Comparative Study of Antibiotic Resistance of "Escherichia coli" Isolates from Human and Faecal Matter of Urban Rats

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Abstract:

In view of the increasing emergence of antimicrobial resistant *E. coli* strains and the on-going discussion about environmental reservoirs, it is important to evaluate antibiotic resistance of human pathogens associated with animals. In the present study, 30 fecal Escherichia coli isolates, recovered from Human (UTI and diarrhea patient) and Rat (*Rattus norvegicus, R. rattus)* fecal matter were studied for susceptibility to nine antimicrobial drugs. The prevalence of strains resistant to routine antibiotics like tetracycline, amikacin, ampicillin, piperacillin, and streptomycin were 72% in human and 40% in rat fecal matter. These findings demonstrate that resistance gene reservoirs are increasing in healthy persons as well as in others mammals. Ingestion of antibiotics is known to provide selective pressure ultimately leading to a higher prevalence of resistant bacteria in urban area, even among who have not taken antibiotics. The source of resistant organisms in our study population is not known, but possible sources are food, water, and person-to-person transfer.

Keywords:
UTI, antibiotics, resistant, diarrhea.

Introduction:

Theodor Escherich first described *E. coli* in 1885, as bacterium coli commune, which is isolated the feces of newborns. It was later renamed *Escherichia coli*. GI tract of most warm blooded animals is colonized by *E. coli* within few hours or after birth. The bacterium is ingested through foods or water or obtained directly from other individuals. The human bowel is usually colonized within 4 hours after birth. *E. coli* can adhere to the mucus overlying the intestine. Once established, an *E. coli* strain may persist for months or years. Resident strains shift over long period (week to month), a more rapidly after an enteric infection or antimicrobial chemotherapy that perturbs the normal flora (1, 2). *E. coli* is the head of large bacterial family, *Enterobacteriaceae*, the enteric bacteria, which are facultative anaerobic, gm – ve rods live in the intestinal tracts of animals. *E. coli* can respond to environmental signals such as chemicals, pH, temperature, as molarity, etc. in a number of very remarkable ways considering single celled organism. *E. coli* is a consistent inhabitant of the human intestinal tract ant it is the predominant facultative organism in the human GI tract (3). *E. coli* is responsible for four

types of infection (4,5,6) i.e. Urinary tract infection (UTI), Gastro Intestinal diseases (Gastroenteritis), Pyogenic Infection and Neonatal meningitis.

Urban rats (*Rattus rattus*) present a global public health concern as they are considered a reservoir and vector of zoonotic pathogens, including *Escherichia coli*. In view of the increasing emergence of antimicrobial resistant *E. coli* strains and the on-going discussion about environmental reservoirs, it is important to evaluate antibiotic resistance of human pathogens associated with animals. *E. coli* is present in rat intestine which is free from any antibiotic treatment (7,8). These strains of *E. coli* which are isolated from Rat Fecal Matter act as probiotic against various pathogens. These bacteria posses the ability to survive in the host depending on their metabolic activity, resistant to gastric acidity, adhesion to the mucosal surface, resistant friendly to the host and thus protect the rat (6.5). In the laboratory, bacteria forms zone of limited growth at the periphery of zone of inhibition during the antibiotic sensitivity test. This zone of limited growth is the probably due to sub-lethal concentration of antibiotic present at the periphery (9,10).

Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control. Normal intestinal flora is a reservoir for resistance genes; the prevalence of resistance in commensal *Escherichia coli* is a useful indicator of antibiotic resistance in bacteria in the community (11,12). Studies with *E. coli* are of particular relevance because this species can occupy multiple niches, including human and animal hosts. In addition, *E. coli* strains efficiently exchange genetic material with pathogens such as *Salmonella, Shigella, Yersinia,* and *Vibrio* species, as well as pathogenic *E. coli* (13,14).
Antibiotic resistance is the ability of bacterial cell to resist the harmful effect of the antibiotic. In order for a bacterium to gain resistance to a given antibiotic there must be either a natural mutation in a gene within the bacterial chromosome or a system that leads to a resistance must be acquired. If the genetic information encoded by a plasmid leads to resistance against a particular antibiotic, this plasmid is known as Resistance Plasmid (15).

Material and methods:

1.1 **Media** – Nutrient agar (M001), Mac Conkey Agar (M081), Eosin Methylene Blue Agar (M118), High Sensitivity Agar (M485), Nutrient Broth (M002), Sugar fermentation medium (M028), Tryptone broth (M463), Glucose phosphate medium (M070), Simmon's citrate agar (M099), Urea base agar (M112), Triple sugar iron agar (MM021).

1.2 **Antibiotics** -Amikacin (AK), Gentamycin (HLG), Piperacillin (PI), Tetracycline (TE), Chloramphenicol (C), Norfloxacin (NX), Cefixime (CFM), Tobramycin (TB), Streptomycin (S), Ampicillin (AMP), Cefoperazone (CPZ),

Cefuroxime (CXM), Ciprofloxacin (CIP), Cefepime (CPM), Cefotaxime (CTX), Imipenum (IMP).

1.3 **Collection of sample** –Samples were collected from the patients suffering from UTI and GI infection from hospitals and pathologies. Samples of Rat feacal matter were collected from different zones of Nagpur city.

1.4 **Isolation of *E. coli* sp.** –*E. coli* was isolated from the clinical samples of patients suffering from UTI and GI infection by enrichment in lactose broth. These cultures were then purified in the lab by streaking on EMB agar plate and picking typical colony of *E. coli* and streaking on nutrient agar slant.

2.5 **Identification of isolates -** Gram staining and motility were performed to know the morphology and motility of *E. coli* isolates. Cultural characteristics were studied on EMB agar medium. Biochemical characteristics of isolates were studied by inoculating into sugar fermentation medium i.e. Glucose, Lactose and Manitol, IMViC test was carried out. Thus, *E. coli* was identified on the basis of morphological, cultural and biochemical characteristics and the results were compared with Bergey's Manual of Determinative Bacteriology 9th edition.

2.6 **Inoculums preparation -** Loopful of culture from slants was inoculated in 5ml sterile nutrient broth and incubated at 37^{0}C for 24hrs. Again loopful of culture from this broth was transferred to 5ml of sterile nutrient broth and incubated at 37^{0}C for 6-8 hrs, this was used as an inoculums.

2.7.1 **Antibiotic Sensitivity of Isolates -** Antibiotic sensitivity of the isolates was carried out by the disc diffusion method with commercially available discs (HiMedia, Mumbai, India). 0.5 ml of inoculums was added in each sterile petri plate, and then 15 ml sterile molten high sensitivity agar media maintained at 50°C was poured into each petri plate. Mix properly to ensure uniform distribution of micro organism into the medium. The plates were allowed to set. Antibiotic disc placed aseptically with the help of sterile forceps and placed on the surface of agar medium and pressed gently. The plates were then kept immediately in refrigerator for 1 hr for proper diffusion of antibiotic into the medium. The plates were removed and incubated at 37°C for 24 hrs. After incubation all plates were examined for zone inhibition. Zone was measured and recorded as sensitive, intermediate or resistant to a particular antimicrobial agent on the basis of the diameters of the inhibitory zones that matched the criteria of the manufacturer's interpretive table, which followed the recommendations of the Performance Standard for Antimicrobial Disk Susceptibility Tests, CLSI (CLSI 2007) (12)

Result and discussion:

Total 30 isolates of suspected *E. coli* were isolated, 10 from suspected cases of UTI, 10 from the suspected cases of diarrhea and 10 from Rat fecal matter. These 30 isolates were identified as *E. coli* on the basis of their

Morphological, Cultural and Biochemical characteristics. The results of antibiogram study of isolates isolated from human and isolated from rat are given in Table 1 and 2 respectively. The isolates isolated from UTI were labeled as HU1, HU2, HU3, HU4, HU5, HU6, HU7, HU8, HU9, HU10 and those isolated from cases of Diarrhea are named as HU11, HU12, HU13, HU14, HU15, HU15, HU16, HU17, HU18, HU19, HU20. Isolates from Rat Fecal Matter labeled as RF1, RF2, RF3, RF4, RF5, RF6, RF7, RF8, RF9, RF10.

Pathogenic *E. coli* isolates from UTI patient i.e. U1 was found to be sensitive to AK, G, C, S, PC TB indermediate to FX where as resistance to T. The isolates U2 is more resistance because it showed resistance pattern against AK, G, C, S, PC, TB, NX, T only in case of FX it gives intermediate zone. Isolate U3 was also found to be resistance against G, C, S, PC, TB and T. It showed sensitivity pattern against AK and NX, where as intermediate to FX. Isolate U4 was found to be sensitive to AK and NX intermediate to G and resistance to C, S, FX, TB T. Isolate U5 was found to be sensitive to AK, G, C. NX, TB, T. It showed intermediate pattern to S and resistance against FX and PC. Isolate U6 was found to be sensitive to AK, G, C, S where as resistant to FX, NX, PC, TB and T. Isolate U7 showed sensitivity pattern against antibiotics named as AK, G, C, S, TB, FX and NX exhibited resistant pattern to only two antibiotics i.e. PC and T. Isolate U8 was found to be sensitive to AK, G, C, S, FX and TB where as resistant to NX, PC and T. Isolate U9 was found to be sensitive to AK, G, C, S, FX, TB and T where as intermediate to NX and PC. Isolates U10 was found to be intermediately susceptible to PC and sensitive to remaining all 9 antibiotics.

Isolate H11 was isolated from stool sample of diarrhea patient was found to be sensitive to AK and C, intermediate to G and FX where as resistant to S, NX, PC TB and T. Isolate H12 was found to sensitive to AK, G and C. Intermediate to FX where as resistant to S, NX, PC TB and T. Isolate H13 was also isolated from stool sample of diarrhea. It was highly resistant because it give resistant pattern to all 9 antibiotics. Isolate H14 was found to be sensitive to AK, G, C, S, NX, TB and T where as resistant to FX and PC. Isolate H15 was found to be sensitive to AK, G, C, S, NX, TB and intermediate to PC where as resistant to FX and T. Isolate H17 was found to be highly sensitive because it showed sensitivity pattern to all antibiotics and give intermediate zone to FX. The isolate H18 and H19 were also demonstrated sensitivity pattern to all antibiotics.

In antibiotic sensitivity study of *E. coli* isolates isolated from rat fecal matter, it was observed that isolate RF1 to RF4 were found to be sensitive and intermediate to all antibiotics. It did not show resistant pattern against all antibiotics. Isolate RF5 to RF10 were found to be resistant and intermediate to most of the antibiotics like human isolates. Beside this, RF1 was resistant against CP and CFM where as intermediate in PI. Isolates RF2 and RF3 were

resistant to CFM, CPZ, CIP, CXM, AMP, and CPM. Isolates RF4 and RF5 were resistant to PI, CFM, and CPZ but intermediate to CIP, CXM and AMP. Isolate RF6 was sensitive for almost antibiotics and shows resistivity only in CFM and CPZ. Isolates RF7 and RF8 shown similar type of resistivity pattern against NX, PI, TE, CFM, CPZ, CIP, CXM, CTX, AMP and CPM. Isolate RF9 was resistant against CFM, CPZ, CIP, CXM, CTX, AMP and CPM. Isolate RF10 was resistant against PI, TE, CFM, CPZ, CTX, CPM.

Piperacillin and Tetracycline resistance was the most common type of resistance observed and 70% of total isolates in human and rat isolates exhibited resistance. This finding is not surprising because tetracycline has been widely used in therapy and to promote feed efficiency in animal production systems since its approval in 1948 (2,14,15). Persistence of tetracycline resistance was reported in animal coliforms a decade after it was no longer used in feed or for treatment.

The chloramphenicol-resistant animal *E. coli* isolates, more than 90% of chloramphenicol-resistant *E. coli* isolates were concurrently resistant to tetracycline. Extended-spectrum beta-lactamase (ESBL)-producing strains account for serious problems in the treatment of infectious diseases in humans and animals as these enzymes confer resistance to nearly all beta-lactam antimicrobial drugs, including third and fourth generation cephalosporins (15,16,17,18). In addition, our data showed an increasing piperacillin, tetracycline and ciprofloxacin resistance trend over a time among animal *E. coli* isolates. Present study also shows that cephalosporins antibiotics are very less effective against *E.coli* sp.

Gentamicin, amikacin, streptomycin was approved for use after 1963. Although gentamycin resistance was rare in human *E. coli* isolates, we found resistance rates <40% among animal *E. coli*. Since 1980, resistance to gentamicin has increased among animal *E. coli* isolates (18,19). Additional data that determine the resistance trend over second, third and fourth-generation cephalosporins which were introduced in the 1980s. But in our study 30% human *E. coli* were resistance to gentamycin and animal E. coli were sensitive to gentamycin.

A small percentage of *E. coli* showed resistance to chloramphenicol, a drug approved in 1947 for human clinical use. Chloramphenicol is not approved for use in food animals in the United States.(20,21,22) Persistence of chloramphenicol resistance in *E. coli* has been observed by other authors. Florfenicol, a closely related drug, was approved for treatment of respiratory diseases in cattle in the United States in 1996. (23,24)

Table. 1–Antibiogram of *E. coli* isolates isolated from human.

E.coli Isolates	Antibiotics															
	AK	**HLG**	**C**	**S**	**NX**	**PI**	**TOB**	**TE**	**CFM**	**CPZ**	**IPM**	**CIP**	**CXM**	**CTX**	**AMP**	**CPM**
HU 1	0	14	25	0	10	0	11	11	0	0	33	0	0	0	0	0
	R	I	S	R	R	R	R	R	R	R	S	R	R	R	R	R
HU 2	0	15	24	0	11	0	12	10	0	0	28	0	0	0	0	0
	R	S	S	R	R	R	R	R	R	R	S	R	R	R	R	R
HU 3	28	30	24	20	14	0	13	10	0	0	32	0	0	0	0	10
	S	S	S	S	I	R	I	R	R	R	S	R	R	R	R	R
HU 4	20	31	19	18	13	0	17	11	0	0	34	0	0	10	0	0
	S	S	S	S	I	R	S	R	R	R	S	R	R	R	R	R
HU 5	0	32	27	18	12	0	10	11	0	0	28	11	0	0	0	0
	R	S	S	S	R	R	R	12	R	R	S	R	R	R	R	R
HU 6	0	12	29	23	11	0	10	10	10	0	30	0	15	0	0	10
	R	R	S	S	R	R	R	R	R	R	S	R	I	R	R	R
HU 7	16	12	30	24	0	18	20	0	11	0	31	0	11	17	0	0
	I	R	S	S	R	I	S	R	R	R	S	R	R	I	R	R
HU 8	28	28	32	11	11	0	11	10	11	0	31	0	12	12	0	0
	S	S	S	R	R	R	R	R	R	R	S	R	R	R	R	R
HU 9	15	34	26	10	10	0	0	11	10	10	30	0	0	0	0	0
	I	S	S	R	R	R	R	R	R	R	S	R	R	R	R	R
HU 10	0	11	27	25	11	0	0	10	12	11	29	0	0	13	0	10
	R	R	S	S	R	R	R	R	R	R	S	R	R	R	R	R
HU 11	29	30	25	21	9	23	20	10	11	12	32	0	15	11	10	10
	S	S	S	S	R	S	S	R	R	R	S	R	I	R	R	R
HU 12	0	0	15	11	0	0	0	0	10	0	34	0	11	10	10	0
	R	R	I	R	R	R	R	R	R	R	S	R	R	R	R	R
HU 13	23	13	0	0	21	0	0	0	9	0	33	10	10	0	0	0
	S	I	R	R	S	R	R	R	R	R	S	R	R	R	R	R
HU 14	20	19	25	14	20	0	18	15	0	0	34	11	0	0	10	0
	S	S	S	I	S	R	S	S	R	R	S	R	R	R	R	R
HU 15	27	26	29	30	0	22	0	9	0	12	31	0	0	11	0	10
	S	S	S	S	R	S	R	R	R	R	S	R	R	R	R	R
HU 16	28	37	32	24	8	0	24	0	10	11	30	10	0	12	0	11
	S	S	S	S	R	R	S	R	R	R	S	R	R	R	R	R
HU 17	29	34	30	26	20	21	16	28	11	0	30	0	0	10	0	11
	S	S	S	S	S	S	S	S	R	R	S	R	R	R	R	R
HU 18	18	38	22	0	23	21	22	27	12	0	29	11	11	9	0	0
	S	S	S	R	S	S	S	S	R	R	S	R	R	R	R	R
HU 19	24	26	21	20	25	10	21	22	0	0	31	12	12	10	0	0
	S	S	S	S	S	R	S	S	R	R	S	R	R	R	R	R
HU 20	26	25	30	24	0	0	19	0	0	13	32	0	0	0	0	0
	S	S	S	S	R	R	S	R	R	R	S	R	R	R	R	R

S- sensitive, R- resistant, I- Intermidiate

Table. 2 –Antibiogram of *E. coli* isolates isolated from rat.

E. coli Isolates	Antibiotics															
	AK	HLG	C	S	NX	PI	TOB	TE	CFM	CPZ	IPM	CIP	CXM	CTX	AMP	CPM
RF 1	20	21	22	18	23	20	22	20	10	0	33	24	15	21	15	20
	S	S	S	S	S	I	S	S	R	R	S	S	I	I	I	S
RF 2	24	22	24	18	21	21	15	20	11	0	33	10	11	17	10	11
	S	S	S	S	S	S	I	S	R	R	S	R	R	I	R	R
RF 3	25	23	25	20	15	22	20	20	10	0	30	11	10	13	10	10
	S	S	S	S	I	S	S	S	R	R	S	R	R	R	R	R
RF 4	31	21	27	19	20	12	18	18	0	9	31	23	15	18	16	19
	S	S	S	S	S	R	S	I	R	R	S	S	I	I	I	S
RF 5	28	22	22	17	11	10	17	10	12	10	33	0	11	12	0	12
	S	S	S	S	R	R	S	R	R	R	S	R	R	R	R	R
RF 6	24	24	24	21	22	22	15	20	11	0	32	24	15	20	16	20
	S	S	S	S	S	S	I	S	R	R	S	S	I	I	I	S
RF 7	25	23	24	20	10	12	18	11	10	0	31	0	10	12	0	0
	S	S	S	S	R	R	S	R	R	R	S	R	R	R	R	R
RF 8	26	25	27	20	11	12	19	10	10	0	30	0	0	13	0	10
	S	S	S	S	R	R	S	R	R	R	S	R	R	R	R	R
RF 9	22	23	25	22	21	22	21	20	0	10	32	0	0	11	10	0
	S	S	S	S	S	S	S	S	R	R	S	R	R	R	R	R
RF 10	20	21	25	18	22	10	18	10	0	0	31	23	15	12	15	10
	S	S	S	S	S	R	S	R	R	R	S	S	I	R	I	R

S- sensitive, R- resistant, I- Intermidiate

Table. 3– Resistivity and Sensitivity pattern of Human and Rat in percentage (%)

Antibiotics	RESISTANCE %		SENSITIVITY %	
	Human	Rat	Human	Rat
AK	40	0	60	100
HLG	30	0	70	100
C	10	0	90	100
S	40	0	60	100
NX	75	40	25	60
PI	80	60	20	40
TOB	55	20	45	80
TE	80	50	20	50
CFM	100	100	0	0
CPZ	100	100	0	0
IPM	0	0	100	100
CIP	100	60	0	40
CXM	100	100	0	0
CTX	100	100	0	0
AMP	100	100	0	0
CPM	100	70	0	30

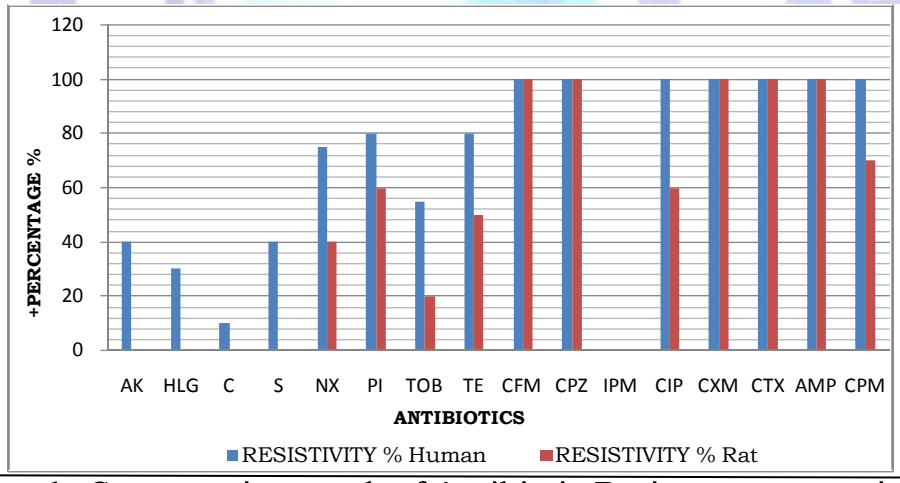

Figure. 1- Comparative graph of Antibiotic Resistant pattern in Human and Rat

Conclusion:

We observed rapid increase in the prevalence of resistance in commensal *E. coli* to most of the older, less expensive antimicrobial drugs used in the management of infections in human. Not only are these strains potential causes of infection, but they are also potential reservoirs of resistance genes that could be transferred to pathogens. For this reason, the trends seen with commensal *E. coli* may also be observed with pathogenic organisms. Our study emphasizes the need to monitor commensal organisms as well as pathogens by susceptibility testing to guide treatment and to understand its prevalence in animal. Control of antibiotic resistance is needed to conserve the usefulness of the remaining drugs. These results are compared with standard *E. coli* isolated

from rat which would not be introduced any antibiotic which revealed that resistant *E. coli* also found their way in rat gut. The present study showed bacterial resistant growing due to continuous intake of antibiotic by human. This analysis provides foundational information for resistance development over time, laying the groundwork for understanding evolution of multidrug resistance in both human as well as in animal.

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