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Variations in antibiotic resistance and plasmid content in Escherichia coli isolates from the normal intestinal flora and clinical sources

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ABSTRACT

INTRODUCTION: Escherichia coli is one of the most important bacteria in the normal intestinal flora. The current study aimed to investigate their possession of antibiotic resistance and plasmids compared with their clinical isolates to determine the extent of their risk and health importance.
METHODS: For this purpose, 400 stool samples were collected from healthy individuals (newborns and healthy adults), as well as clinical samples. Microscopic, cultural, and biochemical diagnostic methods were used, and the diagnosis was confirmed using the Vitek 2 compact system to obtain 12 identified isolates of E. coli for each isolation source.
RESULTS: Antibiotic susceptibility tests revealed that multidrug resistance existed in nearly all isolate sources; for example, multidrug resistance in newborn isolates was 25-100%, while in healthy adult isolates was 25 - 75%, and in patients, isolates were 33.33 - 100%. Moreover, the results showed that the studied isolates possess plasmid bands in varying numbers, in newborn isolates 1- 3 plasmid bands, in healthy adult isolates 0-4 plasmid bands, and patient isolates 0- 3 plasmid bands.
CONCLUSION: We conclude from the current study that E. coli isolated from the normal flora of either newborns or adults, possess high resistance to antibiotics, and they also possess plasmids that support the existence of this resistance, which makes these isolates of high risk as its pathogenic isolates.

Keywords: *Escherichia coli*, Normal flora, Antibiotic resistance, Plasmid

INTRODUCTION

The impact of normal flora, especially intestinal microbiota, on human health and disease has been long acknowledged, which has stimulated many studies worldwide to focus on the significance and the association of human health with these microbiota [1]. Normal flora is one of the most common agents responsible for the transportation of resistance genes with spread between the other normal flora and pathogenic bacteria. The normal flora is the resource of genes that have antibiotic resistance characteristics and is most dependable for the increase of antimicrobial resistance [2]. Bacteria use different strategies for resistance, including phenotypic and genetic mechanisms.

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These mechanisms may involve the absence of an antibiotic target, reduced permeability of the cell wall, or the production of specific enzymes [3].

Drug resistance genes are easily transferred between different bacterial species, including normal flora and pathogenic bacteria in the intestine, accelerating the spread of multidrug resistance [4]. Studies related to the effectiveness of antibiotics against *E. coli* are of great importance because these bacteria act as a storehouse of resistance genes in the intestine, which are later transferred to disease-causing strains by giving or acquiring plasmids or providing a genetic platform for the emergence of resistant strains [5]. Bacterial resistance to commonly used antibiotics poses a public health threat around the world, and this resistance is coupled with the presence of plasmids that constitute the primary tool for disseminating resistance determinants in *E. coli*, which contain the resistance genes [6].

An increase in the drug resistance of *E. coli* strains has been observed due to their tremendous adaptability, enabling them to acquire and confer genes through horizontal gene transfer [7]. E. coli generally harbored a much greater number of resistance genes compared to other bacteria [8]. Therefore, it can take resistance genes from other bacteria and can also pass them on to other bacteria, which may pose a major challenge. *E. coli* strains are characterized by the acquisition of many resistance genes like genes encoding broad-spectrum beta-lactamases, genes encoding carbamates, aminoglycoside resistance, and quinolone resistance genes [9].

The current study was conducted to investigate whether bacteria isolated from the normal flora of the intestine possess antibiotic resistance and plasmid content compared to their clinical isolates, and thus determine the extent of their risk and health importance.

METHODS

Isolation and identification: In this study, 250 stool samples were collected from healthy individuals (100 newborns and 150 Healthy adults of different ages and sexes), as well as 150 clinical samples from patients (including diarrhoea, urinary tract and bacteremia infections). The samples were cultured on MacConkey agar medium, then Eosin Mythelin Blue medium. The isolates expected to be E. coli bacteria were subjected to microscopic testing using Gram stain, oxidase, and catalase tests. Then, they were purified and diagnosed using the Vitek 2 compact system. The isolation and diagnosis step aimed to reach 36 diagnosed E. coli isolates, distributed into 12 isolates for each isolation source (newborns, healthy adults, and patients) [10].

Antibiotic susceptibility test: Susceptibility of isolated E. coli isolates to different antibiotics was determined by Kirby-Bauer disc-diffusion techniques on Mueller Hinton agar (MHA) plates and interpreted according to the National Committee for Clinical Laboratory Standards [11]. Penicillin (P 10 µg), Ampicillin (AM25µg), Vancomycin (VA30µg), Novobiocin (NV30µg), Levofloxacine (LEV 5µg), Trimethoprim (TMP10µg), Ofloxacin (OFX5µg), Cefotaxime (CTX10µg) Trimethoprime Sulphamethoxazol (STX 30µg), Ciprofloxacin (CIP5µg), Tetracycline (TE10µg), and Ceftriaxone (CRO 10 µg).

Plasmid DNA Extraction: The Promega plasmid DNA extraction kit provided by Promega (USA), was used to purify plasmid DNA from bacterial isolates [12]. As per manufacturer instructions, bacteria were suspended in a nutrient broth medium and incubated for 24 hours. Bacteria were collected by centrifugation and resuspended in a cell lysis buffer [12,13]. The mixture was mixed thoroughly and incubated in a water bath at 37°C for 30 minutes. A neutralization solution was added and mixed thoroughly. Then, the mixture was incubated in a water bath at 37°C for 5 minutes. Following that, the mixture was centrifuged again to separate the plasmid DNA from the cell debris. The presence of plasmids in each sample was detected by electrophoresis in 1% agarose gel [14].

RESULTS

Results have shown antibiotic susceptibility patterns of E. coli isolates from different sources. The results of newborn isolates confirmed a high rate of resistance to many antibiotics, including Cefotaxime (100%), Tetracycline (91.6%), Novobiocin (91.6%), Penicillin (83.3%), Ampicillin (75%), and Vancomycin (75%) (Table 1).

Table 1: Antibiotic susceptibility pattern of *E. coli* isolates from newborns

Isolate	Antibiotics											
	P	AM	VA	NV	The LEV	TMP	OFX	STX	CTX	CIP	TE	CRO
1	R	R	R	R	S	R	R	R	R	I	R	S
2	R	R	R	R	R	R	R	R	R	R	R	R
3	R	I	R	R	I	S	S	S	R	S	R	I
4	I	R	R	I	R	R	R	R	R	R	I	R
5	R	R	R	R	R	R	R	R	R	R	R	R
6	R	R	R	R	S	R	I	R	R	I	R	R
7	R	R	I	R	S	S	S	S	R	S	R	I
8	R	S	R	R	S	S	S	S	R	S	R	S
9	I	I	S	R	S	S	S	S	R	S	R	I
10	R	R	R	R	R	I	R	S	R	I	R	S
11	R	R	I	R	I	R	S	R	R	R	R	I
12	R	R	R	R	I	R	S	R	R	I	R	R
% Resistance	83.3	75	75	91.6	33.3	58.3	41.6	58.3	100	33.3	91.6	41.6

Sensitive (S), Intermediate (I), Resistant (R), Penicillin (P), Ampicillin (AM), Vancomycin (VA), Novobiocin (NV), Levofloxacine (LEV), Trimethoprim (TMP), Ofloxacin (OFX), Sulfamethoxazol (STX), Cefotaxime (CTX), Ciprofloxacin (CIP), Tetracycline (TE), and Ceftriaxone (CRO).

Results of healthy adult isolates showed a high rate of resistance to Novobiocin (100%), Vancomycin (83.3%), Penicillin, and Ceftriaxone (75%) (Table 2).

The results also showed a high rate of resistance to Cefotaxime (100%), Penicillin (91.6%), Ampicillin (91.6%), tetracycline (91.6%), Ceftriaxone (91.6%), Penicillin, Ciprofloxacin (75%), and

Table 2: Antibiotic susceptibility pattern of *E. coli* isolates from healthy adults

Isolate	Antibiotics											
	P	AM	VA	NV	The LEV	TMP	OFX	STX	CTX	CIP	TE	CRO
1	I	S	I	R	S	S	S	S	I	S	R	R
2	R	R	R	R	S	R	S	I	R	S	S	R
3	R	S	R	R	S	S	S	S	I	S	I	R
4	R	R	R	R	S	I	R	S	R	I	R	R
5	I	S	R	R	S	R	S	R	R	S	R	R
6	R	S	S	R	S	S	S	S	S	R	I	R
7	R	R	R	R	R	S	R	S	R	R	I	S
8	R	R	R	R	S	R	S	R	R	S	R	R
9	R	R	R	R	S	R	S	I	S	S	R	S
10	I	I	R	R	S	S	S	S	S	S	S	S
11	R	R	R	R	S	R	S	R	R	S	R	R
12	R	R	R	R	S	R	S	I	R	S	S	R
	75	58.3	83.3	100	8.3	50	16.6	25	58.3	16.6	50	75

Sensitive (S), Intermediate (I), Resistant (R), Penicillin (P), Ampicillin (AM), Vancomycin (VA), Novobiocin (NV), Levofloxacine (LEV), Trimethoprim (TMP), Ofloxacin (OFX), Sulfamethoxazol (STX), Cefotaxime (CTX), Ciprofloxacin (CIP), Tetracycline (TE), and Ceftriaxone (CRO).

Table 3: Antibiotic susceptibility pattern of *E. coli* isolates from patients

Isolate	Antibiotics											
	P	AM	VA	NV	The LEV	TMP	OFX	STX	CTX	CIP	TE	CRO
1	R	R	I	R	R	R	R	I	R	R	R	R
2	R	R	S	R	R	R	R	R	R	R	R	R
3	R	R	I	R	R	R	R	R	R	R	R	R
4	R	R	S	R	R	S	I	S	R	R	R	R
5	R	R	R	R	R	R	R	R	R	R	I	R
6	R	R	R	R	R	R	R	R	R	R	R	R
7	R	R	R	R	R	R	R	R	R	R	R	R
8	R	R	R	R	R	R	R	R	R	R	R	R
9	R	R	R	R	S	S	S	S	R	S	R	R
10	R	R	R	R	S	R	S	R	R	I	R	R
11	I	I	R	R	S	S	S	S	R	I	R	I
12	R	R	I	R	I	R	I	S	R	R	R	R
% Resistance	91.6	91.6	58.3	100	66.6	75	58.3	58.3	100	75	91.6	91.6

Sensitive (S), Intermediate (I), Resistant (R), Penicillin (P), Ampicillin (AM), Vancomycin (VA), Novobiocin (NV), Levofloxacine (LEV), Trimethoprim (TMP), Ofloxacin (OFX), Sulfamethoxazol (STX), Cefotaxime (CTX), Ciprofloxacin (CIP), Tetracycline (TE), and Ceftriaxone (CRO).

Sulphamethoxazol (75%) (Table 3).

All the studied isolates possessed multidrug resistance with varying percentages, ranging in newborn isolates (25- 100%), healthy adult isolates (25 - 75%), and patient isolates (33.33 - 100%) (Table 4). All sources of isolation have shown that the plasmid content possesses plasmid bands in varying numbers, ranging in newborn isolates with 1- 3 plasmid bands, in healthy adult isolates with 0 - 4 plasmid bands, and in patient isolates with 0- 3 plasmid bands (Table 5 and Figure 1).

Table 4: Percentage of Multiple Drug Resistance among *E. coli* isolates from different sources

Isolate	% Multiple Drug Resistance		
	Newborns	Healthy adults	Patients
1	75	25	83.3
2	100	58.33	91.6
3	41.6	33.33	91.6
4	75	66.66	66.66
5	100	58.33	91.6
6	75	33.33	100
7	41.6	66.66	100
8	41.6	75	100
9	25	50	58.3
10	66.66	16.66	75
11	66.66	75	33.33
12	75	58.33	66.66

Table 5: Plasmid Bands Number of *E. coli* isolates from different sources.

Isolate	Plasmid Band Number		
	Newborns	Healthy adults	Patients
1	1	2	2
2	1	2	0
3	1	3	1
4	1	1	0
5	2	4	1
6	2	3	1
7	3	1	3
8	2	1	0
9	1	2	1
10	1	2	1
11	1	0	1
12	3	2	0

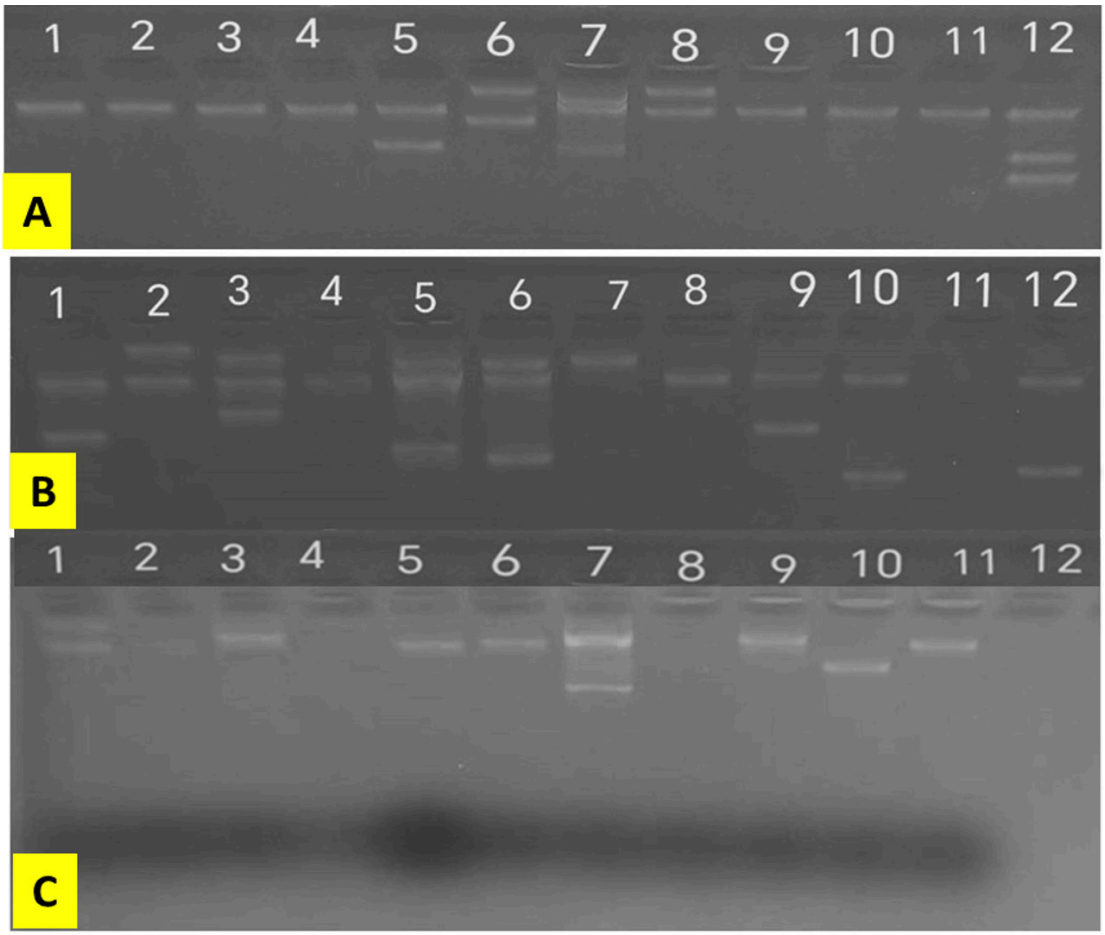

Figure 1. Plasmid profiles of *E. coli* isolates from (A) Newborns (B) Healthy adults (C) Patients.

DISCUSSION

The results of the current research showed that E. coli isolates were clearly resistant to the antibiotics used, and most of these isolates possessed multiple resistances, regardless of whether they were normal flora or pathogenic isolates. This confirms the spread of antibiotic resistance in the intestinal flora, including E. coli. The results also showed that most flora isolates possessed plasmids, and more than the pathogenic isolates possessed plasmids, if we take into account the role of plasmids in conferring antibiotic resistance to bacterial isolates, this confirms the results reached regarding the presence and spread of antibiotic resistance in the studied isolates, especially isolates of normal flora. Resistant bacteria colonize the gastrointestinal tract during the first weeks after birth, even if antibiotics are not used, due to their transmission from the mother to the fetus, whether via vaginal or cesarean delivery [15]. The study conducted by Hall et al. [16] is consistent with our results, which confirmed that the resistance of E. coli to antibiotics is due to its possession of plasmids. The study of Singh et al. [17] also showed that the normal flora in the digestive system of healthy adults contains a large number of antibiotic resistance genes, and the E. coli strains that possess the plasmid have a higher resistance than those that do not possess it. Therefore, it is clear that there is a consistent relationship between the presence of a specific plasmid and resistance to antimicrobial agents, which indicates the possibility of the spread of resistance through conjugation [18]. Globally, there is a clear risk of bacterial species becoming resistant to various antibiotics [19].

Resistance genes are transferred from more virulent pathogenic species to non-pathogenic species [20]. These acquired genes help bacteria resist antibiotics by encoding many of the mechanisms by which bacteria can survive and reproduce in their environment, such as producing

enzymes that degrade antibiotic molecules or changing their structure, changing the target site at which the antibiotic is targeted, or by Reducing the concentration of antibiotic molecules inside the bacterial cell using efflux pumps. The results are also consistent with those of Akter et al. [21], who have shown resistance of E. coli to antibiotics containing beta-lactam ring, ampicillin, and ceftriaxone. Because it produces 1 of 3 enzymes (beta-lactamase) responsible for changing the basic structure of antibiotics, making them ineffective. Another mechanism is the presence of several pumps that release these antibiotics, making the dose insufficient to eliminate bacteria [22]. E. coli, one of the normal flora, contributes and is responsible for the transportation of resistance genes between the other normal flora or pathogenic bacteria. This type of resistance may be responsible for the transport of genetic material within Enterobacteriaceae species [2].

CONCLUSION

E. coli isolated from the normal flora of either newborns or adults, based on the results of the current study, possess high resistance to antibiotics, and they also possess plasmids that support the existence of this resistance, which makes these isolates of high risk as its pathogenic isolates.

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