CME LIVE OPEN ACCESS

CME Journal of Medical Microbiology

Research Article ISSN: 3070-3638

Prevalence of Pap C, Cnf1, Fimh, Blaoxa, Hly Beta Genes and Phylogenetic Analysis of Escherchia Coli Isolated from Oral Ulcer Infections of Post Chemotherapy Patients

Halah Dawood Salman*

Department of Clinical and Laboratory Science, College of Pharmacy, University of Babylon, Babylon, Iraq.

Corresponding Author: Halah Dawood Salman, Department of Clinical and Laboratory Science, College of Pharmacy, University of Babylon, Babylon, Iraq.

Received: 2025 Jun 01 **Accepted:** 2025 Jun 20 **Published:** 2025 Jun 30

Abstract

Background: *Patients in post chemotherapy susceptible to have variable complications or infections, mucositis and oral ulcers one of these changes of oral flora as chemotherapy predispose for these infections.*

Objective: *The current study aimed to investigate Escherchia coli isolates as a causative agent of oral ulcer infections in post chemotherapy patients, as well as to establish the existence and genotype of virulence genes (pap c, cnf1, fimH, blaOXA and hly beta) in E. coli isolates, moreover to detect the relationship of these local isolates to internationally isolates through alignments of aimed genes sequenceing and phylogentic trees of these isolates.*

Subjects and Methods: *In this study, 150 clinical samples were collected from oral ulcer infections in post chemotherapy patients for isolation of E. coli that identified and diagnosed biochemically and molecularly using specific primers to detect virulence genes (pap c, cnf1, fimH, blaOXA and hly beta) in E. coli isolates, the study also included 25 apparently healthy people as a (control group) for collecting oral swabs from oral ulcer infections of post chemotherapy patients. Construction of phylogenetic trees was included.*

Results: *The study found only 43 (28.6 %) of patients infected with E.coli while the rest 107 (71.3 %) of patients not infected with E.coli, only 10 (23.2 %) of the tested isolates have pap c gene , 11 isolates (25.5%) have cnf1 gene, 10 isolates (23.2 %) have fimH gene , 5 isolates (11.6%) have blaOXA gene and 12 isolates (27.9 %) have hly beta gene, moreover, phylogenetic tree of locally gene sequences that required for E.coli isolates showed identical in high percentage comparison to different countries according to the sequence of nucleotide bases derived from GenBank database, control group revealed negative results for the presence of E.coli.*

Conclusions: *The study concluded that E.coli isolated from oral ulcer of patients in post chemotherapy have pap C, cnf1, fimH, blaOXA and hly beta genes which complicate health status of cancer patients through bacterial oral mucositis, moreover, phylogenetic analysis of these strains showed identical ratios according to the sequence of nucleotide bases of different countries derived from GenBank database at NCBI.*

Keywords: Oral Ulcer Infections, Post Chemotherapy Treatment , Pap C, Cnf1, Fimh, Blaoxa, Hly Beta

1. Introduction

Majority of the bacterial species found in oral tissue are commensal as innocent bacteria, however, in ordinary conditions, the oral cavities are in homeostasis status but in patients with cancer, the cancer itself could disturb this sensitive balance based on the level of immunity anticancer therapy or supportive therapies all may participate to change the mouth microflora from principally Gram positive bacteria to Gram negative [1,2]. Mucositis of GIT considered one toxicities that is most popular and debilitating situation correlated with chemoradiation protocol [3]. Mucositis pathogenesis include a sequence of biological events which coupled to impact both on the oral microbiome also on the environment, larger numbers of lines have a consequence to mucositis which are identical in patients undergoing chemotherapy or radiation or even chemoradiation, progress of mucositis can be listed in five steps including:1-initiation, 2-elevation (upregulation) or activation, 3-signal amplification, 4-ulceration and the last 5-healing [4]. Mucositis frequency had been reported as range from 30-40 percent to

nearly 100 percent when all severities of mucositis considered mucositis initiates in nearly 60-85 percent in patients experience hematopoietic stem cell transplantation, while mucositis occurred in only 20-40 percent in patients obtaining traditional chemotherapy, but it develops in about whole numbers of patients receiving radiation therapy for cancer of head and neck [5-8].

Mucositis as a common complication in patients with neutropenia who submitted to chemotherapy, as a result patients having immunosuppression which leading to infections including periodontal and oral ulcers, organisms of oral flora exhibit prolonged mucosal healing in mucositis leading to secondary oral infections this interruption of balance may be correlated to direct cytotoxicity on the oral microflora such as using of antibiotics, acquisition of hospital pathogens and compromised oral hygiene [9,10]. In addition, mucositis affects whole gastrointestinal,tract (GIT) even,mouth of patient, cytotoxic factors result in,mucositis through variety of mechanisms consist of normal barrier damage, minimize of immunological, protection as a result of neutropenia, and elevated inflammation resulting in directly tissue damage, regarding bacterial infections and mucositis correlation to immune, response, knowledge the organisms of oral flora important for choosing the proper therapy to causing microorganisms, normal oral flora composed of more than five hundred species of bacteria that are typically harmless [11,12]. Healthy persons are thought to own reservoir for extra intestinal pathogenic *E.coli* that have high ability for surviving and persistence in human gut and spreading to cause disease [13,14].

E.coli can simply convey virulence and antibiotic resistance genes from another bacteria flora of the intestine, as though, colonization of *E.coli* in sterile areas of the body outside the intestine, that consistently regarded as etiology of pathogenesis, *E.coli* strains produce diseases outside intestine such as UTIs, sepsis, newborn meningitis, pneumonia, osteomyelitis, infections in surgical sites and infections in other sites extra-intestinal, although these infections have low morbidity, but endemic problem of these infections increased in importance as a result of the fatal course and prolonged hospitalization [15,16]. Some *E. coli* strains can cause intestinal or extra-intestinal infections as a result of specific virulence factors [17,18].

E.coli strains that caused infections in extra-intestinal sites have several virulence factors which have a role in infection through allowing the bacterial cells to colonize the host and spread, encoding of virulence factors are either on the bacterial chromosome where they are usually located within pathogenicity islands (PAIs) or on plasmids those factors including: (adhesion molecules, host defense, toxins, iron acquisition and subverting mechanisms [19-21].

Commensal *E. coli* strains could be disseminated as intestinal and Extra pathogenic strains by having of virulence mediators [22]. Extra pathogenic *E. coli* strains display considerable genetic heterogenicity and explain extensive range of virulence correlated factors like adhesins, lipopolysaccharides(LPS), invasions and toxins that are expressed on mobile genetic factors (plasmids, pathogenicity-associated islands in addition to bacteriophages) faecal flora of the hosts provide the most popular origin of infectious *E.coli* strains these bacterial strains are a primary cause for morbidity and mortality both in hospital also infections acquired by community, acquisition of virulence agents besides antimicrobial resistance seem to contribute to globally pandemic of *E.coli* extraintestinal infections in addition to horizontal transfer of ESBL genes that are plasmid mediated evidence suggested that virulence factors help the pathogens of gastrointestinal to compete the commensal microbiota and impair immunity of the host by inducing colonization and resistance in addition to invasion [23-28].

fimH of *E.coli* represent one of type I fimbriae as an filamentous organelles covering bacterial surface gene coding for these organelles composed of cluster as fim gene having consist of fimA, fimH, fimF, and fimG while pilus coding by fimD and fimC, fimH as it pilli tip participate binding between bacteria and glycoproteins [29-31]. Moreover, fimH has the ability for attaching to various kinds of cells as macrophages and erythrocytes; this attachment is believed to performed by variant mechanisms other than epithelial, cells fimH fimbriae motivate attachment to phagocyting cells that followed by phagocyting process when bacteria having fimbriae-1, they undergo phagocytosis, however, opsonization could be not occurred, as a result bacteria can be survive within vacuoles [32-34]. Moreover, expression of P pili enhance colonization in gastrointestinal tract, it has been thought that P-pili evolvement in *E.coli* strains happened to encourage bacterial persistence of *E.coli* in the gut could be conducted by attachment of Galα1g-4Galß-containing receptors on epithelial cells of the gut [35, 36, 37, 38]. P pili assembling occur via proteins cluster of gene (pap) which including papF, papG, papE, papH, papA and papC subunits, other gene of pap such as (papD) are necessary for assembling of fimbriae but are no associated directly to adhesion process [39].

CNF1 is a protein that produced by particular pathogenic strains of E.coli which are more frequently responsible for infections outside the intestine additionally, cnf1 as a subset of cnfs toxins group, is popular determinants of virulence and nearly these determinants are exclusively confined to phylogroup (B2) of *E. coli* which includes the extra-intestinal pathogenic *E.coli* [40-43]. In general, cytotoxic necrotizing factor (CNF)including of cnf1 and cnf3 represent one of two important toxins or factors that have been defined in *E. coli* spp, moreover, CNFs encourage replication of DNA without cytokinesis and encoded by mobile elements such as pathogenicity islands and plasmids [44,45]. Other virulence component of *E.coli* is hemolysin (hly), which is produced by several pathogenic strains of *E.coli* causing infections in and out intestine, however, hemolysin effect on virulence is not completely cleared [46,47]. The current research aimed to investigate *E. coli* isolates as a causative agent of oral ulcer infections in post chemotherapy patients, as well as to establish the existence and genotype of virulence genes (pap c, cnf1 , fimH, blaOXA and hly beta) in *E.coli* isolates, in addition to detect the relationship of these local isolates to internation-

ally isolates through alignments of aimed genes sequenceing and phylogentic trees of these isolates.

2. Methods

2.1. Patients

The current study included collection of 150 swaps of mouth ulcer from post chemotherapy patients from both sexes with age range from 10 to 80 years old, those patients admitted to consult of Babylon Center of Oncology / Babylon Province / Iraq, during the period from May 2024 to November 2024, only 43 of them have *E.coli* isolates. Oral swaps were taken from the surface of oral mucosa ulcers from patients, systematic diseases and other medication were not considered, ulcer was diagnosed by the doctor, most of the lesions are single episodes (one ulcer per person), all swabs were transferred at once to the transport Amies' media, patient information fixed on each swabs after that immediately transport to the research laboratory. Control group: The study also included 50 apparently healthy people for collecting oral swabs that all revealed negative results for the presence of *E.coli*.

2.2. Identification of Bacterial Isolates

The collected samples were inoculated on (MacConkey agar, Eosin methyl blue agar and on chrom agar for Enterobacteriaceae), incubated at 37ºC for 24 hours, *E.coli* isolates was identified preliminary depending on morphological features on culture medium, the culture was examined for their shape, size, color and Gram stain reaction, after that a single pure isolated colony of *E.coli* was transferred to Brain-Heart infusion agar medium for the preservation and to carry out biochemical tests and using of the Vitek 2 system that confirmed the identification of isolates and recognized as *E.coli*.

2.3. Extraction of DNA and Molecular Detection and Determination of (Pap c, cnf1, fimH, blaOXA and hly beta) Ggene

In order to extract the genomic DNA, the bacterial isolates were cultured overnight in 10ml of broth at 37°C. Then the extraction was carried out by using (Promega, USA).

2.4. Conventional Polymerase Chain Reaction (Pcr) Assays

To detect virulence genes (Pap c, cnf1, fimH, blaOXA and hly beta) gene in *E. coli* isolates, conventional polymerase chain reaction was performed. The components of the (PCR) were used (Maxime PCR ABM Kit) and the process was carried out according to company instructions. Extracted DNA from the bacterial isolates and primers Table 1 added to the master mix components of PCR then placed in standard Mix PCR pre Mix tubes including all components needed to PCR reaction like dNTPs, Tris-HCl pH:9.0, Taq DNA polymerase, MgCl2, KCl in addition to loading dye, after that, each tubes of PCR translocated to exispin vortex centrifuge that set in 3000 rpm to one minutes, subsequently, these tubes transferred to PCR Thermo cycler type ABM Canada, these tubes then positioned in the thermal cycler, the conditions of the correct cycling parameters of PCR software which convert with primer used.

2.5. Agarose Gel Electrophoresis

Product of PCR were analyzed depending on the instructions of manufactory of Plus science/ UK by Agarose,gel electrophoresis (1.5%). This method was carried out according to Sambrook and Rusell (2001) ladder used from (ABM Canada).

2.6. Phylogenetic Tree Analysis

Nucleotides sets of (Pap c, cnf1, fimH, blaOXA and hly beta) genes were included to gain the conformity score of this strains comparing to other world reference strains through MEGA10 and NCBI system online after that analyze in order to construction of phylogenetic trees by using of Maximum-Likelihood option ways [48,49].

Gene	Primer Sequence
Pap	F:GACGGCTGTACTGCAGGGTGTGGCG
	R:ATATCCTTTCTGCAGGGATGCAATA
cnf1	F:AAGATGGAGTTTCCTATGCAGGAG
	R:CATTCAGAGTCCTGCCCTCATTATT
Fimh	F:TGCAGAACGGATAAGCCGTGG
	R:GCAGTCACCTGCCCTCCGGTA
blaOXA	F:GGCACCAGATTCAACTTTCAAG
	R:GACCCCAAGTTTCCTGTAAGTG
hlybeta	F:AACAAGGATAAGCACTGTTCTGGCT
	R: ACCATATAAGCGGTCATTCCCGTCA

Table 1: Primers Used to Detect the Five Genes ((Pap C, Cnf1, Fimh, Blaoxa and Hly Beta) of *E.Coli* Isolates

3. Results

In this study, 150 clinical samples were collected from oral ulcer infections in post chemotherapy patients for isolation of *E.coli* that identified and diagnosed biochemically and molecularly using specific primers. The study found only 43(28.6 %) of patients infected with *E.coli* (Figure 1)and (Figure 2)while the rest 107 (71.3 %) of patients not infected with *E.coli* (may be other microbial causes not included in

this research). Agarose gel electrophoresis revealed that the prevelance of the five virulence genes in isolated E.coli as 10 (23.2 %) of the tested isolates have pap c gene, 11 isolates (25.5%) have cnf1 gene, 10 isolates (23.2 %) have fimH gene , 5 isolates (11.6%) have blaOXA gene and 12 isolates (27.9 %) have hly beta, in addition, the study found that the molecular weight of pap was (328 bp), cnf1 was (498 bp), fimh was (508 bp), blaOXA was (564bp) and hly beta (1177 bp) figure (3,4,5,6,7). Alignment of the aimed five genes sequences in isolated *E.coli* with reference gene recorded in National Center for Biotechnology Information showed 98 to 100% similarity (Figure 3) to (Figure 8), moreover, the phylogenetic tree of *E.coli* isolated from oral ulcer showed identical ratios according to the sequence of nucleotide bases of different countries derived from GenBank database. The phylogenetic tree of each five genes in present study (pap c, cnf1, fimH, blaOXA and hly beta genes) of the isolates revealed that they were align-matched up with isolates from different locations, including: MW384885.1- India and X61239.1-Sweden-AF483828.1-U.S.A-OU701449.1-France-OP999010.1-Thailand and CP104949.1- Germany-LM997209. 1-Norway, OU349848.1 -France and CP051632.1 -USA , respectively as demonstrated in (Figure 9) to (Figure 13).

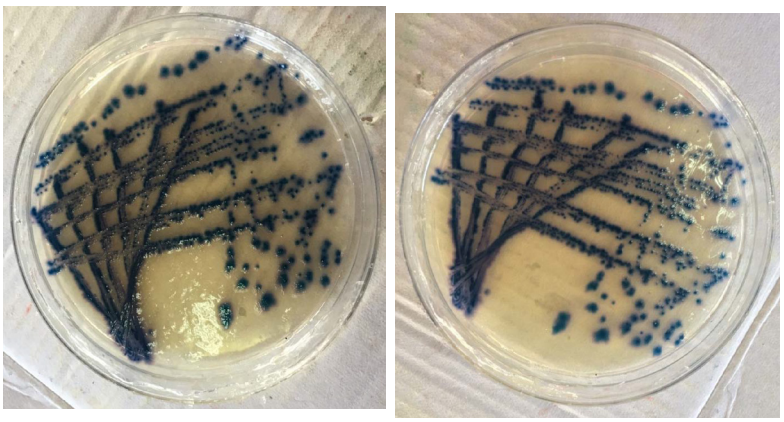


Figure 1: *E.Coli* on Chrom Agar for Enterobacteriaceae

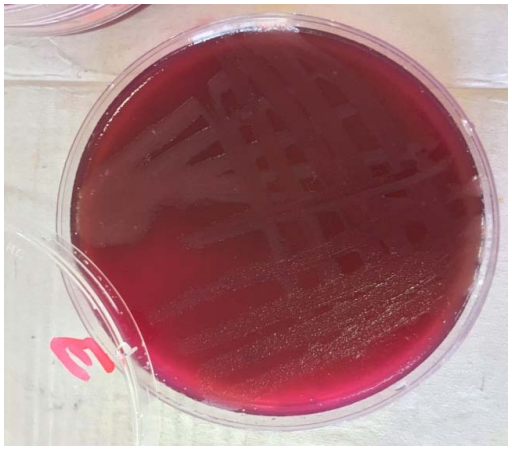


Figure 2: *E.Coli* on Macconkey Agar

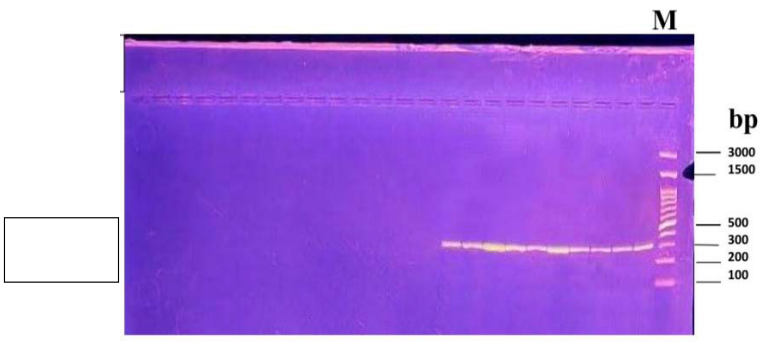


Figure 3: Gel Electrophoresis (1.5%) of PCR Products of pap c Genes of *E.coli* Strains. M: 100 bp DNA Ladder. The Electrophoresis was Performed at 70 volt for 82 min

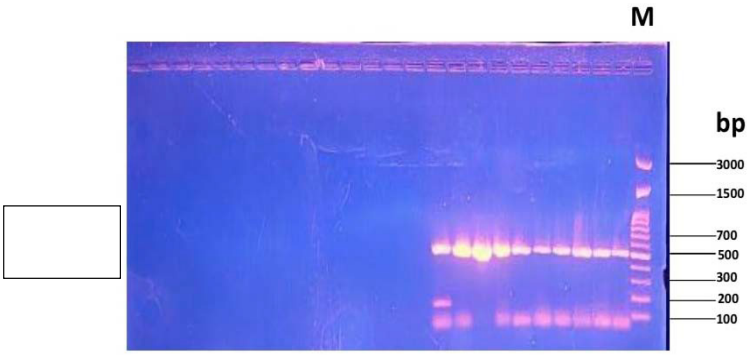


Figure 4: Gel Electrophoresis (1.5%) of PCR Products of Cytotoxic Necrotizing Factor 1 (cnf1) Gene for *E.coli* Strains. M: 100 bp DNA Ladder. The Electrophoresis was Performed at 70 volt for 82 min

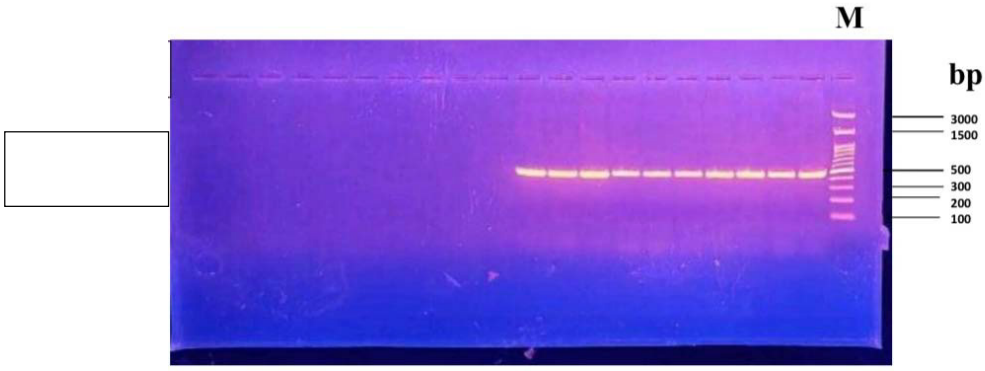


Figure 5: Gel Electrophoresis (1.5%) of PCR Products of Fimh Genes of *E.coli* strains. M: 100 bp DNA Ladder. The Electrophoresis was Performed at 70 volt for 82 min

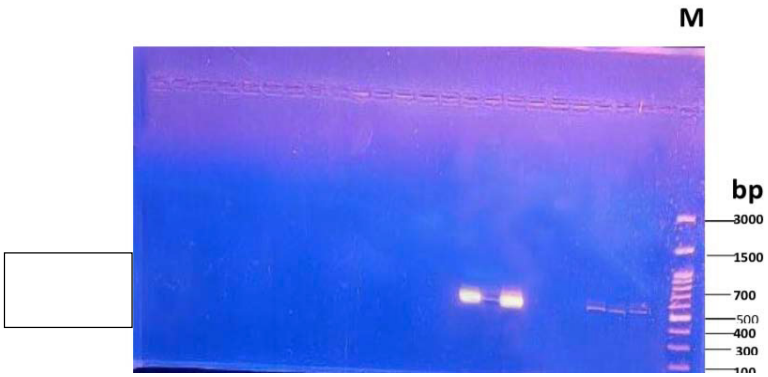


Figure 6: Gel Electrophoresis (1.5%) of PCR Products of Blaoxa gene of *E.coli* strains. M: 100 bp DNA Ladder. The Electrophoresis was Performed at 70 volt for 82 min

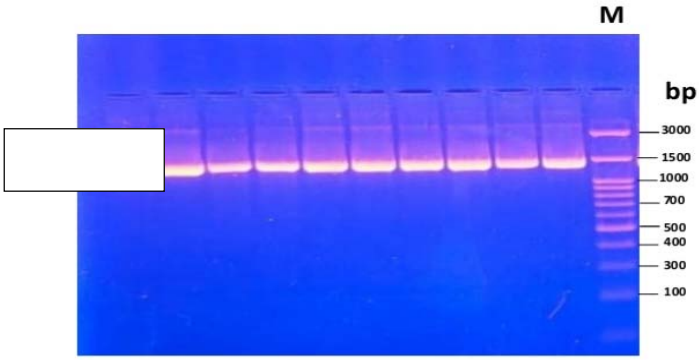
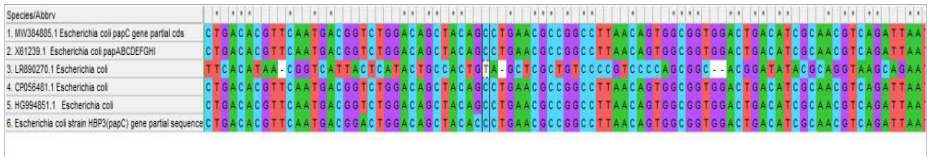
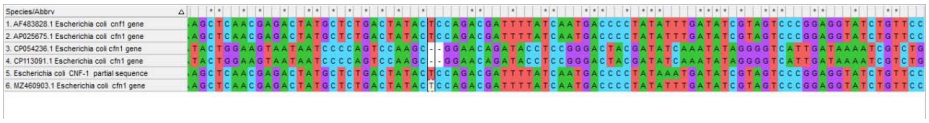


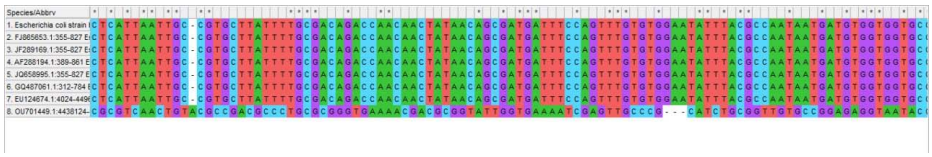
Figure 7: El Electrophoresis (1.5%) of PCR Products of Hly Beta Gene of *E.Coli* Strains. M: 100 bp DNA Ladder. The Electrophoresis was Performed at 70 volt for 82 mina



A- *pap c* sequence

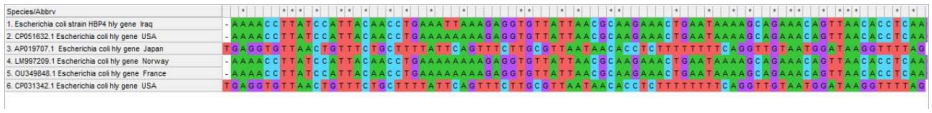


B- *cnf1* sequence



C- *fimH* sequence

D- *blaOXA* sequence



E- *hly beta* sequence

Figure 8: Alignment of The Sequence : (A) Pap C, (B) Cnf1, (C) Fimh, (D) Blaoxa and (E) Hly Beta Genes of Representative Tested Isolates with Reference Escherichia Coli Strain

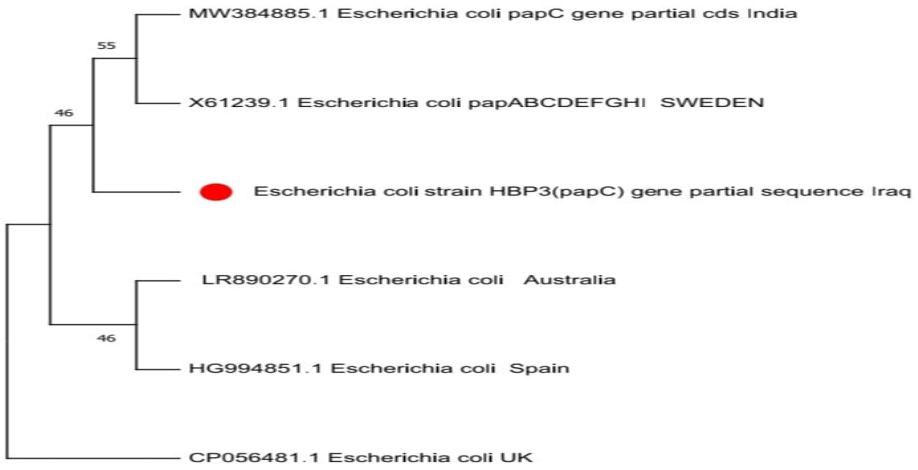


Figure 9: Phylogenetic Tree Analysis Dependent on the (Pap C) Gene Partial Sequence Using for Genetic Correlation Analysis of Local *E.Coli* Strains, Phylogenetic Tree Constructed by Maximum- Likelihood Option Method of Arithmetic Mean (Upgma Tree) in (Mega 10.0 Version)

Citation: Salman, H. D. (2025). Prevalence of pap C, cnf1, fimH, blaOXA, hly beta genes and Phylogenetic Analysis of Escherchia coli Isolated from Oral Ulcer Infections of Post Chemotherapy Patients. *CME J Med Microbiol*, 2(1), 1-13.

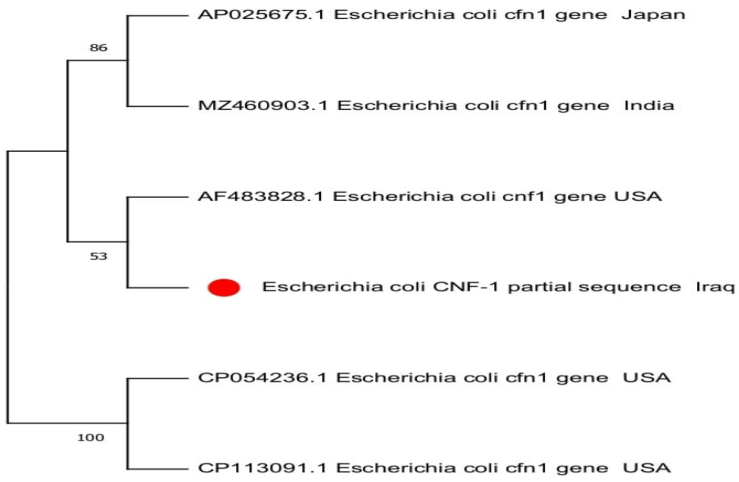


Figure 10: Phylogenetic Tree Analysis Dependent on the (Cnf 1) Gene Partial Sequence Using for Genetic Correlation Analysis of Local *E.Coli* Strains, the Phylogenetic Tree Was Constructed by Maximum- Likelihood Option Method of Arithmetic Mean (Upgma Tree) in (Mega 10.0 Version)

Figure 11: Phylogenetic Tree Analysis Dependent on the (Fim H) Gene Partial Sequence Using for Genetic Correlation Analysis of Local *E.Coli* Strains, the Phylogenetic Tree was Constructed by Maximum- Likelihood Option Method with Arithmetic Mean (Upgma Tree) in (Mega 10.0 Version)

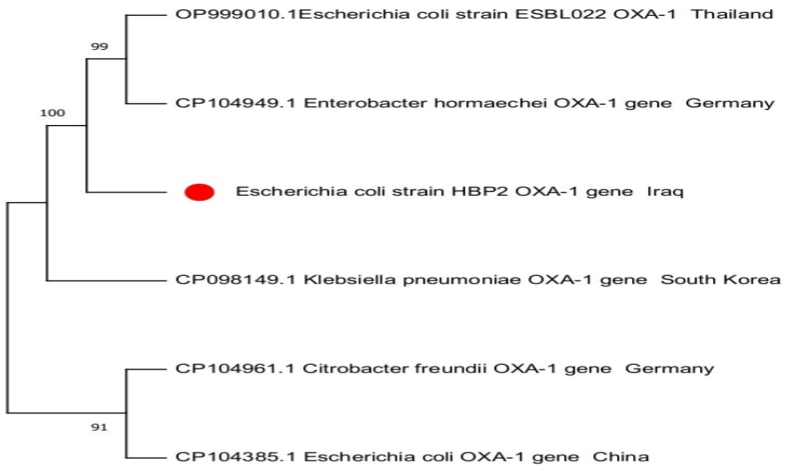


Figure 12: Phylogenetic Tree Analysis Dependent on the (Bla Oxa) Gene Partial Sequence Using for Genetic Correlation Analysis of Local *E.Coli* Strains, the Phylogenetic Tree was Constructed Using Maximum- Likelihood Option Method with Arithmetic Mean (Upgma Tree) in (Mega 10.0 Version)

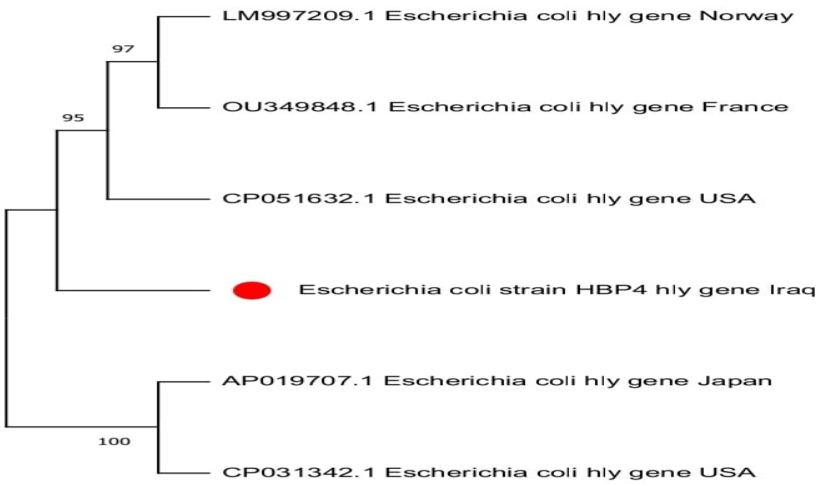


Figure 13: Phylogenetic Tree Analysis Dependent on the (Hly Beta) Gene Partial Sequence that is Using for Genetic Correlation Analysis of Local *E.Coli* Strains, the Phylogenetic Tree was Constructed Using Maximum- Likelihood Option Method with Arithmetic Mean (Upgma Tree) in (Mega 10.0 Version)

4. Discussion

Pathogenicity of *E.coli* strains is determined by the presence of certain virulence factors that assist overcoming the host defenses and help colonization leading to development of intestinal besides extraintestinal disease the importance of pathogenicity determinants in strains of *E.coli* responsible for infections at multiple sites in the body that clarified through molecular and other studies [50-52]. Cancer patients still delicately susceptible to have infection by bacteria of Gram negative group due to mucositis, dysfunction of lymphocyte neutropenia in addition to using of invasive tools [53]. Bacteria of GIT origin are oftentimes reliable for infections in neutropenic patients, agents of broad spectrum beta lactam considered the corner of cancer patients' therapy with suspicious infection with Enterobacteriaceae members, that result in elevating diffusion of carbapenem resistant of Enterobacteriaceae in healthcare system, in addition to bacterial resistance to other beta lactam drugs and that owing an urgent threating moreover, production of ESBL is present in lower than 10% of E. coli bacteria [54,55].

Renard indicated to small decrease in total bacteria but it was significant at two to seven days after the initial chemotherapy course at the sites where of shifting to Gram-negative bacteria, moreover, Minah and Main also noted near findings in their studies as they indicated quantitative increasing in bacterial strains of *E.coli*, Klebsiella sp. and in Gram positive bacteria (Staphylococcus aureus) levels through chemotherapy course all these studies are in coordination with result of present study regarding isolation of *E.coli* as a causative agent [56-58]. Regardless of isolation of other bacteria as the presnt research focusing only on *E.coli* bacteria. Control group of this research denoted no isolation of *E.coli* and that roughly accepted with results of Galili who isolated Enterobacteria in fifteen out of sixteen adult patients during period for chemotherapy while in comparison to control persons, the isolation was only three out of twelve [59].

In this research, only 43 (28.6 %) out of 150 clinical samples collected from oral ulcer of patients with post chemotherapy revealed infection with *E.coli*, these results are congruent with study of Napeñas who introduce a literature review pointed to the predominance of reviewed researches indicated isolation of Gram negative bacteria (rods) in patients submitted to chemotherapy that including *E.coli*, Klebsiella, Enterobacter and Pseudomonas, while Gram positive bacteria were isolated more commonly prior to chemotherapy, same review also pointed to that the researchers were not able to produce inference concerning oral flora changes in those patients as a result of lack the investigations in addition to great extent of variance in the studied groups composed of certain chemotherapy type, chemotherapy duration that received at the time of collecting and culturing methods [60]. The results of current research regarding resistance of isolated *E.coli* (11.6% have blaOXA gene) approaching to other study conducted by Perez who indicated that in cancer patients, rising of multi drug resistance infections of Gram negative bacteria have been reported with increased recognition of certain bacteria including carbapenem resistant enterobacteriaceae, fluoroquinolone resistant *E.coli*, multidrug resistant Acinetobacter baumanni, P. aeruginosa and others, in addition, Wahlin revealed that there are changes in resistance manner of oral bacteria in patients of chemotherapy comparing to control population [61,62]. In study conducted by Tohamy they indicated that multidrug resistant *E.coli* isolated from patients of neutropenia in those exhibited rising resistance for ampicillin, cephradine, ceftriaxone beside cefepime [63].

The experimental treatment of *E.coli* infections, for decades, has been more dependent on the β lactam antibiotics and the wideuse of these antibiotics resulting in the appearance of resistant strains bacteria conferring ESBLs that owing the ability for hydrolyzing a wider range of β lactam antibiotics including(penicillin and oxyimino cephalosporins) and that occur as a result of mutations which alter configuration of amino acid in the encoded proteins [64,65]. Although the

present research deduced that out of forty three *E.coli* isolates only five isolates (11.6%) have blaOXA gene but this ratio of resistance also drawing attention as this effect on this group of patients. Among multidrug resistant *E.coli* strains, antimicrobial resistance caused by ESBL is at most as a result to family of blaCTX-M enzymes, especially blaCTX-M-15 beside14, comparing to less commonly observed families of blaOXA and blaSHV enzymes and that may interpret the results of present study regarding percent of blaOXA gene harboring byisolated bacteria [66,67].

Study of Castanheira, concluded that the prevalence of ESBL *E. coli* strains in the infections of extraintestinal sites is various also differ around geographical regions, blaCTX-M-15 represent broadly popular ESBL genes then gnes of CTX-M, SHV, PER, TEM, GES, BES, VEB, TLA and OXA respectively, Ikram also pointed that the antimicrobial susceptibility of *E. coli* has been shown to vary geographically [68,69]. Concerning fimH , the current research revealed that only ten *E. coli* isolates (23.2 %) out of forty three have fimH gene, Bouckaert mentioned that of fimH alleles, although high conservation of nucleotide was observed as more than 98% , minor differences in sequence have been reported to be correlated with differential binding and adhesion phenotypes, also in the current research, only twelve isolates (27.9 %) have hly beta, in a review performed by Katouli, the reviewer reported average of (12%) from fecal isolates of *E. coli* have ability for production of haemolysin, moreover, these results reinforced with outcomes of other researchers [70,71].

Regarding cnf1, this toxin has been proven to result in direct cytotoxicity in tissues the present research exhibited that only eleven isolates with ratio of (25.5%) have cnf1 gene, however, environmental changes including alterations in temperature or as mutation lead to loss of cnf1 in bacteria [72-74]. Prolonged neutropenic cancer subjects are believed to colonize with Gram negative bacterial rods as those patients are in elevated risk for this infection, nevertheless, there is insufficient studies describing changes in oral flora of those patients submitted to chemotherapy, in addition, results of the studies have been inconstant [75].

Molecular typing relying on multilocus sequence typing (MLST) and multiple PCR, moreover, latterly after this period, whole sequencing of genome have introduce a good understanding comprehension of the phylogenetic system of the *E.coli* moreover, phylogenetic tree system revealed that comparing local sequences of certain genes to globally strains in NCBI of same bacteria may indicated compatible in high percentage, however, variation may interpret that the origin of these genetic changes may be due to mutation or integrons correlating to present results concerning phylogenetic tree of each five genes (pap c, cnf1, fimH, blaOXA and hly beta genes) of *E.coli* isolates concluded that there is matching to isolates from different countries, such as: India, Sweden, U.S.A, France, Thailand, Germany, Norway and France [76,77].

Correlation of these phylogenetic trees is of specific importance, as there is a relation between the genetic information of certain strain and the virulence factors of the same strain in the epidemiology of *E. coli*, there have some major alteration as some clonal strains have become more common in extraintestinal diseases some of these clones disseminate various antibiotic resistance, the hub of this resistance and the principle driver for spreading antibiotic resistance through the community has been established to be the gut [78-87].

Actually, inconsistent data on mucositis act as principle barrier to collect literatures and comparing the results as a result of lacking standardized criteria, variable regimens of therapy and location of tumor, in addition to side effects of cancer treatment, all that lead to make mucositis underreported [88, 89]. Multiple agents acting as a barrier for the current research, factors as dietary system of patients, great variability in patient populations, cultural characteristics, hospital policies, antibiotics and anticancer included in therapy, sample size and molecular detection methods, all of them may have principle impacts on evaluation of virulence factors of isolated *E. coli*. To the best of knowledge, there are too limited and few studies, reviews or articles that focused on bacterial roles in mucositis of post chemotherapy patients especially studies concerned with *E. coli* as etiology of these mucositis cases.

5. Conclusion

Oral ulcer represent one of the dangerous sequelae of cancer therapy and still an important burden for patients having chemotherapy and radiation therapy, cancer patients are at elevated risk for developing oral lesions by bacteria especially Gram negative rods, the current investigation found that *E.coli* have pap C, cnf1, fimH, blaOXA and hly beta genes which can be implicated as principle virulence factors in multiple infections of *E.coli* and complicate health status of cancer patients through bacterial oral mucositis. Moreover, phylogenetic analysis of these strains important to detect and identity causative bacterial strains certainly and predict the prognosis of the infections to prevent any further complications, in order to support those patients who originally suffering from cancer and minimized immunity, further researches and surveillance both in vivo and in vitro are required. Protection of cancer patients from bacterial infection of oral tissues such as *E. coli*, probable to minimize morbidity also mortality of those patients, however it important to detect whether all observed changes resulted from chemotherapy transformation of oral microbiota or antimicrobial agents or resulting from acquisition of nosocomial bacteria.

Acknowledgements

I'd want to offer my heartfelt gratitude and appreciation to all of the patients whose samples were used in this study.

Reference

1. Anitha, M., Monisha, D. M., Ramya, K. M., Chinmayee, Y., & Sulthan, A. M. (2016). A study on the occurrence of bacterial isolation in mouth ulcer patients. *International Journal of Advanced Research in Biological Sciences, 3*, 188-92.
2. Laheij, A. M., de Soet, J. J., von dem Borne, P. A., Kuijper, E.

J., Kraneveld, E. A., van Loveren, C., & Raber-Durlacher, J. E. (2012). Oral bacteria and yeasts in relationship to oral ulcerations in hematopoietic stem cell transplant recipients. *Supportive Care in Cancer, 20*, 3231-3240.

3. Lalla, R. V., Bowen, J., Barasch, A., Elting, L., Epstein, J., Keefe, D. M., ... & Mucositis Guidelines Leadership Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO). (2014). MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer, 120*(10), 1453-1461.
4. Sonis, S. T. (2009). Mucositis: the impact, biology and therapeutic opportunities of oral mucositis. *Oral oncology, 45*(12), 1015-1020.
5. Mortensen, H. R., Overgaard, J., Specht, L., Overgaard, M., Johansen, J., Evensen, J. F., ... & Grau, C. (2012). Prevalence and peak incidence of acute and late normal tissue morbidity in the DAHANCA 6&7 randomised trial with accelerated radiotherapy for head and neck cancer. *Radiotherapy and oncology, 103*(1), 69-75.
6. Keefe, D. M., Schubert, M. M., Elting, L. S., Sonis, S. T., Epstein, J. B., Raber-Durlacher, J. E., ... & Peterson, D. E. (2007). Updated clinical practice guidelines for the prevention and treatment of mucositis. *Cancer: Interdisciplinary International Journal of the American Cancer Society, 109*(5), 820-831.
7. Sonis, S. T., Elting, L. S., Keefe, D., Peterson, D. E., Schubert, M., Hauer-Jensen, M., ... & Rubenstein, E. B. (2004). Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer: Interdisciplinary International Journal of the American Cancer Society, 100*(S9), 1995-2025.
8. Blijlevens, N., Schwenkglenks, M., Bacon, P., D'Addio, A., Einsele, H., Maertens, J., ... & McCann, S. (2008). Prospective oral mucositis audit: oral mucositis in patients receiving high-dose melphalan or BEAM conditioning chemotherapy—European Blood and Marrow Transplantation Mucositis Advisory Group. *Journal of clinical oncology, 26*(9), 1519-1525.
9. Laheij, A. M., & de Soet, J. J. (2014). Can the oral microflora affect oral ulcerative mucositis?. *Current opinion in supportive and palliative care, 8*(2), 180-187.
10. Villa, A., & Sonis, S. T. (2015). Mucositis: pathobiology and management. *Current opinion in oncology, 27*(3), 159-164.
11. Sampson, M. M., Nanjappa, S., & Greene, J. N. (2017). Mucositis and oral infections secondary to gram negative rods in patients with prolonged neutropenia. *IDCases, 9*, 101-103.
12. Stringer, A. M., & Logan, R. M. (2015). The role of oral flora in the development of chemotherapy-induced oral mucositis. *Journal of Oral Pathology & Medicine, 44*(2), 81-87.
13. Nowrouzian, F. L., Wold, A. E., & Adlerberth, I. (2005). Escherichia coli strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. *The Journal of infectious diseases, 191*(7), 1078-1083.
14. Bagger-Skjøt, L., Sandvang, D., Frimodt-Møller, N., Lester, C. H., Olsen, K. E., Jannok Porsbo, L., ... & Hammerum, A. M. (2007). Association between antimicrobial resistance and virulence genes in Escherichia coli obtained from blood and faeces. *Scandinavian journal of infectious diseases, 39*(8), 724-727.
15. Bagger-Skjøt, L., Sandvang, D., Frimodt-Møller, N., Lester, C. H., Olsen, K. E., Jannok Porsbo, L., ... & Hammerum, A. M. (2007). Association between antimicrobial resistance and virulence genes in Escherichia coli obtained from blood and faeces. *Scandinavian journal of infectious diseases, 39*(8), 724-727.
16. Wiles, T. J., Kulesus, R. R., & Mulvey, M. A. (2008). Origins and virulence mechanisms of uropathogenic Escherichia coli. *Experimental and molecular pathology, 85*(1), 11-19.
17. Burdet, C., Clermont, O., Bonacorsi, S., Laouénan, C., Bingen, E., Aujard, Y., ... & COLIBAFI Group. (2014). Escherichia coli bacteremia in children: age and portal of entry are the main predictors of severity. *The Pediatric infectious disease journal, 33*(8), 872-879.
18. Usein, C. R., Papagheorghe, R., Oprea, M., Condei, M., & Strãuț, M. (2016). Molecular characterization of bacteremic Escherichia coli isolates in Romania. *Folia microbiologica, 61*, 221-226.
19. Dale, A. P., & Woodford, N. (2015). Extra-intestinal pathogenic Escherichia coli (ExPEC): disease, carriage and clones. *Journal of Infection, 71*(6), 615-626.
20. Lefort, A., Panhard, X., Clermont, O., Woerther, P. L., Branger, C., Mentré, F., ... & Denamur, E. (2011). Host factors and portal of entry outweigh bacterial determinants to predict the severity of Escherichia coli bacteremia. *Journal of clinical microbiology, 49*(3), 777-783.
21. Mora-Rillo, M., Fernández-Romero, N., Navarro-San Francisco, C., Díez-Sebastián, J., Romero-Gómez, M. P., Arnalich Fernández, F., ... & Mingorance, J. (2015). Impact of virulence genes on sepsis severity and survival in Escherichia coli bacteremia. *Virulence, 6*(1), 93-100.
22. Russo, T. A., & Johnson, J. R. (2000). Proposal for a new inclusive designation for extraintestinal pathogenic isolates of Escherichia coli: ExPEC. *The Journal of infectious diseases, 181*(5), 1753-1754.
23. Rodriguez-Siek, K. E., Giddings, C. W., Doetkott, C., Johnson, T. J., Fakhr, M. K., & Nolan, L. K. (2005). Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology, 151*(6), 2097-2110.
24. Clermont, O., Bonacorsi, S., & Bingen, E. (2000). Rapid and simple determination of the Escherichia coli phylogenetic group. *Applied and environmental microbiology, 66*(10), 4555-4558.
25. Riley, L. W. (2020). Distinguishing pathovars from nonpathovars: Escherichia coli. *Microbiology spectrum, 8*(4), 10-1128.
26. Riley, L. W. (2014). Pandemic lineages of extraintestinal pathogenic Escherichia coli. *Clinical Microbiology and Infection, 20*(5), 380-390.
27. Castanheira, M., Simner, P. J., & Bradford, P. A. (2021). Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC-antimicrobial resistance, 3*(3), dlab092.

28. Kitamoto, S., Nagao-Kitamoto, H., Kuffa, P., & Kamada, N. (2016). Regulation of virulence: the rise and fall of gastrointestinal pathogens. *Journal of gastroenterology, 51*, 195-205.
29. Martinez, J. J., Mulvey, M. A., Schilling, J. D., Pinkner, J. S., & Hultgren, S. J. (2000). Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *The EMBO journal*.
30. Jones, C. H., Pinkner, J. S., Roth, R., Heuser, J., Nicholes, A. V., Abraham, S. N., & Hultgren, S. J. (1995). FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the Enterobacteriaceae. *Proceedings of the National Academy of Sciences, 92*(6), 2081-2085.
31. Dhakal, B. K., Kulesus, R. R., & Mulvey, M. A. (2008). Mechanisms and consequences of bladder cell invasion by uropathogenic Escherichia coli. *European journal of clinical investigation, 38*, 2-11.
32. Hamrick, T. S., Harris, S. L., Spears, P. A., Havell, E. A., Horton, J. R., Russell, P. W., & Orndorff, P. E. (2000). Genetic characterization of Escherichia coli type 1 pilus adhesin mutants and identification of a novel binding phenotype. *Journal of Bacteriology, 182*(14), 4012-4021.
33. Johnson, J. R. (1991). Virulence factors in Escherichia coli urinary tract infection. *Clinical microbiology reviews, 4*(1), 80-128.
34. Goetz, M. B., Kuriyama, S. M., & Silverblatt, F. J. (1987). Phagolysosome formation by polymorphonuclear neutrophilic leukocytes after ingestion of Escherichia coli that express type 1 pili. *The Journal of infectious diseases, 156*(1), 229-233.
35. Wold, A. E., Caugant, D. A., Lidin-Janson, G., de Man, P., & Svanborg, C. (1992). Resident colonic Escherichia coli strains frequently display uropathogenic characteristics. *Journal of Infectious Diseases, 165*(1), 46-52.
36. Moreno, E., Andreu, A., Pigrau, C., Kuskowski, M. A., Johnson, J. R., & Prats, G. (2008). Relationship between Escherichia coli strains causing acute cystitis in women and the fecal E. coli population of the host. *Journal of clinical microbiology, 46*(8), 2529-2534.
37. Tullus, K., Kühn, I., Ørskov, I., Ørskov, F., & Möllby, R. (1992). The importance of P and type 1 fimbriae for the persistence of Escherichia coli in the human gut. *Epidemiology & Infection, 108*(3), 415-421.
38. Nowrouzian, F., Wold, A. E., & Adlerberth, I. (2001). P fimbriae and aerobactin as intestinal colonization factors for Escherichia coli in Pakistani infants. *Epidemiology & Infection, 126*(1), 19-23.
39. Wilson, B. A., Salyers, A. A., Whitt, D. D., & Winkler, M. E. (2011). *Bacterial pathogenesis: a molecular approach* (No. Ed. 3, pp. xiv+-526).
40. Boquet, P. (2001). The cytotoxic necrotizing factor 1 (CNF1) from Escherichia coli. *Toxicon, 39*(11), 1673-1680.
41. El-Aouar Filho, R. A., Nicolas, A., De Paula Castro, T. L., Deplanche, M., De Carvalho Azevedo, V. A., Goossens, P. L., ... & Berkova, N. (2017). Heterogeneous family of cyclomodulins: smart weapons that allow bacteria to hijack the eukaryotic cell cycle and promote infections. *Frontiers in cellular and infection microbiology, 7*, 208.
42. Brotherton, C. A. (2016). *Investigations of the biosynthesis and structure of colibactin, a cytotoxin made by human-associated Escherichia coli* (Doctoral dissertation).
43. Nougayrède, J. P., Homburg, S., Taieb, F., Boury, M., Brzuszkiewicz, E., Gottschalk, G., ... & Oswald, E. (2006). Escherichia coli induces DNA double-strand breaks in eukaryotic cells. *Science, 313*(5788), 848-851.
44. Pérès, S. Y., Marchès, O., Daigle, F., Nougayrède, J. P., Hérault, F., Tasca, C., ... & Oswald, E. (1997). A new cytolethal distending toxin (CDT) from Escherichia coli producing CNF2 blocks HeLa cell division in G2/M phase. *Molecular microbiology, 24*(5), 1095-1107.
45. Hacker, J., Hentschel, U., & Dobrindt, U. (2003). Prokaryotic chromosomes and disease. *Science, 301*(5634), 790-793.
46. Mainil, J. (2013). Escherichia coli virulence factors. *Veterinary immunology and immunopathology, 152*(1-2), 2-12.
47. Bien, J., Sokolova, O., & Bozko, P. (2012). Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage. *International journal of nephrology, 2012*(1), 681473.
48. Sambrook, J. (2001). russell DW (2001) Molecular cloning: a laboratory manual. *Cold Spring Harb Lab Press Cold Spring Harb NY*, *999*.
49. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution, 28(*10), 2731-2739.
50. Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic escherichia coli. *Nature reviews microbiology, 2*(2), 123-140.
51. Albert, M. J., Faruque, S. M., Faruque, A. S., Bettelheim, K. A., Neogi, P. K. B., Bhuiyan, N. A., & Kaper, J. B. (1996). Controlled study of cytolethal distending toxin-producing Escherichia coli infections in Bangladeshi children. *Journal of Clinical Microbiology, 34*(3), 717-719.
52. Hoffmann, C., & Schmidt, G. (2004). CNF and DNT. *Reviews of physiology, biochemistry and pharmacology*, 49-63.
53. Safdar, A., & Armstrong, D. (2011). Infections in patients with hematologic neoplasms and hematopoietic stem cell transplantation: neutropenia, humoral, and splenic defects. *Clinical infectious diseases, 53*(8), 798-806.
54. Jacob, J. T., Klein, E., Laxminarayan, R., Beldavs, Z., Lynfield, R., Kallen, A. J., ... & Cardo, D. (2013). Vital signs: carbapenem-resistant Enterobacteriaceae. *Morbidity and mortality weekly report, 62*(9), 165.
55. Gudiol, C., Tubau, F., Calatayud, L., Garcia-Vidal, C., Cisnal, M., Sánchez-Ortega, I., ... & Carratalà, J. (2011). Bacteraemia due to multidrug-resistant Gram-negative bacilli in cancer patients: risk factors, antibiotic therapy and outcomes. *Journal of antimicrobial chemotherapy, 66*(3), 657-663.
56. Renard, K. W., Marling-Cason, M., Sheehan, R. G., & Mackowiak, P. A. (1986). Effects of cancer chemotherapy on the human aerobic oropharyngeal flora. *Infection, 14*(5), 237-242.
57. Minah, G. E., Rednor, J. L., Peterson, D. E., Overholser, C. D., Depaola, L. G., & Suzuki, J. B. (1986). Oral succession of gram-negative bacilli in myelosuppressed cancer pa-

tients. *Journal of clinical microbiology, 24*(2), 210-213.
58. Main, B. E., Calman, K. C., Ferguson, M. M., Kaye, S. B., MacFarlane, T. W., Mairs, R. J., ... & Welsh, J. (1984). The effect of cytotoxic therapy on saliva and oral flora. *Oral Surgery, Oral Medicine, Oral Pathology, 58*(5), 545-548.
59. Galili, D., Donitza, A., Garfunkel, A., & Sela, M. N. (1992). Gram-negative enteric bacteria in the oral cavity of leukemia patients. *Oral surgery, oral medicine, oral pathology, 74*(4), 459-462.
60. Napeñas, J. J., Brennan, M. T., Bahrani-Mougeot, F. K., Fox, P. C., & Lockhart, P. B. (2007). Relationship between mucositis and changes in oral microflora during cancer chemotherapy. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 103*(1), 48-59.
61. Perez, F., Adachi, J., & Bonomo, R. A. (2014). Antibiotic-resistant gram-negative bacterial infections in patients with cancer. *Clinical Infectious Diseases, 59*(suppl_5), S335-S339.
62. Wahlin, Y. B., Granstro, S., Persson, S., & Sjo, M. (1991). Multivariate study of enterobacteria andPseudomonas in saliva of patients with acute leukemia. *Oral surgery, oral medicine, oral pathology, 72*(3), 300-308.
63. Tohamy, S. T., Aboshanab, K. M., El-Mahallawy, H. A., El-Ansary, M. R., & Afifi, S. S. (2018). Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations. *Infection and drug resistance*, 791-803.
64. Thakuria, B., & Lahon, K. (2013). The beta lactam antibiotics as an empirical therapy in a developing country: an update on their current status and recommendations to counter the resistance against them. *Journal of clinical and diagnostic research: JCDR, 7*(6), 1207.
65. Jena, J., Sahoo, R. K., Debata, N. K., & Subudhi, E. (2017). Prevalence of TEM, SHV, and CTX-M genes of extended-spectrum β-lactamase-producing Escherichia coli strains isolated from urinary tract infections in adults. *3 Biotech, 7*, 1-7.
66. Nicolas-Chanoine, M. H., Blanco, J., Leflon-Guibout, V., Demarty, R., Alonso, M. P., Caniça, M. M., ... & Johnson, J. R. (2008). Intercontinental emergence of Escherichia coli clone O25: H4-ST131 producing CTX-M-15. *Journal of Antimicrobial Chemotherapy, 61*(2), 273-281.
67. Alhashash, F., Weston, V., Diggle, M., & McNally, A. (2013). Multidrug-resistant Escherichia coli bacteremia. *Emerging infectious diseases, 19*(10), 1699.
68. Castanheira, M., Simner, P. J., & Bradford, P. A. (2021). Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC-antimicrobial resistance, 3*(3), dlab092.
69. Ikram, R., Psutka, R., Carter, A., & Priest, P. (2015). An outbreak of multi-drug resistant Escherichia coli urinary tract infection in an elderly population: a case-control study of risk factors. *BMC infectious diseases, 15*, 1-7.
70. Bouckaert, J., Mackenzie, J., De Paz, J. L., Chipwaza, B., Choudhury, D., Zavialov, A., ... & Knight, S. D. (2006). The affinity of the FimH fimbrial adhesin is receptor-driven and quasi-independent of Escherichia coli pathotypes. *Molecular microbiology, 61*(6), 1556-1568.
71. Katouli, M. (2010). Population structure of gut Escherichia coli and its role in development of extra-intestinal infections. *Iranian journal of microbiology, 2*(2), 59.
72. Hacker, J., & Kaper, J. B. (2000). Pathogenicity islands and the evolution of microbes. *Annual Reviews in Microbiology, 54*(1), 641-679.
73. Yamamoto, S. (2007). Molecular epidemiology of uropathogenic Escherichia coli. *Journal of infection and Chemotherapy, 13*(2), 68-73.
74. Watts, R. E., Totsika, M., Challinor, V. L., Mabbett, A. N., Ulett, G. C., De Voss, J. J., & Schembri, M. A. (2012). Contribution of siderophore systems to growth and urinary tract colonization of asymptomatic bacteriuria Escherichia coli. *Infection and immunity, 80*(1), 333-344.
75. Meurman, J. H., Pyrhönen, S., Teerenhovi, L., & Lindqvist, C. (1997). Oral sources of septicaemia in patients with malignancies. *Oral Oncology, 33*(6), 389-397.
76. Clermont, O., Gordon, D., & Denamur, E. (2015). Guide to the various phylogenetic classification schemes for Escherichia coli and the correspondence among schemes. *Microbiology, 161*(5), 980-988.
77. Salman M. N., Kzar A. J.and Hamzah A. S. (2022)." Study Genotyping of cnf, hly, aer, pap genes in Uropathogenic Escherichia coli from Chronic kidney patients", BNIHS, ISSN: 1343-4292 Volume 140, Issue 02
78. Escobar-Páramo, P., Clermont, O., Blanc-Potard, A. B., Bui, H., Le Bouguénec, C., & Denamur, E. (2004). A specific genetic background is required for acquisition and expression of virulence factors in Escherichia coli. *Molecular biology and evolution, 21*(6), 1085-1094.
79. Bidet, P., Mahjoub-Messai, F., Blanco, J., Blanco, J., Dehem, M., Aujard, Y., ... & Bonacorsi, S. (2007). Combined multilocus sequence typing and O serogrouping distinguishes Escherichia coli subtypes associated with infant urosepsis and/or meningitis. *The Journal of infectious diseases, 196*(2), 297-303.
80. Bert, F., Johnson, J. R., Ouattara, B., Leflon-Guibout, V., Johnston, B., Marcon, E., ... & Nicolas-Chanoine, M. H. (2010). Genetic diversity and virulence profiles of Escherichia coli isolates causing spontaneous bacterial peritonitis and bacteremia in patients with cirrhosis. *Journal of clinical microbiology, 48*(8), 2709-2714.
81. Mahjoub-Messai, F., Bidet, P., Caro, V., Diancourt, L., Biran, V., Aujard, Y., ... & Bonacorsi, S. (2011). Escherichia coli isolates causing bacteremia via gut translocation and urinary tract infection in young infants exhibit different virulence genotypes. *Journal of Infectious Diseases, 203*(12), 1844-1849.
82. Bengtsson, S., Naseer, U., Sundsfjord, A., Kahlmeter, G., & Sundqvist, M. (2012). Sequence types and plasmid carriage of uropathogenic Escherichia coli devoid of phenotypically detectable resistance. *Journal of Antimicrobial chemotherapy, 67*(1), 69-73.
83. Gibreel, T. M., Dodgson, A. R., Cheesbrough, J., Fox, A. J., Bolton, F. J., & Upton, M. (2012). Population structure, virulence potential and antibiotic susceptibility of uropathogenic Escherichia coli from Northwest England. *Journal of Antimicrobial Chemotherapy, 67*(2), 346-356.
84. Alhashash, F., Weston, V., Diggle, M., & McNally, A. (2013). Multidrug-resistant Escherichia coli bacteremia. *Emerg-*

ing infectious diseases, 19(10), 1699.

85. Clermont, O., Christenson, J. K., Daubié, A. S., Gordon, D. M., & Denamur, E. (2014). Development of an allele-specific PCR for Escherichia coli B2 sub-typing, a rapid and easy to perform substitute of multilocus sequence typing. *Journal of microbiological methods, 101*, 24-27.
86. Carlet, J. (2012). The gut is the epicentre of antibiotic resistance. *Antimicrobial resistance and infection control, 1*, 1-7.
87. Woerther, P. L., Angebault, C., Jacquier, H., Clermont, O., El Mniai, A., Moreau, B., ... & Andremont, A. (2013). Characterization of fecal extended-spectrum-β-lactamase-producing Escherichia coli in a remote community during a long time period. *Antimicrobial agents and chemotherapy, 57*(10), 5060-5066.
88. Sonis, S. T. (2007). Pathobiology of oral mucositis: novel insights and opportunities. *The journal of supportive oncology, 5*(9 Suppl 4), 3-11.
89. Murphy, B. A. (2007). Clinical and economic consequences of mucositis induced by chemotherapy and/or radiation therapy. *The journal of supportive oncology, 5*(9 Suppl 4), 13-21.