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Eschershia coli integrons and cfr, procfr genes identified from samples of autistic children's stools

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***Abstract*—Escherichia coli is a Gram-negative Bacilli belonging to the Enterobacteriaceae family. It has a rod-shaped coliform . A number of neuropsychiatric conditions, likeanxiety, schizophrenia, autism, bipolar disorder, and depressive disorder, Have been reported to be associated with the microbiome. Autism spectrum disorder (ASD), is a neurodevelopmental disturbance. A sample of fifteen E.coli bacteria were isolated *E. coli*isolates From ASD children's stools.ASD children. The isolates were identified by phenotypic identification using API 20 E. The molecular analysis was processed for Integron I,II,I, cfr, *procfr* genes.These resultsshowed Integron II less prevalence Than in Integron*I and III.* I,III while *pro-cfr*, more prevalence than *cfr*gene.Multidrug-resistant in *E. coli* has widespread.**

Keywords*: Autism, Integron I, II,I ,*cfr*, *procfr genes

I. INTRODUCTION PRESENCE

The microbial population of the human body known as the "microbiota," which includes bacteria, viruses, archaea, protozoans, and fungi (Bakhtiar *et al.*, 2013). Several neuropsychiatric conditions, such as schizophrenia, anxiety, bipolar , autism,and depressive disorder, have beenReported to be related to the microbiotato the microbiota. (Inserm, 2018). According to Abbasi et al. (2021), altered gut microbiota may be crucial in the development of autism. ASD is the name given to a class of neurodevelopmental diseases that are characterized by genetic and phenotypic variation in affected persons ~~affected~~. ASD sufferers exhibit a range of problems in their social and adaptive functioning, language, and cognitive abilities. (lord*et al.*,2018). *E. coli. ~~It~~ is a rod-shaped coliform that can ferment lactose at 44°C and can grow and exhibit a color reaction on recognized types of culture media, distinguishing it from the bulk of other coliforms. It produces pink colonies on MacConkey agar after fermenting lactose. A metallic green media with dark purple colonies on an Eosin Methylene blue (EMB) culture of E. coli suggests a successful outcome.* (Kavitha and Devasena, 2013). E. coli that is multidrug resistant (MDR) has emerged as one of the biggest threats to both human and animal health. (Aslam *et al.*, 2018).The ExPEC group includes uropathogenic E. coli (UPEC), neonatal meningitis E. coli(NMEC), sepsis-associated E. coli (SEPEC), and avian pathogenic E. coli (APEC) .ExPEC E. colihave many virulence-associated factors, including adhesins, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, and invasins, which are usually encoded on pathogenicity islands (PAIs), plasmids, and other mobile genetic elements (Köhler andDobrindt.,*2011*). Urinary tract infection is one of the most common infectious diseases(Tan and Chlebicki, 2016).

The propagation of resistant genes in bacteria has been linked to a variety of acquired resistance mechanisms, such as bacteriophages, transposons, plasmids, and integrons (Domingus *et al.*,2012).Integrons may also contribute to the more spread incidence and spread of antibiotic resistance (Chaly *et al.*, 2017). These components have the capacity to seize, incorporate, and mobilize antibiotic resistance gene cassettes. Based on the genetic similarity of the integrase intI gene sequence, the integrons were divided into three significant classes: I, II, and III. Class Iintegrons are ~~more~~the most prevalent in GNB.(Jonus –Dias *et al.*, 2016) . A derivative of chloramphenicol called florfenicol, which is solely used to treat infections in animals, is effective against isolates that are resistant to chloramphenicol. (Apley *et al.*, 2015). With the wide using of florfenicol in veterinary medicine and agriculture, resistance to florfenicol has increased rapidly (Zhao *et al.*, 2016).

II. MATERIALS AND METHODS

Fifteen *E.coli* isolates from the stool of ASD children.Phenotypic were diagnosed with API 20E. The study's ethical management was carried out in accordance with guidelines obtained from the College of Science at the University of Thi-Qar.All of the bacterial isolates used in this investigation were taken from patients of the Thi-Qar Autistic Disorders Rehabilitation Center with the consent of the patient and the doctor, who gave their formal clearance. Genetically, engineered organisms and prohibited biological elements were excluded from the study.

Bacterial DNA extraction protocol (Geneaid,USA) was used. Estimation of DNA quality wasdone usingNanodrop that determined the amount of DNA (ng/µL). Then, By measuring the absorbance between 260 and 280 nm, DNA purity was determined. The method has been proceeded according to the manufacturer's instructions. Primers used in this work are listed in Table 1. According to manufacturer instructions, PCR reaction done and the PCR products were analyzed by agarose gel electrophoresis (Cleaver Scientific Co., U.K.).

TABLE 1 PRIMERS SEQUENCES USED FOR GENES AMPLIFICATION

Primer	Primer Sequences (5'-3')	Products ize bp	References
cfr	FTGAAGTATAAAGCAGGTTGGGAGTCA	768	Lei *et al.*, 2018
	RACCATATAATTGACCACAAGCAGC		
pro-cfr	F- CAAATTGTGAAAGGATGAAAG	1073	Zhua, et *al.*,2020
	R-CTATTGGCTATTTTGATAATTAC		
IntI-I	F-TCTCGGGTAACATCAAGG	243	Ahangarkani *et al* .,2015
	R-AGGAGATCCGAAGACCTC		
Intl-II	F-CACGGATATGCGACAAAAAGG	788	Kargar *et al.*, 2014
	R-TGTAGCAAACGAGTGACGAAATG		
IntI-III	F-AGTGGGTGGCGAATGAGTG	600	Goldstein *et al.*,2
	R-TGTTCTTGTATCGGCAGGTG		

III. RESULT

A -Integrons of E. coli

A total of fifteen *E.coli* isolates were examined for Integrons classes I, II, III by Conventional PCR, and the result showed Int- I and Int-III present in all isolates percentage 100% while Int-II present in 8 isolates percentage 53.3% showed in table 2, Figures(1,2,3).

TABLE 2 -INTEGRONS OF *E. COLI*

Type of int.	No. of Isolates	Frequency	%
Int.1	15	15	100
Int.2	15	8	53.33
Int.3	15	15	100
X^2	16.57		
P value	0*		

* Significant difference at P<0.05

B-Resistance of Florfenicol in E.coli

Fifteen *E. coli* isolate were examined for *cfr* gene two primers (pro-cfr and cfr) by Conventional PCR, and the result showed frequency in 6(40%) isolates for cfr primer while 11(75.33) for pro-cfr primer. The results were showed in Table (3) and Figures(4,5).

TABLE (3) RESISTANCE FLORFENICOL IN *E. COLI* (FREQUENCY AND PERCENTAGE)

Type of gene	No. of Isolates	Frequency	%
cfr (cfr)	15	6	40
Cfr (pro-cfr)	15	11	75.33
X^2	0		
P value	1*		

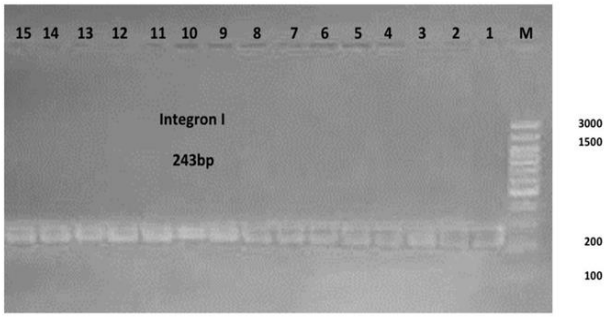

Fig. 1. Agarose gel electrophoresis (1.5%) showing Conventional PCR product of Integron classI. Lane M represents the DNA ladder. Lane (1-15) represents Conventional PCR product (243 bp). 70 volt for 45 min.

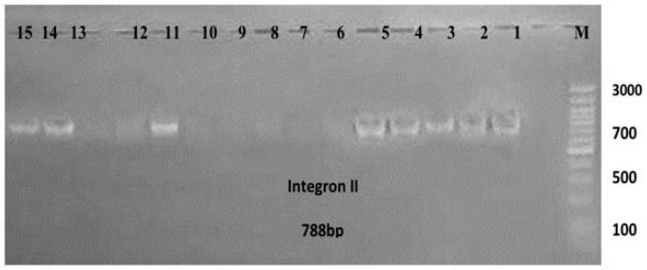

Fig. 2. Agarose gel electrophoresis (1.5%) showing Conventional PCR product of Integron class II. Lane M represents the DNA ladder. Lane (1-15) represents a Conventional PCR product (788 bp).70 volt for 45 min.

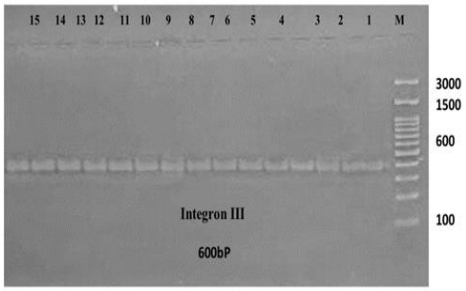

Fig. 3. Agarose gel electrophoresis (1.5%) showing Conventional PCR product of Integron class III. Lane M represents the DNA ladder. Lane (1-15) represents a Conventional PCR product (600 bp).70 volt for 45 min

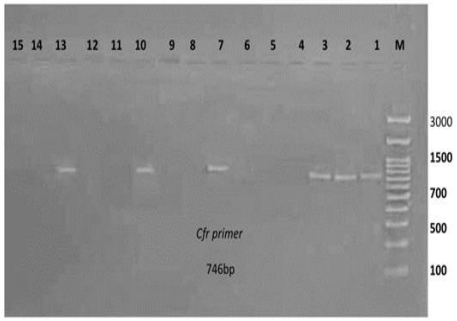

Fig. 4.

Agarose gel electrophoresis (1.5%) showing Conventional PCR product of cfr gene. Lane M represents the DNA ladder. Lane (1-15) represents Conventional PCR product (746 bp).70 volt for 45 min

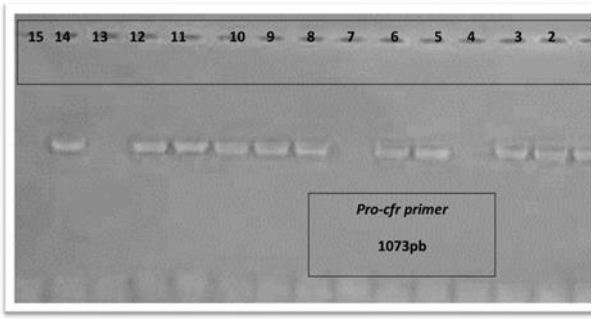

Fig. 5.

Agarose gel electrophoresis (1.5%) showing Conventional PCR product of cfr gene ((pro-cfr).Lane M represents DNA ladder. Lane (1-15) represents Conventional PCR product (1073bp). 70 volt ,45 min.

IV. DISCUSSION

A serious public health issue is the spread of illnesses due to the resistance of bacteria to antibiotics. Previous studies have shown that regardless of the pattern of antibiotic usage, antibiotic resistant genes can spread between bacterial populations. The development of multidrug-resistant bacteria currently relies heavily on the horizontal transmission of resistance genes (MDR strains) (Kargar*et al.*,2014).

This work aimed to investigate the role of class I, II, and III integrons in developing antibiotic resistance in *E. coli* isolates. The study was done for 15 isolates, and theresults showed the prevalence of integrons I, II and III frequency 15 (100%),8(53.33), and 15(100%), respectively. According to the previous study, *E. coli* isolates have a comparatively high incidence of class 1 integrons. (Karimi *et al.*, 2020).

Staji *et al.* (2018) have discovered that 23 strains (36.5 %) contained at least one integron encoding gene, with Class I integron being the most common type detected.

In another previous investigation, 26.5 % of the *E. coli* isolates had class1 integrons, a significant proportion. There was no proof of class 2 or 3 integrons in this investigation. (Huang *et al.*, 2020).

According to the previous study, 78.26% and 76.81%, respectively, of MDR isolates, Class 1 and Class 2 integrons were discovered.Aside from that, a class 3 integron was found in 26.09 % of MDR isolates (Kargar *et al.*, 2014). Abbassi *et al.* (2021) found from classes 1 and 2, respectively, One isolate had both of the integrons in 57 and 2 as well. Class 2 integrons only had one gene cassette array, compared to class 1 integrons' seven.

B. Florofinicol resistance*E. coli*

The present study examined 15 *E.coli* isolates for the cfr gene; two primers, pro-cfr and cfr, showed only 6 (40%) and 11(73.33%) respectively, favorable comparison with the previous study, 2.22% showed cfr gene-positive results. At the same time, no other known florfenicol-resistant gene was detected (Li et al., 2020). Cfr gene positivity was found in *E. coli* isolates, From 1.6% in 2014 to 29.1% in 2017, there was a considerable increase in the prevalence of the cfr gene. (Ma *et al.*, 2021). Zhao *et al.* (2020) mentioned a high resistance for florfenicol in *E.coli* at 95.1%.

V. CONCLUSION

Multidrug-resistant (MDR) in *E. coli*is widespread.This work showed high prevalence of IntegrionsI, III while II less prevalence and flourofincol resistance was appearance

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