Determination of Antibiotic Susceptibility of Hospital Escherichia coli Isolates

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Abstract

This research was conducted in 2015, Azad Jammu and Kashmir (AJK) to check the antibiotic susceptibility testing of hospital *Escherichia coli* isolates. Urine samples were collected from DHQ hospital of kotli, Azad Jammu and Kashmir. *E. coli* are isolated by using MacConkey agar as a selective media. The antibiotics which were used Amikim, Cipiroxin, Cefradin, Ceftriaxon, Ofloxacin, Sulphmethoxazole, Norfloxacin, Augment, Gentamycin and Meropenem (MRP). Maximum susceptibility (100%) was toward MRP, Ciproxin, Gentamycin and Amikim. Susceptible of about 88% was found in case of Oflloxacin and Ceftriaxon. Maximum resistance (50%) was shown toward the Cefradin.

Key Words: Sensitivity and resistance

1 INTRODUCTION

E. coli was discovered by the Theodor Escherichia in 1985 which is facultative anaerobic and has ability to make ATP by aerobic respiration, if oxygen is present. *E. coli* is gram negative and non speculating. Some of the species of *E. coli* which are inhabitant of just intestinal track of warm blood animals provide a portion of microbiology and derived vitamin K for their host. *E .coli* is classified as part of enterobacteriaceae family of gamma proteobacteria. *E. coli* is typed on the base of surface of antigen i.e. O antigen, H antigen, K antigen and R antigen (O'Shea *et al*., 2012). Enterohaemorrhagic *E. coli* (EHEC) found in humans, cattle's and goats. The most famous member of this virotype is strain O157; H7, which causes blood diarrhea and no fever. EHEC can cause hemolytic Uraemic syndrome and sudden kidney failure and it usually uses bacterial fimbriae for attachment. (Croxen *et al*., 2010). Cattle serve as the natural reservoirs for EHEC and causes Gastrointestinal diseases (Wells *et al* ., 2009).

The primary habitat of *E. coli* is the Gastrointestinal track of warm blood animals as well as man. The most abundant facultative anaerobic microorganism in GIT of mammals and human is the *E.coli*. These bacteria appear in body just after few hours of birth and form mutuality relationship with its host (Grant *et al.*, 2010). Naturally it occurs in colon of large intestine as well as grows in large intestine and play an important role in maintaining the normal flora of human. Most of strain of *E. coli* doesn't harm, when present in body but when they move in the blood or another site of body then cause infection such as urinary track infection (UTI). *E. coli* cycles between two principal habitats intestine of warm animals and water. Elementary calculation shows that *E. coli* produce in the intestine and spent half of its life in intestine and than excrete into the earth surface when it spend second half of life and then it dies or harm new colonies in the host (Ewald *et al.*, 1994). *E. coli* can live on a wide variety of substrate. It uses mixed-acid fermentation in anaerobic condition, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the level of hydrogen to be low, as is the case *E. coli* also lives together with hydrogen-consuming organisms, such as methanogens or sulphate - reducing bacteria (Dethlefsen *et al.*, 2006).

Luria – Bertaniborth supports *E. coli* growth to an optical density 600nm of 7 steady state growth of bacteria ceases of an optical 600 nm at 0.3 and growth rate become slow down due to which cell mass decreases. Catabolize amino acid are the carbon source of *E. coli* in Luria – Bertaniborth sugar not act as carbon source in this Luria – Bertain broth (Qin *et al*., 2006). The carbon storage regulator (Csr) global regulatory system of *E.coli* comprises of four components. This system was first described by T. Romeo in 1993. In *E. coli* Csr is composed of 61 amino acid Csr-A protein and two small, non – coding RNA Csr-B and Csr-C. Csr-B and Cs-rC regulate the

activity of Csr-C protein by requesting the 18 molecule of Csr-C and preventing it to bind with the target mRNA. *E. coli* reproduce by two means; Cell division and transfer of genetic material through a sex pilus (Samaranayake. 2011).

Primarily it is transmitted by taking contaminated food such as under cooked ground meats products, contaminated raw vegetable by contact with person and by drinking water during summer season were identified as risk factor (Slifko *et al.*, 2000). Transmission of *E. coli* may be through fecal oral route (Mayer et al, 1996. Shiga toxin produce *E. coli* (STEC) is transmitted by flies. Transmission of *E. coli* may be through irrigation of crop with raw sewage and by feral pig on crop land (Rangel *et al.*, 2005).

It causes enormous number of disease in population of whole World. Some uropathogenic strain of *E. coli* are characterized by the virulence factor because theses factor help the organisms to overcome host defense and invade the urinary track by cause the infection. *E. coli* infection in digestive system destroy red blood cells by producing the toxic substance and it injured the kidneys and the condition is known as Hemolytic uremic syndrome (Scheiring *et al.*, 2008).

About 83 % of human infection can be preventing by using *E. coli* vaccine on cattle. Cattle vaccine reduces the number of 015:147 in manure by a factor of 1000, to about 1000 pathogenic bacteria per gram of manure. Vaccine can reduce *E. coli* level by more than 50 % in most cattle and by 75 % in super shedder (Chas *et al.*, 2008). Rifaxmin is a gastrintestinal selective antibiotic with a broad spectrum of antimicrobial activity, an excellent safety profile, minimal drug interactions and negligible impact on the intestinal micro brome and it is currently approved in the United states for the treatment of travelers, diarrhea caused by non-invasive diarrheagenic *E. coli* and its approved in more than 30 other countries for a variety of gastrointestinal disorders (Koo *et al.*, 2010).

Antibiotic are common type of medication which are useful for treat of bacterial infection but these are not effective against viral illness and have been overused by years. Because of this use we have to deal with the antibiotic resistance which mean that the antibiotic which we use are no longer effective to treat the bacteria (Singh *et al.*, 2014). Antimicrobial peptides are the antibiotics which are potent and have broad spectrum. These are demonstrated to kill gram positive and gram negative bacteria and some of strain are resistant to these peptides. These peptides are also called defend peptides which are part of innate immune response (Zasloff *etal*., 2002).

Some of the proteinaceous toxins given off by bacteria to inhibit the growth similar bacterial strain called bacteriocins. Resistance pattern of microorganism vary from country to country, state to state, large hospital to small hospital and hospital to community. In Pakistan the problem of antibiotics resistance is compounding because of overuse and misses use of antibiotics (Erah *et al.*, 2003).

2 MATERIALS AND METHODS

Urine samples were collected from DHQ hospitals of Kotli (A.K). Sterilized bottles were used to bring the samples to laboratory. The 100 µl of each urine sample was spread on MacConkey agar medium plates. These plates were incubated at 37^{o}C for 48 hours. After 48 hours of incubation, pink color colonies were grown on the MacConkey agar medium plate. Eight strains were randomly isolated from above mentioned sources. All *E. coli* strains designated as ST1, ST2, ST3, ST4, ST5, ST6, ST7 and ST8 as shown in table 3. *E. coli* were identified on the basis of morphological characteristics and cultural characteristics. After culturing of *E. coil* on MacConkey agar, these are introduced on nutrient agar on which antibiotics were applied to check sensitivity and resistance as shown in fig 1 and 2.

3 RESULTS AND DISCUSSION

Different strains show different behavior against different antibiotics. The antibiotics used are shown in table 1.

Table1: Antibiotics used for antibiotic susceptibility testing of *E.coli* strains

Antibiotics	SYMBOLS	CONCENTRATION (µg)
AUGEMENT	AMC	20
AMIKIM	AK	5
GENTAMYCIN	CN	10
CIPIROXIN	CIP	5
CEFRADIN	CE	20
CEFTRIAXON	CRO	25
OFLOXACIN	OFX	10
SULPHMETHOXAZOLE	SXT	30
NORFLOXACIN	NOR	25

Table 3: Zone of inhibition in millimeters (mm) of E. coli strains against different antibiotics (symbol X shows completely resistance against antibiotic)

Strain No	Antibiotics									
	AK	SXT	AMC	OFX	NOR	CRO	CE	CIP	MRP	CN
ST1	18mm	5mm	12mm	18mm	7mm	X	X	15mm	17mm	18mm
ST2	16mm	17mm	8mm	10mm	10mm	14mm	X	17mm	16mm	8mm
ST3	17mm	X	X	14mm	6mm	18mm	6mm	8mm	17mm	20mm
ST4	22mm	23mm	14mm	12mm	11mm	10mm	20mm	12mm	21mm	22mm
ST5	19mm	8mm	X	X	18mm	X	X	20mm	21mm	22mm
ST6	15mm	X	12mm	14mm	12mm	14mm	X	16mm	20mm	10mm
ST7	13mm	18mm	16mm	15mm	X	19mm	14mm	13mm	16mm	12mm
ST8	17mm	20mm	18mm	19mm	15mm	18mm	10mm	18mm	19mm	17mm

Table 2: Percentage of resistance and susceptibility of E. coli strains against different antibiotics

Antibiotics	Susceptibility%	Resistance%
Amikim	100	0
Augment	75	25
Cefradin	50	50
Ofloxacin	88	12
Sulphmethoxazole	75	25
Norflaxcin	88	12
Gentamycin	100	0
Cipiroxin	100	0
Ceftriaxon	88	12
MRP	100	0

The strains which do not show any zone of inhibition show completely resistance against the antibiotic while the strain which form the zone of 10mm-15mm was consider as weakly susceptible against antibiotic. Some strains have zone of 20mm and are highly susceptible to such antiobiotics. In 1919, Thielman and Guerrant reported that low level of resistanes was absorbed for ciproflaxin and gentamycin (Schmitt *et al*., 2011). Among non-β lactams gentamycin shows good activity with 100% isolates found susceptible in this study which is more than recorded in Israel and India (Ullah *et a*l.,2009). During this study maximum sensitivity was shown by MRP 100% and Amikim 100% against strains of *E. coli* (ST1, ST2, ST3, ST4, ST5, ST6, ST7 and ST8). Gentamycin and ciproxin also show the susceptibility of 100% against all the strains of *E. coli* (ST1, ST2, ST3, ST4, ST5, ST6 and *E. coli* ST8). Sulphimethoxazole shows 75% susceptiblity against 6 the strains of *E. coli* (ST1, ST2, ST4, ST5, ST7, ST8). Out of 8 strains 6 strains of *E. coli* (ST1, ST2, ST4, ST7, ST8) show the 75% of susceptibilty against the Augment . While out of 8 strains 7 strains of *E. coli* (ST1, ST2, ST3, ST4, ST5, ST6 and ST8) show the 88% of susceptibilty against the Norfloxacin. In

case of Cefradin 4 strain of *E. coli* (ST3, ST4, ST7 and ST8) show 50% 0f susceptibility. This sensitivity and resistance has shown in table 3.

In 2004, 3,641 samples were received by the pathology laboratory of Ayub Medical complex of Pakistan. In this study of 2004, it was demonstrated that *E. coli* was found highly susceptible to ceftriaxone and ceftazidime (Rashid, 2004). In our study, 4 strains of Cefradin of *E. coli* (ST1, ST2, ST5 and ST6) show 50% resistance. Sulphimethoxazole shows 25% resistance against 2 strains of *E. coli* (ST3 and ST6). While out of 8 strains 1 strain of *E. coli* (ST7) shows the 12% of resistance against the Norfloxacin. Out of 8 strains 2 strains of *E. coli* (ST3 and ST5) show the 25% of resistance against the Augment . All these strains of *E. coli* (ST1, ST2, ST3, ST4, ST5, ST6, ST7, and ST8) do not show resistance against cipiroxin ceftriaxon, MRP and gentamycin as shown in table 2.

Conclusion

From present study it is concluded that antibiotics show variations in resistance and sensitivity to microorganisms.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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