10%)	10 (20%)	0 (0%)	15 (30%)
Ciprofloxacin	5 (10%)	2 (4%)	0 (0%)	7 (14%)

Table 5: Antibiotic sensitivity pattern of specimens referred from Medical Ward.

Antibiotics	Resistant Count(%age)	Sensitive Count(%age)	Intermediate Count(%age)	Total Count(%age)
Amoxicillin	47 (94%)	3(6%)	0 (0.0%)	50 (100%)
Ceftazidime	43 (86%)	7 (17%)	0 (0.0%)	50 (100%)
Ceftrixone	45 (90%)	5 (10%)	0 (0.0%)	50 (100%)
Tazobactum	18 (36%)	32 (64%)	4 (0.0%)	50 (100%)
Sulbactum-cefparazone	22 (44%)	28 (56%)	0 (0.0%)	50 (100%)
Amikacin	6 (12%)	22 (44%)	1 (2%)	30 (60%)
Imipenem	3 (6%)	29 (58%)	3 (6%)	35 (70%)
Polymyxin B	1 (2%)	28 (56%)	0 (0.0%)	31 (62%)
Tobramycin	7 (14%)	17 (34%)	2 (4%)	26 (52%)
Piperacillin	7 (14%)	5 (10%)	0 (0.0%)	12 (24%)
Levofloxacin	8 (16%)	9 (18%)	0 (0.0%)	17 (34%)
Norfloxacin	2 (4%)	3 (6%)	0 (0%)	5 (10%)
Ciprofloxacin	3 (6%)	7 (14%)	1 (2%)	10 (20%)

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Table 6: Antibiotic sensitivity pattern of specimens referred from Neonatal Intensive Care Unit.

Antibiotics	Resistant Count(%age)	Sensitive Count(%age)	Intermediate Count(%age)	Total Count(%age)
Ampicillin	23 (92%)	3(12%)	0 (0.0%)	25 (100%)
Ceftazidime	23 (92%)	2 (8%)	0 (0.0%)	25 (100%)
Ceftriaxone	24 (96%)	1 (4%)	0 (0.0%)	25 (100%)
Tazobactum	8 (32%)	15 (60%)	2 (8%)	25 (100%)
Sulbactum-cefparazone	6 (24%)	19 (76%)	0 (0.0%)	25 (100%)
Amikacin	3 (12%)	10 (40%)	0 (0.0%)	13 (52%)
Imipenem	2 (8%)	19 (76%)	0 (0.0%)	21 (84%)
Polymyxin B	1 (4%)	18 (72%)	0 (0.0%)	19 (76%)
Tobramycin	8 (32%)	5 (20%)	2 (8%)	15 (60%)
Piperacillin	5 (20%)	3 (12%)	0 (0.0%)	8 (32%)
Levofloxacin	15 (60%)	6 (24%)	0 (0.0%)	20 (80%)
Norfloxacin	1 (4%)	3 (12%)	0 (0%)	4 (16%)
Ciprofloxacin	5 (20%)	6 (24%)	0 (0.0%)	11 (44%)

Table 7: Antibiotic sensitivity pattern of specimens referred from Out Patient Department

Antibiotics	Resistant Count(%age)	Sensitive Count (%age)	Intermediate Count(%age)	Total Count(%age)
Ampicillin	42 (84%)	8 (16%)	0 (0.0%)	50 (100%)
Ceftazidime	40 (8-%)	10 (20%)	0 (0.0%)	50 (100%)
Ceftrixone	42 (84%)	8 (16%)	0 (0.0%)	50 (100%)
Tazobactum	15 (30%)	34 (68%)	1 (2%)	50 (100%)
Sulbactum-cefparazone	13 (26%)	35 (70%)	2 (4%)	50 (100%)
Amikacin	20 (40%)	20 (40%)	5 (10%)	45 (90%)
Imipenem	14 (28%)	28 (56%)	0 (0.0%)	42 (84%)
Polymyxin B	12 (24%)	30 (60%)	0 (2%)	42 (84%)
Tobramycin	25 (50%)	18 (36%)	2 (8%)	45 (90%)
Piperacillin	14 (28%)	20 (40%)	4 (1%)	35(70%)
Levfloxacin	30 (60%)	14 (28%)	2 (0.0%)	44 (88%)
Norfloxacin	7 (14%)	10 (20%)	0 (0.0%)	17 (34%)
Cipfloxacin	8 (16%)	14 (28%)	0 (0.0%)	22 (44%)

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Table 8: Antibiotic sensitivity pattern of specimens referred from Surgical Ward

Antibiotics	Resistant Count(%age)	Sensitive Count (%age)	Intermediate Count(%age)	Total Count(%age)
Ampicillin	40 (88.8%)	5 (11.1%)	0 (0.0%)	45 (100%)
Ceftazidime	39 (86.6%)	6 (13.3%)	0 (0.0%)	45 (100%)
Ceftrixone	41 (91%)	4 (8.8%)	0 (0.0%)	45 (100%)
Tazobactum	7 (15.5%)	35 (77.7%)	3 (6.6%)	45 (100%)
Sulbactum-cefparazone	10 (22.2%)	33 (73.3%)	2 (4.4%)	45 (100%)
Amikacin	4 (8.8%)	28 (62.2%)	0 (0.0%)	32 (71.1%)
Imipenem	3 (6.6%)	27 (60%)	1 (02.2%)	31 (68.8%)
Polymyxin	3 (6.6%)	29 (64.4%)	0 (0.0%)	32 (71.1%)
Tobramycin	11 (24%)	13 (28.8%)	0 (0.0%)	24 (53.3%)
Piperacillin	5 (11.1%)	2 (4.4%)	0 (0.0%)	7 (15.5%)
Levofloxacin	19 (42.2)	7 (15.5%)	1 (2.2%)	27 (60%)
Norfloxacin	4 (8.8%)	4 (8.8%)	0 (0.0%)	8 (17.7%)
Ciprofloxacin	4 (8.8%)	6 (13.3%)	0 (0.0%)	10 (22.2%)

Discussion

A number of studies have reported the prevalence of ESBL producing organisms, particularly *E. coli* isolates. It was found in this study that male and female patients were near equally infected, i.e., with the percentage value of 52% and 48% respectively. Growth rate was found to be higher in patients of 1-10 years of age as compared to other groups as also observed in a likewise study (Kamberovik et al; 2006). ESBL production was determined for all the 220 *E. coli* isolates and only 17.% were found negative ESBL as similarly observed in another setting (Chaikittisuk et a;l 2007). *E. coli* the most prevalent Gram negative bacilli from clinical samples of pus, blood, urine etc were found predominant as also observed in cases similar where E. coli was seen widely distributed in environment and cause a variety of infections in community and hospitalized settings (Donnenberg et al; 2005). The prevalence of infected males was slightly higher than infected females. Noticeable resistance was found among the isolates against one of the class of beta lactam antibiotics. These results are comparable to related outcomes in a study. (Philippon and Arlet; 2006). The marked resistance of *E. coli* was viewed against penicillin group like that of amoxicilline-clavulanic acid as seen also in another study (Zehra et al 2011).

The most effective drugs established in this setting were sulbactum-cefoparazone, amikacin and papircilline-tazobactum. Almost all the isolates were sensitive to imipenem which is as likewise reported (Patrícia et al., 2010). In conclusion, a relatively high antibiotic resistance was observed among the isolated *E. coli* strain and no doubt that growing problem of antimicrobial resistance has become a growing public health concern, especially in developing countries. This phenomenal increase in drug resistance is greatly contributed to by the misuse of antibiotics that have led to the prevailing alarming situation (Marwa et al; 2012, Ruifang et al; 2006, Rahbar et al; 2007). Selection of drug of choice in any condition especially in chronic diseases is not easy. In case of infectious diseases, we have to pay attention to microbial sensitivity and resistance pattern observation for various antimicrobials (Asti; 2013). Moreover, monitoring ESBL production and antimicrobial susceptibility

testing are necessary to reduce the burden of increasingly resistant pathogens (Mohanalakshmi et al; 2014). Therefore, the antibiotic stewardship guidelines need to be recommended and strictly adopted in a comprehensive control program to reduce the high levels of bacterial antibiotic resistance (Gautam et al; 2013). In the perspective of this study, the patients' sufferings with proper public health plans can be minimized with a positive direction to their health care by developing strategies to prevent the emergence and spread of drug resistant *E. coli* strains in the clinical environment.

Conclusion

It was concluded that to ensure adequate treatment of infections arising especially from urinary pathogens and controlling spread of bacterial resistant strains, the continuous monitoring by bacterial susceptibility testing is essential.

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