



Correlation of Bacteria Diversity and Drug Resistance with Colorectal Cancer Patients

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Abstract: Colorectal cancer (CRC) is the leading cause of illness and mortality worldwide. The aims of the study is investigation and detection of some bacterial interfering with CRC occurrence and progression . The study conducted between September 2022 till February 2023, a total of 50 specimens were collected from confirmed CRC patients. In addition , 50 stool specimens were collected from healthy volunteers as a control. All specimens were gathered from medical city hospital and gastro-intestinal hospital. Isolation and identification of bacteria in all collected specimens were done by using cultural and differential media (Blood agar, macconkey agar, mannitol salt agar and Pfizer agar), as well the Vitek- 2 compact system. The bacterial species, in the specimens of control were(*Escherichia coli* 50 (86.20%), *Klebsiella Pneumonia* 3(5.17%), *Salmonella typhi* 2(3.44%), *Staphylococcus aureus* 1(1.72%), *Proteus mirabilis* 1(1.72%) and *Pseudomonas aeruginosa* 1(1.72%), while in the specimens of CRC and polyp were (*Escherichia coli* 30(38.69%), *Streptococcus uberis* 6(7.79%), *Enterobacter cloacae* 4(5.19%), *Proteus mirabilis* 11(14.28), *Streptococcus constellatus pharyneis* 1(1.29%), *Micrococcus luteus* 1(1.29%), *Staphylococcus pseudintermedius* 1(1.29%), *Streptococcus thoraltensis* 1(1.29%), *Citrobacter freundii* 1(1.29%), *Streptococcus mutans* 1(1.29%), *Enterococcus faecium* 5(6.49%), *Enterococcus faecalis* 4(5.19%), *Granulicatella elegans* 1(1.29%), *Enterococcus gallinarum* 2(2.59%), *Serratia marcescens* 1(1.29%), *Streptococcus sanguinis* 1(1.29%), *Staphylococcus lentus* 1(1.29%), *Comamons testosteroni* 1(1.29%), *Morganella morgani* 1(1.29%), *Pseudomonas aeruginosa* 1(1.29%), *Klebsiella pneumonia* 2(2.59%). The bacteria which has been shown to be associated and more abundance in the specimens of CRC tissues are *Escherichia.coli* 30(38.96%), *Streptococcus uberis* 6(7.79%), *Enterobacter cloacae* 4(5.19%), *Enterococcus faecium* 5(6.49%), *Enterococcus faecalis* 4(5.19%). The relative abundance of (*Escherichia coli*, *Streptococcus uberis*, *Enterobacter cloacae*, *Enterococcus faecium*, *Enterococcus faecalis*) among CRC specimens. The biofilm formation capability for the tested isolates revealed that (*Escherichia coli*, *Streptococcus uberis* and *Enterococcus faecalis*) were moderate intensity production, while (*Enterobacter cloacae* and *Enterococcus faecium*) were weak intensity. The antibiotics susceptibility test (AST) showed that *Streptococcus uberis*. It was concluded as an Extensive drug resistance (XDR), while (*Escherichia coli*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Enterococcus faecium*) were considered as Multi Drug resistance (MDR).

Keywords: CRC, *S. uberis*, *E. coli*, *E. cloacae*, *E. faecalis*, and *E. faecium*.

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Introduction

The third most frequent cancer and the fourth most common cause of cancer-related death is colorectal cancer (CRC). It is the most widespread and serious health problem on a global scale due to its increasing frequency. The risk of developing colorectal cancer (CRC),

is associated with several risk factors, including dietary habits (less fiber/excess red meat), smoking, obesity, alcohol consumption, diabetes, and lifestyle. The incidence of CRC is increasing annually. have been connected to the start and development of colon cancer (1). The gut microbiota

is important in this context, because dysbiosis conditions can cause colonic carcinogenesis via a chronic inflammatory process. Moreover, elevated amounts of reactive oxygen species (ROS) have been discovered in almost all malignancies, and they are believed to be crucial to the development and spread of cancer.

Although these highly reactive ions and molecules are produced during a cell's normal metabolism, cancer cells produce more of them due to increased metabolic activity, mitochondrial dysfunction, peroxisome activity, increased cellular receptor signaling, oncogene activity, increased activity of oxidases, cyclooxygenases, lipoxygenases, and thymidine phosphorylase, or through crosstalk with infiltrating immune cells. And the role of the gut microbiota in cancer biology. According to two different theories, colon cancer is thought to develop from two different types of precursor polyps. Conventional adenoma by the conventional adenoma-to-carcinoma sequence and serrated adenoma by the serrated adenoma-to-carcinoma hypothesis. The adenomatous polyposis coli (APC) gene is mutated in conventional adenomas, and colon cancer develops over a number of years. Invasive carcinomas come next in the multi-step process leading to colon cancer (2), as shown figure (1,2). The bacteria that cause this multiphase process, for example, include *Streptococcus uberis*, *Enterobacter cloacae complex*, *Enterococcus faecium*, *Enterococcus faecalis* and *Escherichia coli*. Colon rectal cancer is caused by mutations. *Streptococcus uberis* is a Gram-positive bacteria specific beta-hemolytic.

Associated with intramammary infection and mastitis in ruminants. It is occasionally described as a human

pathogen, recently, there were few reports existed regarding *Streptococcus uberis* as a human pathogen. Report *Streptococcus uberis* as a pathogenic agent of human urinary tract infections. According to the European Food Safety Authority (EFSA), *Streptococcus uberis* is classified as being in risk group1 (3).

A major contributor to mastitis in dairy cattle is the principal environmental bacterium *Streptococcus uberis*. Chronic *Streptococcus uberis* subclinical mastitis infections are very expensive and challenging to treat. We also believe that more detailed studies are necessary to further illuminate the pathogenesis of human infection, its relation to milk and milk products, the infection of various human body sites, as well as the significance of safe and effective storage of milk and milk products for public safety. After stimulation with *Streptococcus uberis* epithelial cells express more important enzymes in the mitochondrial apoptotic pathway. Reactive oxygen species (ROS) are produced in excess, which causes oxidative stress in host cells and slows the progression of disease. ROS generation is strongly correlated with mitochondrial dysfunction since they are a major source of ROS (4), and the capacity of *Streptococcus uberis* to create biofilms is one of the elements that contributes to its pathogenicity.

Enterococcus faecalis is a commensal bacterium that has the potential to mutate colonic cells, leading to intestinal lesions and *Enterococcus faecalis* translocation into the bloodstream (4). *Enterococcus faecium* is also be pathogenic cause diseases such as neonatal meningitis or endocarditis Enterococci play a vital role, especially *Enterococcus faecalis* and *Enterococcus faecium*.

Additionally, the likelihood of an epidemic is increased by the

virulence traits and multi-drug resistance of enterococci, particularly in hospital environments. Recent research has shown their harmful role in the growth of colon cancers (5).

Enterococcus faecium strains may possess traits that facilitate colonization of portions of the GI tract, Cell surface protein, Enterococcal surface protein (Esp), which is implicated in biofilm formation. Previously it was shown that *Enterococcus faecium* is able to adhere to human intestinal mucus. A study showed that hydrogen peroxide is produced by *Enterococcus faecium* at quantities that harm cells. Oxygen radicals ability to disrupt membranes may make surrounding intestinal epithelial cells more susceptible to cellular harm, according to research on *Enterococcus faecium* transposon insertion mutants. Evidence suggests that the DNA of colonic epithelial cells is harmed by the formation of extracellular superoxide and hydrogen peroxide(6). *Enterobacter cloacae* are opportunistic microorganisms and multiresistant bacterial pathogens for humans. *Enterobacter cloacae* its capacity to create biofilms and secrete different cytotoxins (pore-forming toxins, hemolysins, and enterotoxins) are crucial to its pathogenicity (7).

Escherichia coli are intestinal flora. *Escherichia coli*, as a commensal, more frequently than in healthy colonic tissue, pathogenic *Escherichia coli* has been discovered in the colon tissue of patients with adenocarcinomas. *Escherichia coli* infection promotes the production of the macrophage-inhibitory cytokine-1 (MIC-1) according to in vitro research.

Pathogenic *Escherichia coli* strains produce several toxins called cyclomodulins including the cytotoxic necrotizing factor (CNF), cycle

inhibiting factor (Cif), colibactin, and cytolethal distending toxins (CDTs). The colibactin is a toxin produced by polyketide synthetase (pks). Cyclomodulins are attracting growing attention due to their ability to influence cellular differentiation, apoptosis, and cell proliferation by disrupting the eukaryotic cell cycle and/or promoting DNA damage (8).

Materials and methods

Bacterial isolation and identification

The period from September 2022 till February 2023, a total of 7 specimens of stool and biopsy without formalin were collected from confirmed CRC patients and 43 specimens of polyp patients admitted to hospitals in Baghdad (Medical city Hospitals, Gastrointestinal Hospital).

Also, fifty stool specimens were collected from healthy volunteers with no personal or familial history or diagnosis of colorectal disease as control group. All patients underwent Fecal Occult Blood Test and laparoscopic (colonscopy) and surgery. All specimens were transported to the laboratory without delay.

Histopathologically for 7 specimens of colorectal cancer were differentiated as adenocarcinoma, and 43 specimens were diagnosis as polyp, as shown in Figures (1,2). Bacteria isolation were detected by cultural characterization on the (blood agar media, macconkey agar, mannitol salt agar and Pfizer selective enterococcus agar) at 37°C under aerobic condition and in anaerobic gas jar condition.

All the necessary examinations for preliminary diagnosis of the isolated bacteria were carried out, and the confirmatory diagnosis of these isolates was done by Vitek- 2 compact system.



Figure (7): Colonrectal cancer polyp



Figure (2): After excision of polyp

Biofilm formation test

A colorimetric microtiter plate assay was used to measure biofilm formation quantitatively (9):

1. All isolates were raised at 37°C in brain heart infusion broth for 24h. Thereafter, 100 µl of bacterial growth were transferred into a tube of 2 ml of normal saline, and then the turbidity was adjusted to McFarland 0.5.
2. A volume of 180 µl of brain heart infusion broth contained 1% glucose were added to sterile flat-bottomed 96-well polystyrene microtiter plates.
3. A volume of 20 µl of bacterial suspension (from normal saline) was added to three wells of sterile flat-bottomed 96-well polystyrene microtiter plates. A total of three wells with bacteria-free brain heart infusion broth were considered as a negative control.
4. The dishes were sealed with their lids and left undisturbed for 24 hours at 37°C in an aerobic environment. All plates were lightly washed three times with distilled water after incubation, then dried.
5. Each well received 200 µl of methanol for the fixation of the biofilms, which was done for 15 minutes at room temperature. The wells were then cleaned and allowed to air dry.

6. 200 μ l of a 0.1% crystal violet solution was used to color the plates for 15 minutes at room temperature. In addition, wells were cleaned and drained for about 30 minutes at 37°C.
7. Resolving the dye in 200 μ l of pure ethanol mixed with acetic acid glacial (1:1) for 10 min.
8. Using a microtiter plate reader, the optical density (OD) of each well was measured at 630 nm. Three standard deviations more than the mean OD of the negative control was designated as the cut-off OD (OD_c). Based on four classifications, all isolates were divided into OD_c value: non-producer, weak biofilm producer, moderate biofilm producer, and strong biofilm producer, as shown in Table (1).
9. All data statistically were analyzed by using SPSS, version 29, 2023, IBM, USA.

Table(1): Interpretation of biofilm production

OD Value	Biofilm intensity
$OD \leq ODC^*$	Non – producer
$ODC < OD \leq 2 \times ODC$	Weak
$2 \times ODC < OD \leq 4 \times ODC$	Moderate
$4 \times ODC < OD$	Strong

*ODC= three standard deviation above the mean OD of the negative control.

Antibiotics susceptibility test(AST) by Vitek- 2 system

The procedure was done according to (10), as follow:

1. The 0.5 McFarland bacterial suspension was diluted to 1.5×10^8 CFU/ml by using DensiCHEK plus meter in 0.45% saline.
2. Cards were automatically filled by bacterial suspension diluted.
3. Cards were loaded into the Vitek- 2 system for incubation and reading after 8h.

Results and discussion

Bacterial identification

The current study revealed, the total counts of bacterial species isolation which has been shown in the specimens of control group were 58 isolate. The species distributed into *Escherichia coli* 50(86.20%), *Klebsiella pneumonia* 3(5.17%), *Salmonella Typhi* 2(3.44%), *Staphylococcus aureus* 1(1.72%), *Proteus mirabilis* 1(1.72%) and *Pseudomonas aeruginosa* 1(1.72%), while those in the specimens

of colon rectal cancer and polyps were 77 isolate, distributed into (*Escherichia coli* 30(38.69%), *Streptococcus uberis* 6(7.79%), *Enterobacter cloacae* 4(5.19%), *Proteus mirabilis* 11(14.28%), *Streptococcus constellatus pharyneis* 1(1.29%), *Micrococcus luteus* 1(1.29%), *Staphylococcus pseudintermedius* 1(1.29%), *Streptococcus thoralensis* 1(1.29%), *Citrobacter freundii* 1(1.29%), *Streptococcus mutans* 1(1.29%), *Enterococcus faecium* 5(6.49%), *Enterococcus faecalis* 4(5.19%), *Granulicatella elegans* 1(1.29%), *Enterococcus gallinarum* 2(2.59%), *Serratia marcescens* 1(1.29%), *Streptococcus sanguinis* 1(1.29%), *Staphylococcus lentus* 1(1.29%), *Comamons testosteroni* 1(1.29%), *Morganella morganii* 1(1.29%), *Pseudomonas aeruginosa* 1(1.29%), and *Klebsiella pneumonia* 2(2.59%), as shown in Table (2,3). *Streptococcus uberis* it is referred to as a human pathogen. A major contributor to

mastitis in dairy cattle is the principal environmental bacterium *Streptococcus uberis*. Chronic *Streptococcus uberis* subclinical mastitis infections are very expensive and challenging to treat. Also certain animals are numerous streptococcal species, including pathogens, can coexist with their host in an asymptomatic carriage condition (11). *Enterococcus faecalis* As well as commensal bacteria found in the gastrointestinal tracts of mammals including humans, has the ability to breach the intestinal wall and cause systemic infections like infective endocarditis. One of the most frequent sources of infection in the aged population is Enterococci, and malignancy is the most common comorbidity. It has not yet been determined whether *Enterococcus faecalis* endocarditis and CRC are related. According to some evidence, *Enterococcus faecalis* may even be to blame for the mutagenesis of colonic cells, which results in intestinal lesions and cancer, before *Enterococcus faecalis* is transferred into the bloodstream. May cause the intestinal lesion and colonic cell mutation that result in intestinal cancer. Because its metabolic products can harm the DNA of intestinal epithelial cells, the *Enterococcus faecalis* has been identified as a bacterium associated with colorectal cancer(6). *Enterococcus faecium* it can coexist with humans and animals in the gastrointestinal tract. yet it could also be harmful, causing conditions like endocarditis or newborn meningitis. A considerable role is played by Enterococci. *Enterococcus faecium* is a minor member of the intestinal microbiota that primarily lives in mature human's colons and cecums(12). *Enterobacter cloacae* Due to their widespread character *Enterobacter cloacae* are nosocomial

pathogens that can be acquired through the urinary tract, skin, gastrointestinal system, or externally. Being an opportunistic pathogen, this organism preys on vulnerable individuals, such as the young, the elderly, or those who have a serious illness like the human immunodeficiency virus. This organism most frequently causes nosocomial infections, meaning it can spread through medical settings like the Intensive Care Unit (ICU). Numerous items, including palms of staff, blood products, endoscopes, albumin, stethoscopes, digital thermometers, and devices for measuring intra arterial pressure, have been found to contain *Enterobacter cloacae*. Additionally, illnesses caused by *Enterobacter cloacae* include bacteremia, infections of the lower respiratory tract, infections of the skin and soft tissues, endocarditis, infections of the urinary system, infections of the abdomen, osteomyelitis, septic arthritis, and infections of the eyes (7). *Escherichia coli* gut flora containing *Escherichia coli* According to research, patients with colon cancer have an elevated *Escherichia coli* burden in their intestines, and their intestinal isolates pathogenic processes and types of toxins differ from those of healthy individuals (13). *Proteus mirabilis* is commonly associated with urinary tract infections (UTIs) in humans, especially in patients with catheter-associated UTIs or hypo-immunity (14). Some researchers suggested that *Proteus mirabilis* is an opportunistic pathogen(14). However, more and more food poisoning incidents associated with *Proteus mirabilis* were reported worldwide. *Klebsiella pneumonia* It is most commonly associated with pneumonia and is a common cause of infections in the urinary tract, lower biliary tract, and surgical wound sites.

Pyogenic liver abscess (PLA) is caused by bacteria and depending on the geographical data, most commonly isolated bacteria from pyogenic liver abscesses are *Escherichia coli* for the western countries and *Klebsiella pneumonia* for the eastern populations. Since the 1990s, there are more reports showing that *Klebsiella pneumonia* is the most common cause of PLA within western countries as well (15). There

are a number of case reports showing a positive correlation between PLA caused by *Klebsiella pneumonia* and CRC. *Pseudomonas aeruginosa* as the opportunistic pathogen in the management of human cancers, Although. *Pseudomonas aeruginosa* is a major pathogen in cystic fibrosis (CF) causing significant morbidity and mortality (16).

Table (2): Types of bacterial isolates in colonrectal cancer and polyp specimens

N	Type of bacterial isolate	Number (%)
1	<i>Enterobacter cloacae</i>	4(5.19%)
2	<i>Streptococcus uberis</i>	6(7.79%)
3	<i>Escherichia coli</i>	30(38.96%)
4	<i>Proteus mirabilis</i>	11(14.28%)
5	<i>Streptococcus constellatus pharyneis</i>	1(1.29%)
6	<i>Micrococcus luteus</i>	1(1.29%)
7	<i>Staphylococcus pseudintermedius</i>	1(1.29%)
8	<i>Streptococcus thoraltensis</i>	1(1.29%)
9	<i>Citrobacter freundii</i>	1(1.29%)
10	<i>Streptococcus mutans</i>	1(1.29%)
11	<i>Enterococcus faecium</i>	5(6.49%)
12	<i>Enterococcus faecalis</i>	4(5.19%)
13	<i>Granulicatella elegans</i>	1(1.29%)
14	<i>Enterococcus gallinarum</i>	2(2.59%)
15	<i>Serratia marcescens</i>	1(1.29%)
16	<i>Streptococcus sanguinis</i>	1(1.29%)
17	<i>Staphylococcus lentus</i>	1(1.29%)
18	<i>Comamons testosteroni</i>	1(1.29%)
19	<i>Morganella morganii</i>	1(1.29%)
20	<i>Pseudomonas aeruginosa</i>	1(1.29%)
21	<i>Klebsiella pneumonia</i>	2(2.59%)
Total		77

Table (3): Types of bacterial isolates in control specimens

N	Type of bacterial isolate	Number
1	<i>Escherichia coli</i>	50(86.20%)
2	<i>Klebsiella pneumonia</i>	3(5.17%)
3	<i>Salmonella Typhi</i>	2(3.44%)
4	<i>Staphylococcus aureus</i>	1(1.72%)
5	<i>Proteus mirabilis</i>	1(1.72%)
6	<i>Pseudomonas aeruginosa</i>	1(1.72%)
Total		58

The bacteria which has been shown to be associated and more abundance in the specimen of CRC

tissues are *Escherichia.coli* 30(38.96%), *Streptococcus uberis* 6(7.79%), *Enterobacter cloacae* 4(5.19%),

Enterococcus faecium 5(6.49%), and *Enterococcus faecalis* 4(5.19%), as shown in (Figures 3,4,5,6 and 7).

Escherichia coli exists in symbiosis in the human intestine, several studies have shown the association between the binding of this bacterium to the intestinal mucosa and its role in the incidence of CRC (17).

Streptococcus uberis is globally recognized as one of the most significant environmental pathogens implicated in intramammary infections, pathogenic *Streptococcus uberis* cause a variety of diseases states a cross range of animal hosts as well as human. As an opportunistic pathogen *Streptococcus uberis* can live in different ecological niches due to its nutritional flexibility indicating that it can adapt to different types of environments as an opportunistic pathogen(18). Recent data suggests that *Enterococcus faecalis* is inherently associated to colorectal cancer (CRC),

despite the fact that it has historically been thought as a normal component of the gut microbiome. According to a recent study CRC patients stool and nearby tissues contained an abundance of *Enterococcus faecalis* (4).

Enterococcus faecium strains may possess traits that facilitate colonization of portions of the GI tract, Cell surface protein (Esp), which is implicated in biofilm formation. Previously it was shown that *Enterococcus faecium* is able to adhere to human intestinal mucus (6).

Enterobacter Cloacae Complex *E. Cloacae* are opportunistic microorganisms. The strains of *Enterobacter cloacae* are incredibly diversified in terms of their genetic make up and ecological role, and the species appears as commensal microflora in the intestinal tracts of huma. Recently, *Enterobacter cloacae* has become a significant nosocomial pathogen (7).



Figure (3): Bacteria *streptococcus uberis* on Blood agar



Figure (4): Bacteria *Escherichia coli* on Macconkey agar

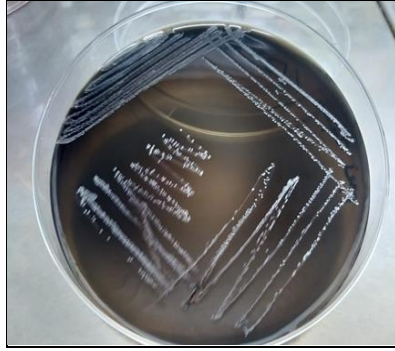


Figure (5): Bacteria *Enterococcus faecalis* On Pfizer selective agar



Figure (6): Bacteria *Enterobacter cloacae complex* on Macconkey agar

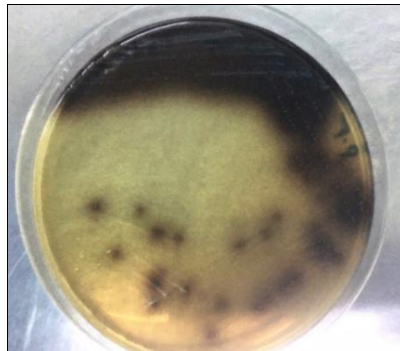


Figure (7): Bacteria *Enterococcus faecium* on Pfizer agar

The notice cultural characteristics isolated from colonrectal cancer and polyps on blood agar media, macconkey agar, and Pfizer agar, has been shown fermenter lactose on macconkey agar 36(46,75%), non fermenter lactose on macconkey agar 4(5.19%), β hemolytic on blood agar 5(6.49%), α hemolytic on blood agar 4(5.19), γ hemolytic on blood agar 1(0.77%), colony color from dark brown to black on Pfizer agar 2(2.59%), were carried out as listed in the (Table 4).

Colonies of *Enterococcus faecalis* on macconkey agar from non lactose fermenters are colorless. *Enterococcus faecalis* on Pfizer Selective Enterococcus Agar. The color range of the colony morphology varies from dark brown to black (20). *Enterococcus faecium* the colonies that are created have a moist appearance and are typically 1-2 mm in size. Pfizer Selective Enterococcus agar also supports the growth of *Enterococcus faecium*. The colony shape ranges in color from dark brown to black (21),

Although the results of *Enterobacter Cloacae* on macconkey agar are unclear, lactose positive colonies are depicted in literature as pink to red. *Enterobacter cloacae* ferments lactose.

Clusters of somewhat mucoid (22). *Escherichia coli* on macconkey agar colonies are pink in color, which is crucial for separating *E. coli* from other bacteria in the specimens (23).

Table (4): Characteristics in cultures of colonrectal cancer and polyps

Characteristics in cultures	%
Fermenter lactose on macconkey agar	36(46,75%)
Non Fermenter lactose on macconkey agar	4(5.19%)
β hemolytic on blood agar	5(6.49%)
α hemolytic on blood agar	4(5.19)
γ hemolytic on blood agar	1(0.77%)
colony color from dark brown to black on Pfizer agar	2(2.59%)

The notice cultural characteristics isolated from control specimens on blood agar media, macconkey agar, and Pfizer agar, has been shown fermenter lactose on macconkey agar 53(91.37%), non fermenter lactose on macconkey agar 1(1.72%), β hemolytic on blood agar 1(1.72%), were carried out as listed in

the (Table 5). *Klebsiella pneumonia* it appears as a mucoid lactose fermenter on macconkey agar. *Proteus mirabilis* it has swarming motility, do not usually ferment lactose. *Pseudomonas aeruginosa* is some strains hemolysis blood completely (β - hemolysis) by producing hemolysis toxin.

Table (5): Characteristics in cultures of control specimens

Characteristics in cultures	%
Fermenter lactose on macconkey agar	53(91.37%)
Non Fermenter lactose on macconkey agar	1(1.72%)
β hemolytic on blood agar	1(1.72%)

Biofilm formation test for the five selected species from colon rectal cancer and polyps showed a moderate intensity for (*Streptococcus uberis*, *Enterococcus faecalis*, *Escherichia coli*), while (*Enterobacter cloacae* complex, *Enterococcus faecium*) gaved weak intensity, as shown in (Table 6) and (Figures 8,9). *Streptococcus uberis* is its ability to form biofilm, communities of bacteria bound together by an extracellular polymeric matrix . Biofilms are common in nature, and it has been estimated that 99% of bacterial cells coexist in biofilm and only 1% live in a free or planktonic state, and the bacteria are protected by this matrix, which makes it challenging to remove,

increases their antibiotic resistance, and renders them immune to host defenses. A lot of these things help the organism be able to colonize , evade or subvert the host's defense systems, damage or penetrate host cells, and cause an inflammatory response that leads to clinical disease (24). *Enterococcus faecium* is the Enterococcus virulence traits and likelihood of medication resistance increase the possibility of an epidemic, particularly in hospitals environments, and recent years increase morbidity and mortality due to infections with multi drug resistant (25). *Enterobacter cloacae* its capacity to create biofilms and produce different cytotoxins (pore-forming toxins,

hemolysins, and enterotoxins) are crucial to its pathogenicity (7). *Enterobacter cloacae* in actuality, they have the ability to produce excessive amounts of ampC beta-lactamases by either derepressing a chromosomal gene or acquiring a transferable ampC gene on plasmids that confers antibiotic

tolerance. *Escherichia coli* the main bacterial component of human intestinal infections is found to be biofilms, and the dense bacterial cells in biofilms interact with one another through the quorum sensing chemical signaling pathway (QS).

Table (6): Biofilm forming capacity of (*Streptococcus uberis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterobacter cloacae* complex, *Escherichia coli*) isolates in this study

Isolate	OD630	SD	Result
<i>Streptococcus uberis</i>	0.240	0.030	Moderate
<i>Enterobacter cloacae</i> complex	0.142	0.018	Weak
<i>Enterococcus faecium</i>	0.116	0.023	Weak
<i>Enterococcus faecalis</i>	0.289	0.020	Moderate
<i>Escherichia coli</i>	0.279	0.290	Moderate
Control	0.058	0.005	

*OD and SD represent optical density and standard deviation, respectively.

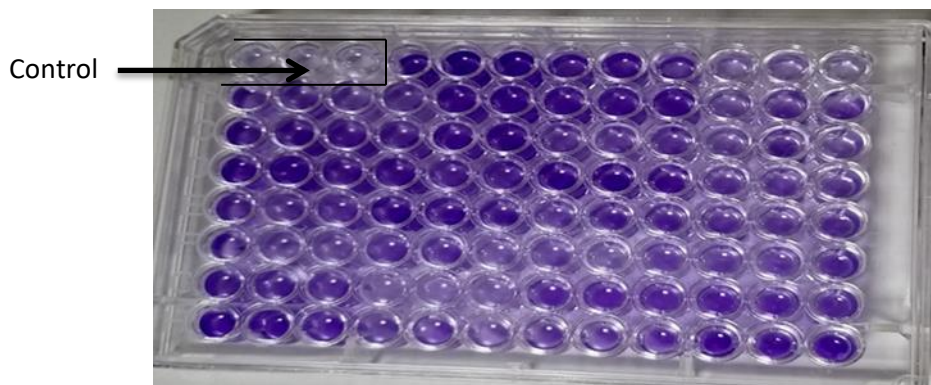


Figure (8): Microtiterplate showing biofilm formation.

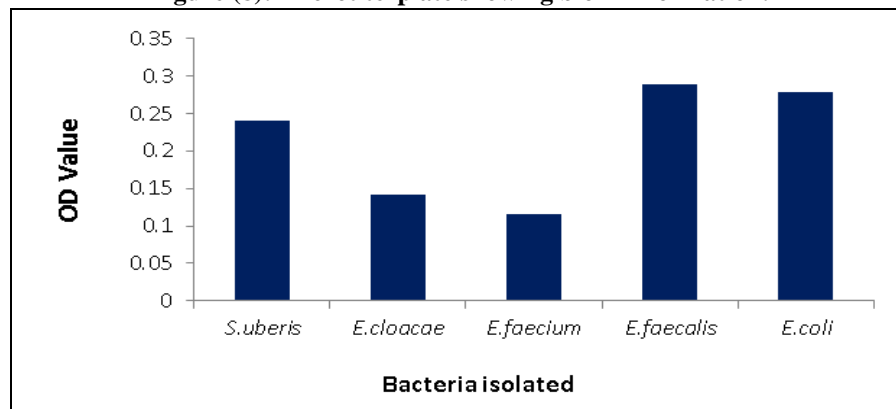


Figure (9): Diagram showing the intensity of Biofilm for isolated bacteria.

Antibiotics susceptibility test (AST) by using 16 different antibiotics agent belong to 11 classes (categories), these results discovered that species *Streptococcus uberis* as Extensive

Drugs Resistance (XDR), while the bacteria species *Enterococcus faecium*, *Enterococcus faecalis*, *Enterobacter cloacae* complex, *Escherichia coli* as Multi Drugs Resistance (MDR), as

shown in (Tables 7,8,9,10 and 11) and (Figures 10, 11, 12, 13 and 14). An important aspects that determines the course of a disease is whether or not its pathogenic etiological agents possesses a virulence factors. Recent research has shown that some strains of *Streptococcus uberis* can form a biofilm structure as early as two hours after incubation, but mature biofilm does not form until 48 hours. The enzymes and toxins produced by bacteria or their capacity to form biofilm help microorganisms survive in infected tissues either directly by impacting host stromal cells or by impacting host defense mechanisms. The ability of bacteria to form biofilms and the use of antibiotics are closely related. Biofilms also shield microorganisms from these agents and from phagocytosis and sanitizers.

Enterococcus faecalis is hypothesized to have a pathogenic role in the development of biofilms on medical devices such intravascular and urinary catheters (5). Ampicillin, Aminoglycosides, and Vancomycin are among the antibiotics that they exhibit high levels of resistance against them.

Enterococcus faecium many nosocomial infections, have been linked to strains, which are challenging to treat because the persistent rise in antibiotic resistance of these microbes and their capacity to create potent biofilms (5). Infections brought on by Vancomycin Resistant *Enterococcus faecium* (VRE) pose the greatest threat. *Enterobacter cloacae* producing strains make up a sizable fraction of clinical bacterial isolates. *blaTEM*, *blaCTX-M*, and *blaSHV* are the three main genes that have been identified as being responsible for this mechanism of resistance to beta lactam antibiotics (penicillins, cephalosporins, and monobactams) (7).

Escherichia coli biofilm makes it difficult for conventional antibiotics to penetrate the cells, decreasing their susceptibility to the antibiotics. *Escherichia coli* appears to be a major factor in the development of CRC cancer in general. Worldwide reports of *Escherichia coli* antimicrobial resistance have been made. The development of resistance to most first line antimicrobial agents has made treating *Escherichia coli* infections more challenging (8).

Table (7): Antibiotic susceptibility test for *Streptococcus uberis* isolates by Vitek-2 system

No.	Antimicrobial	<i>S. uberis</i>
1	Benzylpenicillin	R
2	Oxacillin	R
3	Gentamicin	S
4	Tobramycin	S
5	Levofloxacin	R
6	Moxifloxacin	R
7	Teicoplanin	R
8	Vancomycin	R
9	Erythromycin	R
10	Rifampicin	R
11	Nitrofurantoin	I
12	Sulfamethoxazole\Trimethoprin	R
13	Tetracycline	R
14	Tigecycline	S
15	Fusidic acide	R
16	clindamyin	R

*R= Resistant *I=Intermediate *S=Susceptibl

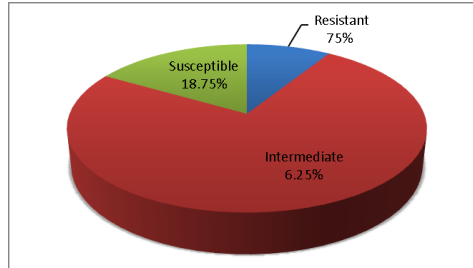


Figure (10): Antibiotic susceptibility test for *Streptococcus uberis* isolates

Table (8): Antibiotic susceptibility test for *Enterococcus faecalis* isolates by Vitek-2 system

No.	Antimicrobial	<i>Enterococcus faecalis</i>
1	Levofloxacin	R
2	Teicoplanin	R
3	Vancomycin	R
4	Erythromycin	R
5	Nitrofuratoin	R
6	Tigecycline	S
7	Tetracycline	R

*R= Resistant *I=Intermediate *S=Susceptible

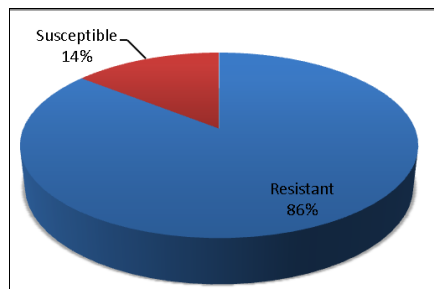


Figure (11): Antibiotic susceptibility test for *Enterococcus faecalis* isolates

Table (9): Antibiotic susceptibility test for *Enterococcus faecium* isolates by Vitek-2 system

No	Antimicrobial	<i>Enterococcus faecium</i>
1	Levofloxacin	R
2	Teicoplanin	R
3	Vancomycin	R
4	Erythromycin	R
5	Nitrofurantonin	R
6	Linezolid	S
7	Tetracycline	S
8	Tigecycline	S

*R= Resistant *I=Intermediate *S=Susceptible

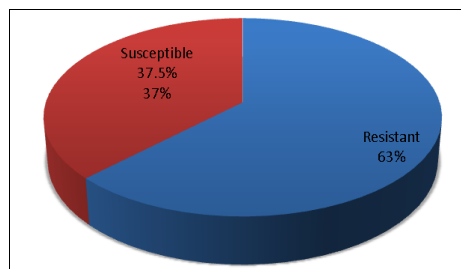
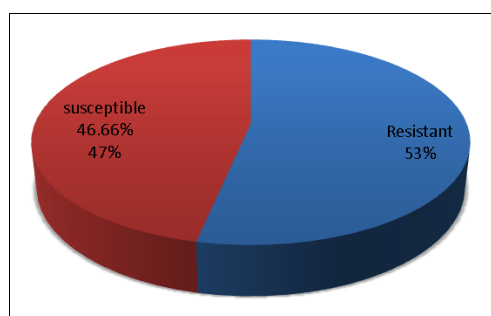


Figure (12): Antibiotic susceptibility test for *Enterococcus faecium* isolates

Table (10): Antibiotic susceptibility test for *Enterobacter Cloacae* isolates by Vitek-2 system

No	Antimicrobial	<i>Enterobacter Cloacae</i>
1	Amoxicillin\ Clavulanic acid	R
2	Piperacillin\Tazobactam	S
3	Cefotaxime	R
4	Cefazidime	R
5	Cefepime	R
6	Ertapenem	S
7	Imipenem	S
8	Meropenem	S
9	Amikacin	S
10	Gentamicin	S
11	Ciprofloxacin	R
12	Norfloxacin	R
13	Nitrofurantoin	R
14	Fosfomycin	S
15	Trimethoprim\Sulfamethoxazole	R

*R= Resistant *I=Intermediate *S=Susceptible

**Figure (13): Antibiotic susceptibility test for *Enterobacter Cloacae* isolates.****Table (11): Antibiotic susceptibility test for *Escherichia coli* isolates by Vitek-2 system**

No.	Antimicrobial	<i>E. coli</i>
1	Ampicillin	R
2	Amoxicilline\ Clavulanic acid	I
3	Piperacilline\Tazobactam	I
4	Cefotaxime	R
5	Ceftazidime	R
6	Cefepime	R
7	Ertapenem	S
8	Imipenem	S
9	Meropenem	S
10	Amikacin	S
11	Gentamicin	S
12	Ciprofloxacin	R
13	Norfloxacin	R
14	Nitrofurantoin	I
15	Fosfomycin	S
16	Trimethoprin\Sulfamethoxazole	R

*R= Resistant *I=Intermediate *S=Susceptible

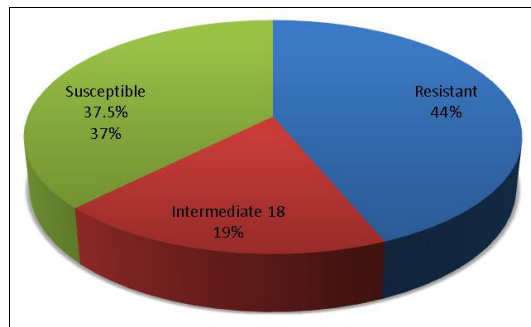


Figure (14): Antibiotic susceptibility test for *Escherichia coli* isolates

Conclusion

Biofilm activity which demonstrated their capacity to form biofilms, three of which isolates were moderate (*Streptococcus uberis*, *Escherichia coli* and *Enterococcus faecalis*), and two isolates were weak (*Enterobacter cloacae* and *Enterococcus faecium*). Antibiotics Susceptibility Test (AST) these results showed that this species *Streptococcus uberis* were considered as Extensive Drugs resistant (XDR) bacteria, and these results showed that this species (*Enterobacter cloacae complex*, *Enterococcus faecium*, *Enterococcus faecalis* and *Escherichia coli*) were considered as a Multi Drugs Resistant (MDR) bacteria.

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