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# Phylogenetic Detection of Patho Genic Escherichia coli Isolated from Different Sources in Najaf Hospitals

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#### Authors' contributions

This work was carried out in collaboration between both authors. Authors RN and JM managed the research. Authors RN and JM done the research and wrote the main manuscript text. Authors RN and JM prepared tables and wrote a part of manuscript text. All authors read and approved the final manuscript.

#### Article Information

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#### ABSTRACT

**The Aim :**The training intended to quarantine bacteria Escherichia coli from urinary tract infection, wound, burns patients and investigating the genes ChuA, yjaA, TspE4.C2 of the phylogenetic groups.

**Study Design:** 100 samples were collected from patients with urinary tract infection, wounds and burns, for a period from the twenty-seventh of July 2022 to the eleventh of December 2023, and after diagnosing the samples in the laboratory by means of selective media, the addition of the VITEK-2 compact.

**Place and Duration of Study:** All samples collected from July to December 2023. Samples were cultured on a MacConkey and Eosin Methylene Blue agar (EMB) and VITEK2 compact.

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**Methodology:** A total of 100 sample from bacteria *Escherichia coli* sample Isolated from urine, wounds, burns Collected from several hospitals in city of Najaf included (AI-Sadr Teaching Hospital, AI-Najaf Teaching Hospital, AI-Furat AI-Awsat Hospital, AI-Hakim General Hospital, AI-Zahra Teaching Hospital, Burns Center, Public Health Laboratory).

**Results:** The results showed that the incidence of urinary tract infection 91%, and wound was 7%, while burns was 2%. the genes ChuA, yjaA, TspE4.C2 of the phylogenetic groups isolates were investigated using the PCR technique.

**Conclusion:** they were classified into four groups according to the presence or absence of these genes, so the results for group B1 was (44%), B2 (43%), D (12%), and A (1%).

Keywords: Genes; ChuA; yjaA; TspE4.C2; Escherichia coli.

#### 1. INTRODUCTION

Escherichia coli belong family to the Enterobacteriaceae, it is found as normal flora in the intestines of mammals, negative gram's stain, facultative anaerobic, rod shaped, cause many diseases such as urinary tract infection (UTI), genital infection, wound infections, respiratory infections [1]. The genus Escherichia has species (E. coli, E. hermanii, E. blattae, E. fergosonii, E. vuneris), and these species differ among themselves in terms of biochemical interactions, and the species E. coli is the most common in causing diseases [2]. Escherichia coli is one of the most common isolates and classified into three major groups, according to their biological significance to humans, groups is 1- extraintestinal pathogenic strains 2- intestinal pathogenic strains 3- commensal strains [3]. Commensal strains belong to phylogenetic groups A or B1, while extraintestinal pathogenic strains belong to phylogenetic groups B2 or D, which possess more virulence factors than commensal [3]. Clermont and Colleagues developed a triplex PCR assay to detect the genes chuA, TspE4.C2, yjaA in 2000 [4].

Clermont and colleagues found 100% of the ChuA gene present in group D and B2, the function of this gene being heme-transporting in E.coli O157:H7 and not found in group A and B1 [5]. and found the TspE4.C2 gene in group А and missing present in group B1, and the yjaA gene identified in The beginning of the sequence E. coli K-12 which is found in group B2 and missing in group D[4].

#### 2. MATERIALS AND METHODS

#### 2.1 Bacterial Strains

Overall of 100 sample from bacteria *Escherichia coli* sample Isolated from urine, wounds, burns Collected from several hospitals in city of Najaf included (AI-Sadr Teaching Hospital, AI-Najaf Teaching Hospital, AI-Furat AI-Awsat Hospital, AI-Hakim General Hospital, AI-Zahra Teaching Hospital, Burns Center, Public Health Laboratory) collected from July to December 2023. Samples were cultured on a MacConkey and Eosin Methylene Blue agar (EMB) and VITEK2 compact, it is shown in Fig. 1.



Fig. 1. E. coli isolated on MacConkey and EMB agar

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Target gene	Primer sequence	Product size (bp)	References
TspE4.C2	GAGTAATGTCGGGGCATTCA F	152	Clermont et
	CGCGCCAACAAAGTATTACG R		al., (2000)
YjaA	F TGAAGTGTCAGGAGACGCTG	211	
	ATGGAGAATGCGTTCCTCAAC R		
ChuA	F GACGAACCAACGGTCAGGAT	279	
	R TGCCGCCAGTACCAAAGACA		

Table 1. primer sequences used in the study

### 2.2 Phylogenetic Detection

All isolates were assigned to one of the four main E.coli phylogenetic groups (A, B1, B2, D) by use of the multiplex PCR [4]. DNA was extracted for all isolates by boiling method. phylogenetic groups was determined by PCR using primers TspE4.C2, YjaA, ChuA. Primer sequences are 1. PCR Table amplifications shown in under the following conditions: TspE4.C2 initial denaturation at 93°C for 5 min and denaturation 93°C for 30 sec and annealing 54°C for 30 sec and extention 72°C for 30 sec and cycles 35 and final elongation 72 °C for 5 min. while viaA initial denaturation at 95 °C for 5 min and denaturation 95°C for 30 sec and annealing 56°C for 30 sec and extention 72°C for 30 sec and cycles 35 and final elongation 72°C for 5 min. ChuA initial denaturation at 94°C for 5 min and denaturation 94°C for 30 sec and annealing 55°C for 30 sec and extention 72 °C for 30 sec and cycles 35 and final elongation 72°C for 5 min.

#### 3. RESULTS

#### 3.1 Distribution of Phylogenetic Groups in Pathogenic and Commensal Strains

The distribution of phylogenetic groups isolates from urine, wound and burns: B1(44%), B2(43%), D(12%), A (1%), respectively, shown in Fig. (2), Table (2). It found 100% of the *ChuA* gene present in group D and B2, the function of this gene being heme-transporting in *E. coli* O157:H7 and not found in group A and B1 [5 ].and found the *TspE4.C2* gene present in group A and missing in group B1, and the *yjaA* gene identified in The beginning of the sequence *E.coli* K-12 which is found in group B2 and missing in group D., It is shown in Fig. (2).



Fig.	2.	shows	genes	ChuA,	yjaA,	TspE4.	C2
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Table 2. phylogenetic	; groups of E	.coli isolated from	different sources
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Phylogenetic groups	Urine	Wound	Burns
A	1%	0	0
B1	41%	1%	2%
B2	38%	5%	0
D	11%	1%	0

## 4. DISCUSSION

The current study showed, the percentage of phylogenetic groups for infection with urinary tract infection, wounds and burns, where the percentage of group B1 (44%), followed by group B2 (43%), then D (12%), and A (1%), and this pattern is contradicts with Which was observed in a previous study of pathogenic bacteria Escherichia coli outside the intestine associated with urinary tract infection of Russian females, where the percentage of group A was (55%) followed by B1 and D (23%), and there was no isolate representing B1[6]. In previous studies, the resistant bacteria Escherichia coli involved in UTI showed a shift in evolution from group B2 to group A [7,8]. Some studies showed that group A is dominant [9,10,6] and there are other studies that showed that group D is dominant [11,12]. And it contradicted with phylogenetic analyzes, which showed that pathogenic strains outside the intestine mostly belong to group B2 and less than group D [13,14,15]. This study agreed with another study, where the percentage of group B1 was the highest, reaching 21% and lower the group D and B2 [16-22].

#### **5. CONCLUSION**

The consequences exhibited that the incidence of urinary tract infection 91%, and wound was 7%, while burns was 2%. The genes ChuA, yjaA, TspE4.C2 of the phylogenetic groups isolates were investigated using the PCR technique. They were classified into four groups according to the presence or absence of these genes, so the results for group B1 was (44%), B2 (43%), D (12%), and A (1%)

# CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

#### ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

#### REFERENCES

- 1. Oliveira RV, Oliveira MC, Pelli A. Disease Infection by Enterobacteriaceae Family in Fishes: A Review. J Microbiol Exp. 2017;4(5):00128.
- Olowe BM, Oluyege JO, Famurewa O, Ogunniran AO, Adelegan O. (Molecular Identification of *Escherichia coli* and New Emerging Enteropathogen, *Escherichia fergusonii*, from Drinking Water Sources in Ado- Ekiti, Ekiti State, Nigeria. J Microbiol Res. 2017;7(3):45-54.
- 3. Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J Infect Dis. 2000;181:1753-1754.
- 4. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;66:4555-4558.
- King-sun Y. Epidemiology and Virulence Characteristics of Multidrug Resistant -*Escherichia coli* from Women with Acute Uncomplicated Cystitis. Thesis. Master of Philosophy, University of Hong Kong; 2007
- Grude N, Potaturkina-Nesterova NI, Jenkins A, et al. A comparison of phylogenetic group, virulence factors and antibiotic resistance in Russian and Norwegian isolates of *Escherichia coli* from urinary tract infection. Clin Microbiol Infect. 2007;13:208-211.
- Skurnik D, Le Menac'h A, Zurakowski D, et al. Integron-associated antibiotic resistance and phylogenetic grouping of *Escherichia coli* isolates from healthy subjects free of recent antibiotic exposure. Antimicrob Agents Chemother. 2005;49:3062-3065.
- 8. Mahmood NA, Jawad S. Preparation, Spectral Characterization, Thermal Study, and Antifungal Assay of (Formazane -Mefenamic acid)- Derivatives., Egyptian Journal of Chemistry. 2022;411 65(2).
- DOI: 10.21608/EJCHEM.2021.88727.4266
  9. Rijavec M, Starcic Erjavec M, Ambrozic Avgustin J, et al. High prevalence of multidrug resistance and random distribution of mobile genetic elements among uropathogenic *Escherichia coli* (UPEC) of the four major phylogenetic groups. Curr Microbiol. 2006;53:158-162.
- 10. Romanus II, Eze AT. Antibiotics susceptibility patterns and clonal relatedness of uropathogenic *Escherichia*

*coli* in Abakaliki, Ebonyi state. Canad J Pure Appl Sci. 2011;5(2):1475–1479.

- Derakhshandeh A, Firouzi R, Motamedifar M, Arabshahi S, Novinrooz A, Boroojeni AM, et al. Virulence characteristics and antibiotic resistance patterns among various phylogenetic groups of Uropathogenic Escherichia coli isolates. Jpn J. Infect Dis. 2015;68:428–431. DOI: 10.7883/yoken.JJID.2014.327.
- 12. AL-Fatlawi HY, Jwad SM. Assessing the level of a trial natriuretic peptide and some biochemical parameters in men with type 2 diabetic nephropathy. Uttar Pradesh Journal of Zoology. 2022;43(9):1-13.
- Themphachana M, Kongpheng 13. S, Rattanachuay Ρ, Khianngam S, Κ, Sukhumungoon Singkhamanan Ρ. Molecular characterization of virulence and antimicrobial susceptibility profiles of uropathogenic Escherichia coli from patients in a tertiary hospital, southern Thailand. Southeast Asian J Trop Med Public Health. 2016;46(6):1021-1030.
- Hussein A. Detection of role the enzyme adenosine deaminase in leishmaniasis as biomarkers during of infection . Al-Salam Journal for Biochemical and Medical Science. 2022;1(2):9–18. Available:https://doi.org/10.55145/ajbms.2 022.1.2.002
- 15. Gao Q, Zhang D, Ye Z, Zhu X, Yang W, Dong L, et al. Virulence traits and pathogenicity of uropathogenic Escherichia coli isolates with common and uncommonserotypes. Microb Pathog. 2017;104:217e224.

DOI: 10.1016/j.micpath.2017.01.027

16. Basu S, Mukherjee SK, Hazra A, Mukherjee M. Molecular characterization of uropathogenic *Escherichia coli:* nalidixic acid and ciprofloxacin resistance, virulent factors and phylogenetic background, Journal of Clinical and Diagnostic Research. 2013;7(12):2727-2731.

- Mahmood A M A, Mahmood SJ. Estimation of the protective efficacy of the alcoholic extract of Lepidium sativum seeds on the level of erythropoietin and some physiological parameters of albino male rats dosed with acetaminophen., Uttar Pradesh Journal of Zoology. 2022; 43(7):42-51.
- Raad M, Ahmed AH, Ahmed F. Identification of MRSA (methicillin resistant Staphylococcus aureus) by mecA gene. Al-Salam Journal for Biochemical and Medical Science. 2022;1(2):25–30. Available:https://doi.org/10.55145/ajbms.2 022.1.2.004
- Moreno E, Andreu A, Pigrau C, Kuskowski MA, Johnson JR, Prats G. Relationship between Escherichia coli strains causing acute cystitis in women and the fecal E. coli population of the host, Journal of Clinical Microbiology. 2008;46(8):2529– 2534.
- 20. Nada AM, Younan MA. Dapagliflozin improves cardiovascular risk factors in Emirati patients with T2DM. *Therapeutic* Advances in Endocrinology and Metabolism. 2021;12: 2042018821995364
- 21. Takahashi S. Kanamaru, H. Kurazono et al., "Escherichia coli isolates associated with uncomplicated and complicated cystitis and asymptomatic bacteriuria possess similar phylogenies, virulence genes, and O-serogroup profiles. Journal of Clinical Microbiology. 2006;44(12): 4589–4592.
- 22. Mojaz-Dalfardi N, Kalantar-Neyestanaki D, Hashemizadeh Z, Monsouri S. Comparison of virulence genes and phylogenetic groups of Escherichia coli isolates from urinary tract infections and normal fecal flora. Journal pre-proof; 2020.

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