Ministry of Higher Education and Scientific Research
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Study of *Escherichia Coli* Bacteria

A Search

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By Students

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بِسْم الله الرّحْمنِ الرَّحِيمِ

وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي

فَضَّلَنَا عَلَىٰ كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ

صَدَقَ الله العَلي العَظِيم

سورة النمل الآية الخامسة عشر

اقرار المشرف

اشهد ان اعداد هذا البحث الموسوم:

Study of *Escherichia Coli* Bacteria

الذي تقدم به طلبة الدبلوم التقني كل من:

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قد جرى تحت اشرافي في قسم تقنيات المختبرات الطبية / المعهد التقني كوت كجزء من متطلبات نيل درجة الدبلوم التقني في تقنيات المختبرات الطبية وعليه ارشحه للمناقشة

المشرف

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/ / 2024

اقرار لجنة المناقشة

نحن اعضاء لجنة المناقشة نشهد باننا اطلعنا على البحث الموسوم

Study of *Escherichia Coli* Bacteria

المقدم من الطلبة:

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والمقدم الى مجلس قسم تقنيات المختبرات الطبية / المعهد التقني كوت.

وقد ناقشنا الطلبة في محتوياته وفي ما له علاقة به. ونقر بانه جدير بالقبول كجزء من متطلبات نيل درجة الدبلوم التقني في تقنيات المختبرات الطبية.

اعضاء اللجنة العلمية

عضوا **عضوا**

عضوا **عضوا**

رئيس اللجنة

م. د. طارق مسلم مهدي

رئيس القسم

Dedication

To my parents

more than anyone else

Who are responsible for

what I have become

Dearest brothers and sisters

with loyalty....

Abstract

Escherichia coli are normal inhabitants of the gastrointestinal tract of animals and humans of which only some strains have become highly adapted to cause diarrhoea and a range of extra-intestinal diseases. *Escherichia coli* are routinely characterised by serological identification of somatic O, flagellar H and capsular K antigens. However, while some serotypes correlate closely with certain clinical syndromes, differentiation of pathogenic strains from the normal flora depends on the identification of virulence characteristics.

It has been recognised that some diarrhoegenic strains of *E. coli* produce toxins that have an irreversible cytopathic effect on cultured Vero cells. Such verocytotoxigenic *E. coli* (VTEC) have been shown to belong to over 100 different serotypes. They are also described as Shiga toxin-producing *E. coli* (STEC) due to the similarity demonstrated between verocytotoxins (VT) and Shiga toxins (Stx) of Shigella dysenteriae. In the past two decades, VTEC O157:H7 has increased in importance world-wide as a public health problem.

The number of *E. coli* isolates from 325 stool specimens was 145 (44.62%), while E. coli was not detected in 180 (55.38%). The absence of E. coli in high percent due to either the most patients exclusively in this study received antibiotics before admission to hospital or the disease caused by other diarrheal microbial agents.

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Chapter One

Introduction

1.1. Introduction

Escherichia coli is a member of the intestinal family Enterobacteriaceae, a rod-shaped bacterium, motile or non-motile, aerobic or facultative anaerobics, fermenting the sugar lactose, most of which are fermenting the sugar ramenose and sorbetole. (Wanger *et al*., 2017)

The optimum temperature for its growth is (36-37) negative the oxidase enzyme and Positive for the catalase test. Producing indole and not consuming citrate. Positive for the methyl red test. and negative for Vogase-Proskauer. (Jawetz *et al.*, 2016; Hemraj *et al.*, 2013)

They live naturally in the intestines of humans and animals, and at the same time they are opportunistic pathogens that cause many diseases such as diarrhea, meningitis, sepsis, and bacteremia. They are among the most common bacterial species causing urinary tract infections, as they cause about (90%) of urinary tract infections in the world, and they are more common in childhood. (Hadi *et al.*, 2014; suardana., 2014)

The pathogenicity of this bacterium is due to the possession of many virulence factors, and one of these factors is the possession of Siderophores for iron and Colisin cytotoxic necrotizing factor chelators. It has surface structures such as flagella, capsules, and polysaccharides, which give bacteria antigenic properties by producing lipopolysaccharides (LPS), which give bacteria antigenic properties by producing antigen, somatic antigen 0, flagellar antigen H, and capsular antigen K. They also possess cilia. (fimbreae) or (pilli), which help them adhere to the host tissues, giving them the ability to form a biofilm E.coli bacteria are characterized by their multidrug resistance. (Terlizz *et al*., 2017; Zowawi *et al*., 2015)

1.2 Aims of the study:

To study the *Escherichia coli* including its types, morphological identification and general characteristics.

Chapter Two

Literature Review

2.1 The Enterobacteriaceae Family

The Enterobacter family includes a large and diverse group of bacterial species that live naturally in the intestines of humans and animals. Its individuals are rod-shaped, gram-negative, aerobic or facultative anaerobic, and can cause many diseases in humans and animals, including wound infections, hospital-acquired infections, nosocomial infections, respiratory system infections, urinary tract infections, and respiratory infections, and they contain factors Various virulence such as toxins. (Oliveira *et al.*, 2017)

Genital infections and reproductive enzymes also ferment a wide range of carbohydrates, most of which are fermentable to the sugar lactose. They are also negative for the oxidase test and positive for the catalase test. They have the ability to reduce nitrate to nitrite for the purpose of production. Most of the energy is moved by flagella 37 Non-spore forming and the optimum temperature for their growth, flagella. (Jawetz et al., 2016)

2.2. Bacteria *Escherichia Coli*

It is one of the most important members of the intestinal family, and grows as a normal flora in the digestive system. It is also considered an opportunistic pathogen, causing diarrhea and diarrheal diseases called Diarrheagenic *E. coli* (DEC), in addition to many diseases outside its natural habitats, including meningitis in children. Neonatal meningitis, sepsis uropathogenic *E.coli* is called urinary tract infection and urinary tract infections (UPEC) as it causes about 90% of urinary tract infections and can be easily transmitted from the anal area to the urinary tract and bladder, which is about 14 times more common in females than in males due to Urethral shortening in females. (Levinson, 2016).

2.3. Classification of *Escherichia Coli*

E.coli bacteria were first identified by the German scientist Theodore Escherich in (1885) during his study of the natural bacteria in the intestines in the feces of infants as commensal bacteria. It settles in the intestines immediately after birth. In 1945 AD, the scientist Bray found that a strain of *E. coli* bacteria it was the main cause of diarrhea in infants in England and was called Enteropathogenic *E.coli*. (Bray, 1945)

The genus Escherichia is closely related to other genera of the family Enterobacteriaceae, especially the genus Shigella this genus includes five species (*E.*

fergosonii, E. hermanii, E. blattae, E. coli). *E. vuneris* differ from each other by some biochemical reactions and after *E. coli* the most important and common type *E. coli*. (Olowe *et al.,* 2017)

Human pathogenesis *E. coli* bacteria are classified in the Bergeys Manual world classifier within the Enterobacteriaceae family:

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *coli* (*Garrity et al.,* 2005)

2.4. General Characteristics of *Escherichia Coli*

They are gram-negative bacilli that move with peritrichous flagella that surround the entire body and do not form spores. Their colonies are smooth, smooth, slightly convex, moist, not mucous or mucous when they have a capsule structure, with a complete sharp edge, shiny pink on the middle of MacConkey agar. Bright green metallic sheen on Eosin Methylene Blue (EMB) agar, and pink colonies also form on Cromagar Orientation agar medium. Cellulobios sugar is not fermented, and more than 80 % of it is fermented to rhamnose sugar, and more than 90% of which is fermented sugar sorbetole. It is also non-degrading to gelatin and does not produce hydrogen sulfide gas (H22S) in Triple Sugar and Iron Agar (TSI). (Wanger *et al*., 2017)

Most of them are the enzyme (B-glucuronidase (GUD), does not grow in the presence of potassium cyanide (KCN), and grows at a pH ranges between (4.4-9) and the optimum temperature (37-36) for its growth is negative for the oxidase test. It is also positive for the catalase test. and positive for the indole test, which is the best test that distinguishes it from other members of the intestinal family. In addition, it does not consume citrate as the sole source of carbon. It is also positive for the methyl red test and negative for the Vogase-Proskauer test. (Hemraj *et al.,* 2013)

2.5. Types of bacteria *Escherichia Coli*

Diarrheagenic *E. coli* (DEC) bacteria that cause diarrhea are divided into types based on its characteristics, specific virulence factors and the mechanism of action of these factors into:

1. Escherichia hemorrhagic *E. coli* (EHEC)
2. Shiga Toxin Producing *E. coli* (STEC)
3. Entero-pathogenic *E. coli* (EPEC)
4. Entero-toxigenic *E. coli* (ETEC)
5. Enteroinvasive *E. coli* (EIEC)
6. Enteroaggregative *E. coli* (EAEC)
7. Diffusely Adhering *Escherichia coli* (DAEC) (Rivas *et al.,* 2018; Malema *et al.,* 2015)

2.6. Morphological Identification

Escherichia coli bacterial isolates were initially identified based on their phenotypic characteristics, after growing them on MacConkey agar medium, Eosin Methyline Chromagar medium, Chromagen blood agar medium, Blue agar (EMB) Orientation medium, and Hekton intestinal agar medium and Enteric agar. The results showed that the bacteria were fermenting the sugar lactose, and produced smooth, shiny, pink colonies with a sharp edge on the differential agar medium, which contains bile salts and crystal violet dye, which allows the growth of gram-negative bacteria, including the Entericidae family, and inhibits the growth of gram-positive bacteria. The isolates are shiny, metallic green colonies on the blue eosin-methylene agar medium. This characteristic is one of the distinguishing characteristics of the *E.coli* bacteria from other members of the Enterobacteriaceae family, as a result of the medium containing the dyes eosin and methylene. (Wanger *et al.,* 2017)

Chapter Three

Materials and Methods

Materials and Methods

In the present study, a total of 325 fecal specimens were collected from children with diarrhea from three hospitals in Al- Kut city.

3.1. Materials

3.1.1. Laboratory Equipments

Tools and Equipment	Company	Origin
Refrigerator	Kelon	korea
ELISA	Biorad	US
Electrophoresis system	Optima	Japan
Water distillatory	GFL	Germany
Bench centrifuge	Diham	Korea
PCR	Bioneer	Korea
Incubator	Mennert	Germany
Water bath	Diham	Korea
Laminar air flow (hood)	Labtech	Korea
Light microscope	Kruss	Germany
Autoclave	Webco	Germany
Ph meter	Martini	USA
Micropipette	Brand-W	Germany
Hot air oven	DLTG	China
Magnetic stirrer	Stuart	UK
Electronic balance	Denver	Germany

3.1.2. Chemical Materials

Chemicals	Company	Origin
Agarose	Biobasic 1 NK	Canada
Hydrogen peroxidase	BDH	England
Tetramethyl-p-phenylene diamine dihydrochloride	DIFECO	England
H2SO4	BDH	England
Seder oil	BDH	England
Ethanol	BDH	England
Absolute ethanol	Bioneer	Korea

3.1.3. Culture Media

Culture media	Company	Origin
Methyl red voges proskauer	Himedia	India
Blood agar base	Himedia	India
Chromagar	Oxiod	England
MacConkey agar	Oxiod	Holland
Urea agar base	Himedia	India
Eosin methylene blue agar	Oxiod	England
Trypton soy agar	Oxiod	England
Simmon citrate agar	Himedia	India
Muller-hinton agar	Oxiod	England
Hekton enteric agar	Oxiod	England
Nutrient agar	Oxiod	England
Pepton water	Himedia	India
Brain heart infusion broth	Oxiod	England

3.1.4. Solutions, Stains and Reagents

Chemicals	Company	Origin
Loading dye	Geneaid	Thailand
Ethidium bromide	Biobasic INK	Canada
Methyl red reagent	Himedia	India
Kovac's reagent	Vac and seralnst	Iraq
Voges-proskauer (VP1) (VP2)	Vac and seralnst	Iraq
Crystal violet, safranin,iodine	Biobasic INK	Switzerland
Tris-borate-EDTA buffer	Biobasic INK	USA
Phosphate buffer saline	Chemical point	Germany
Normal saline	schuchard	Germany

3.2. Methods

3.2.1. Morphological Identification

E.coli bacterial isolates were initially identified based on their phenotypic characteristics, after growing them on MacConkey agar medium, Eosin Methyline Chromagar medium, Chromagen blood agar medium, Blue agar (EMB) Orientation medium, and Hekton intestinal agar medium. Enteric agar The results showed that the bacteria were fermenting the sugar lactose, and produced smooth, shiny, pink colonies with a sharp edge on the differential agar medium, which contains bile salts and crystal violet dye, which allows the growth of gram-negative bacteria, including the Entericidae family, and inhibits the growth of gram-positive bacteria. The isolates are shiny, metallic green colonies on the blue eosin-methylene agar medium. This characteristic is one of the distinguishing characteristics of the E. *coli* bacteria from other members of the Enterobacteriaceae family, as a result of the medium containing the dyes eosin and methylene. (Sharmin *et al.*, 2016)

The blue color that precipitates in the acidic environment after bonding together gives a metallic green sheen, which indicates that the bacteria produced organic acids as a result of fermenting the sugars lactose and sucrose. As for the chromagen agar medium, the bacteria produce glucuronidases-enzymes that work to decompose the conjugated chromogen and release the B-glucuronid chromagenic substrate, or what is called the chromagenic conjugate, the chromaphore, thus giving a pink color to the bacterial colonies. (Wanger., 2017; Bhattacharyya *et al*., 2015)

When growing bacterial colonies on intestinal hectone agar medium, it was found that they showed yellow to orange colonies as a result of them being lactose-fermenting bacteria. This medium is one of the differentiation media that distinguishes between gram-negative bacteria that are lactose-fermenting and non-lactose-fermenting. (Jawetz *et al.*, 2010; Sharmin *et al.*, 2010)

3.2.2. Microscopic Identification

Staining was performed with Gram's stain by preparing a smear of the colony growing on agar medium aged (18-24) hours. The cells of the bacteria, Bacillus brevis, appeared to be Gram-negative and non-spore forming. (Levinson., 2016)

3.2.3. Biochemical Identification

Biochemical tests were performed on all isolates, and they were all positive for the catalase test, as the *E. coli* bacteria had decomposed the hydrogen peroxide

reagent into water and oxygen. The bacteria were negative for the oxidase test because the color of the colonies did not turn purple when the reagent was added, as the bacteria do not possess the enzyme cytochrom oxidase as a hydrogen acceptor, and negative for the urease test because the color of the medium did not change, which confirms that the bacteria are not consuming urea because they do not have the enzyme. Urease as for the results of the IMViC group tests, the bacteria were positive for both the indole test as a result of the appearance of a red ring on the surface of the medium in the isoamyl alcohol layer as a result of the decomposition of the amino acid tryptophan by the tryptophase enzyme. This detection is important in distinguishing between E. *coli* bacteria and individuals. Other Enterobacteriaceae, after it was detected using Kovac's reagent and it was positive for the methyl red test due to the presence of the resulting acid from the bacteria's consumption and fermentation of glucose and peptose, which led to the production of acid and a change in the pH of the medium, which led to the color of the medium turning red. **(**Brown., 2017)

The bacteria were also negative for the Voges-Proskauer test as a result of the appearance of a yellow to brown color in the liquid medium, which is due to the bacteria not converting the glucose sugar glucoe into acetylmethylcarbinol (acetoine), which leads to the lack of reaction of acetoine with acetone (VP22) potassium hydroxide and (VP11) alphanaphthol, the resulting reagents, were negative for the citrate test, which indicates that the bacteria do not use citrate as the sole source of carbon as a result of not having the citrate permease enzyme. The result was that the color of the medium did not change to bluish-green, because citric acid was not produced and the pH did not change.

Chapter Four

Results

Results

The number of *E. coli* isolates from 325 stool specimens was 145 (44.62 %), while *E. coli* was not detected in 180 (55.38 %). The absence of *E. coli* in high percent due to either the most patients exclusively in this study received antibiotics before admission to hospital or the disease caused by other diarrheal microbial agents.

Table (4.1): Sero-typing identification of EPEC isolated from infant patients with diarrhea in Kut city

E. coli isolates	Sex	Age/ month	percentage%
E. coli S1	M	10	0.1 %
E. coli S2	M	14	0.14%
E. coli S3	F	2	0.2 %
E. coli S4	M	7	0.7 %
E. coli S5	M	4	0.4 %
E. coli S6	M	8	0.8 %
E. coli S7	M	12	0.12%
E. coli S8	M	6	0.6 %
E. coli S9	F	10	0.1 %
E. coli S10	M	11	0.11 %
E. coli S11	F	3	0.3 %
E. coli S12	F	3	0.3 %
E. coli S13	M	4	0.4 %
E. coli S14	F	4	0.4 %
E. coli S15	F	8	0.8 %
E. coli S16	M	12	0.12 %
E. coli S17	M	7	0.7 %
E. coli S18	F	3	0.3 %
E. coli S19	M	20 days	0.2 %
E. coli S20	M	6	0.6 %
E. coli S21	F	7	0.7 %
E. coli S22	F	7	0.7 %
E. coli S23	M	2	0.2 %
E. coli S24	M	5	0.5 %
E. coli S25	M	3	0.3 %
E. coli S26	F	9	0.9 %
E. coli S27	M	5	0.5 %
E. coli S28	F	24	0.24%

In the present study, a total of 325 fecal specimens were collected from children with diarrhea from three hospitals in Al- Kut city. It was observed that the number of patients was higher in females 167 (51.38 %) compared to males 158 (48.61 %).

Higher infection of diarrhea was recorded at age group 7 to 12 months (46.15 %) followed by age group since birth up to 6 months (42.77 %), while the lowest age group with diarrheic patients was 19 to 24 months (3.4%).

The reason of higher infection in these two groups of infants may be due to low immunity, as the amount of tranceplantal antibodies of the child starts dwindling after 6 months of age. Those infants also may not have been breast-fed but bottle-fed instead, which is a source of infection and contamination. This becomes a matter of public health importance because such children may serve as a source of infection in the community. With the time, the child is exposed to pathogens and gets gradual resistance against infection.

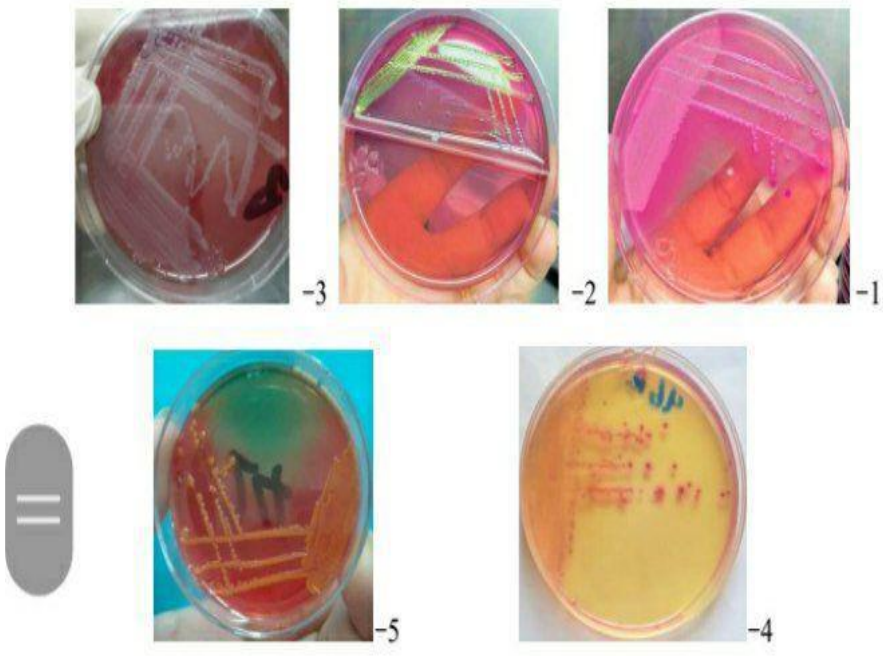

Figure (4.1): Phenotype of E coli bacteria on several diagnostic media

Conclusions

&

Recommendations

Conclusions

E. coli infection depend on various factors such as the strain involved, the severity of symptoms and the affected individual's health status. In general, E. coli infections can range from mild gastroenteritis to severe illnesses such as hemolytic uremic syndrome (HUS) or urinary tract infections. Preventative measures like proper food handling and hygiene are crucial in reducing the risk of E. coli infections. Treatment typically involves supportive care, hydration, and in some cases, antibiotics may be prescribed under medical supervision.

Recommendations

The following suggestions are recommended:

1. Food safety such as good meat cook, avoiding consuming unpasteurized dairy products.

2. Wash hands frequently, especially before eating, and after contact with animals.

3. When traveling with area with poor sanitation use boiling water to drinking and cooking.

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