

# Study the Bacterial Activity Isolated from Colon and Rectal Cancer Biopsy in Cell Lines Culture

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Colorectal cancer (CRC) is the most common disease and cause of death globally. The aim 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, considers as control group. Isolation and identification of bacteria in all collected specimens were done by using cultural and differential media (blood agar, macconkey agar and Pfizer agar), as well as 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 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 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%). Cell-line culture techniques for the five species showed a cellular viability, sequentially *Streptococcus uberis* (16.12%), *Enterococcus faecium* (16.39%), *Enteococcus faecalis* (9.48%), *Enterobacter cloacae* (15.11%) and *Escherichia coli* (17.61%). The results statistically studied by using SPSS, which showed excellent or (highly) significant (p-value is in the range of  $\leq 0.001$ ).

**Keywords:** Colorectal cancer, Cell line culture.

The third most common and second-leading cause of death common type of cancer is colorectal cancer (CRC) prevalent cancer,<sup>1</sup> its occurrence is expanding at such a rapid rate that it is becoming by far common and important health

problem on a global level. Around 4% to 5% of people will acquire colorectal cancer, and various risk factors, such as diet (little fiber and excessive red meat), smoking, obesity, drinking, diabetes, and lifestyle, increase the likelihood of getting the

disease and have been connected to the start and creation of CRC. <sup>2</sup> <sup>3</sup> The human digestive system, particularly one's colon, is home to a diverse bacteria population that counts between  $10^{13}$ - $10^{14}$  bacteria. <sup>t</sup> Because dysbiosis situations can result in colonic carcinogenesis by means of a chronic inflammatory process, the gut microbiota is crucial in this situation. A multitude of factors, including nutrition, antibiotics, and stress, can contribute to dysbiosis. <sup>u</sup> Dysbiosis can alter immune responses, damage the intestinal barrier, and change metabolism, which can result in disease. By host DNA damage, the creation and maintenance of an inflammatory milieu, and interference with cellular immunological reaction, the bacteria in gut primarily influences the occurrence and progression of cancer. The development of cell culture had a profound impact on the field of life sciences and greatly advanced medical research. Utilizing cell lines in research is a crucial step in the modeling of diseases, the study of cancer, and the development of treatments. <sup>v</sup> Primary cancer cell lines are tissue sample derived *ex vivo* cell populations that have been surgically removed; these tissue samples are typically taken from autopsy tissues, resections, pleural effusions, fine needle aspirates, core biopsies, <sup>w</sup> <sup>x</sup> And since these samples capture the organic milieu of the tumor and maintain the distinctive interplay between cancerous and healthy cells. These interpersonal behaviors play a role in how cancer develops, spreads, and metastasizes as well as how the body reacts to treatments. <sup>x</sup> <sup>y</sup> In order to support the growth of rat and human pluripotent stem cells, rat embryonic fibroblasts (REF) are used. REF not only provide a substrate for the growth of pluripotent stem cells, but they also secrete vital growth factors that help to preserve stem cell pluripotency. Early passages use REF that have been isolated from rat embryos. <sup>p</sup> REF must be irradiated or given mitomycin C to stop cell growth to fulfill the role of feeder cells. Moreover, the modified conditioned media can be created by cell for the development of feeder free pluripotent stem cells.

## 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, 50 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 (colonoscopy) 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! 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

### Supernatant extracts preparations

#### Preparation of bacterial crude supernatant<sup>11</sup>

5 mL of BHI were used to make a bacterial suspension, which was then incubated at 37! for 24 hours. 50mL of LB broth (Luria Bertani) was inoculated with a 200 $\mu$ L rate of the suspension, and it was then incubated for 48 hours at 31!  $\pm$ 1 with 200 rpm of shaker agitation. For bacterial cell sedimentation, the resulting broth was centrifuged at 10,000g for 4 minutes. The supernatant was then separated from the precipitated and filtered at 0.22 $\mu$ m. In order to employ the supernatant in the cell-line cultures later, it was placed in freezer at -20!.

#### Cell line culture assay

The procedure was done according to Cell line culture method described by <sup>12</sup>. The test was achieved in Al- Nahrain university\ Biotechnology research center, under a supervision of the laboratory staff.

1- Cell line culture of (REF) were prepared to the experiment by thawing separately in a water-path at 37!.

2- The cells were placed in a 25 cm<sup>2</sup> diameter animal cell culture vessel.

3- Incubation of the container containing REF cell lines in an incubator of 5% CO<sub>2</sub> at a temperature of 37! for 24 hours, after a day of incubation, and

when ensuring that there is growth in cell cultures and that they are clean from contamination, secondary cultures were conducted.

4- An inverted microscope was used to inspect the cells to make sure they were healthy, clean, and had multiplied to the necessary amount (10,000-10,000,000) cells/μL.

5- Cells were transferred to the container and the used cultural medium was discarded.

6- The cells were given enough trypsin enzyme (2–2.5 μL), which they then incubated with for 30–60 seconds at room temperature 22-26! until they had detached from the flask, and then monitored until it changed from a single cell layer to single cells. Cells were further dispensed by pipetting in the growth medium.

7- Cells were distributed from (10.000-10.000.000) in each one wall of sterile 96-well microtitration plate.

8- A volume of 200 μL of Rpmi media (Roswell park memorial institute), were added to serial flat-bottom 96-wall polystyrene microtiter- plate.

9- A volume of 20μL of a prepared bacterial supernatant, was added to three wells of sterile flat bottomed 96-well polystyrene microtiter- plate. A total of six wells with Rpmi media were considered as control (without bacterial supernatant).

10-The plates were covered with their parafilm and incubated of 5% Co2 at temperature of 37! for 24 hours, after a day of incubation all plates were gently washed thrice with PBS and dried.

**The MTT assay.<sup>13</sup>**

The assay measures the percentage of cellular viability depending on the colorimetric variation occurred in the cell-line culture. The procedure was done as follow.

1-A volume of 10μL of MTT indicator was added to the whole tested microtiter-plate of cell-line culture, and then the plate was supernatant (*Escherichia coli*, *Streptococcus uberis*, *Enterobacter cloacae*, *Enterococcus faecium*, *Enterococcus faecalis*).

2-The optical absorbance valves were carried out at a wavelength of 620nm. Using the ELISA reader device (Bio-rad Germany).

3-All resultants data of the optical density valves were analyzed statistically by using SPSS (Statistical Package for the Social Science) (version 29, IBM, USA.2023).

4-The cellular viability percentage of the test calculated according to the following equation.<sup>14</sup>

$$\text{Cellular Viability \%} = \frac{\text{mean of OD sampl}}{\text{mean of OD contri}} \times 100\%$$

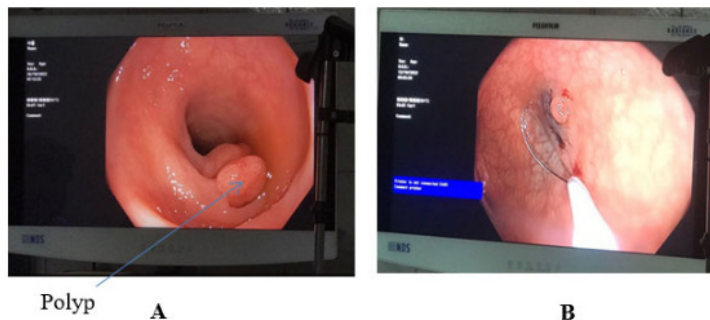


Fig. 1. A, B. Colonrectal cancer polyp



Fig. 2. After excision of polyp

**RESULTS**

**Bacterial identification**

The identification of bacteria isolated from total specimens of CRC patients as well as healthy volunteers (control group), showed numerous different bacterial species with different percentage. The bacterial diversity in CRC patients distributed as shown in Table 1, while in control group as shown in Table 2.

The notice cultural characteristics isolated from colorectal cancer and polyp on blood agar media, macconkey agar, mannitol salt agar and Pfizer agar, has been shown fermenter lactose on macconkey agar 36(46,75%), non-fermenter lactose on macconkey agar 4(5.19%), a hemolytic on blood agar 5(6.49%), a hemolytic on blood agar 4(5.19%), a hemolytic on blood agar 1(0.77%), colony color from dark brown to black on Pfizer agar 2(2.59%), as shown in Table 3.

**Table 1.** Types of bacterial isolates in colorectal 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 thoralensis</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 morgani</i>	1(1.29%)
20	<i>Pseudomonas aeruginosa</i>	1(1.29%)
21	<i>Klebsiella pneumonia</i>	2(2.59%)
	Total	77

**Table 2.** 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 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%), a hemolytic on blood agar 1(1.72%), as shown Table 4.

**Bacterial selection**

The results showed that among different bacterial species diagnosed in this study, the bacteria (*Streptococcus uberis*, *Escherichia coli*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Enterococcus faecium*), were the most highly frequents in the specimens group of CRC patients, their percentages were (*Streptococcus uberis* 6(7.79%), *Escherichia coli* 30(38.96%), *Enterococcus faecalis* 4(5.19%), *Enterobacter cloacae* 4(5.19%) and *Enterococcus faecium* 5(6.49%)) respectively. In addition the species (*Streptococcus uberis*, *Escherichia coli*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Enterococcus faecium*) were not found among species diagnosed in the control group. These species were selected as a chosen bacteria to be study their effectiveness in the cell-line culture technique.

**Cell-line cultures viability**

The study clarified and revealed the actions and impacts of the bacterial supernatants extracts as an inhibition factors of cellular growth on REF cell-line. The results data of the cellular viability percentage showed, for *Streptococcus uberis* (16.12%), *Enterobacter cloacae* (15.11%), *Enterococcus faecium* (16.39 %), *Entreococcus faecalis* (9.48%) and *Escherichia coli* (17.61%), as shown in Table 5 and Figure 3 showed the

**Table 3.** Characteristics in cultures of colorectal cancer and polyps

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

cellular viability. Figures 5,6,7,8 and 9 showed the impacts of bacterial supernatant extracts on cell-line cultures comparable with Figure 4, which represents the control state.

#### Statistical analysis

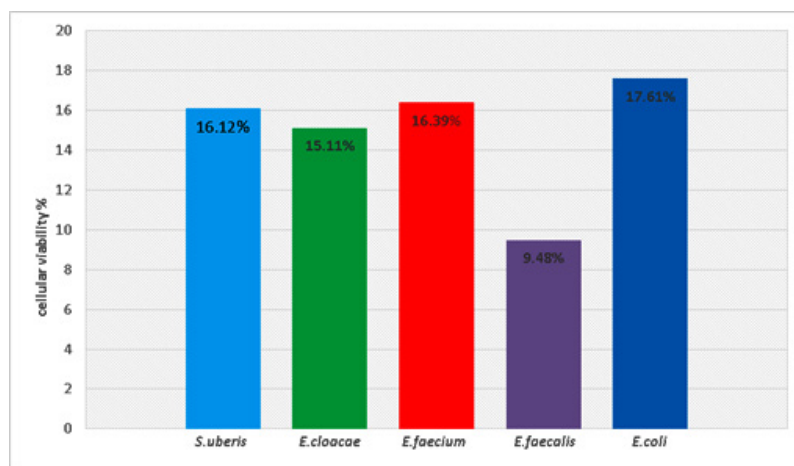
Statistically the P-values recorded in this study to the whole bacterial species in comparable with control were  $\leq 0.001$  which referred to a state of excellent or highly significant, as shown in Table 6.

**Table 4.** 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%)
$\beta$ hemolytic on blood agar	1(1.72%)

**Table 5.** Cellular viability on REF cell line culture for five isolates (*Streptococcus uberis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterobacter cloacae complex*, *Escherichia coli*)

NO	Bacterial species	Cellular viability %
1	<i>Streptococcus uberis</i>	16.12%
2	<i>Enterobacter cloacae complex</i>	15.11%
3	<i>Enterococcus faecium</i>	16.39%
4	<i>Enterococcus faecalis</i>	9.48%
5	<i>Escherichia coli</i>	17.61%



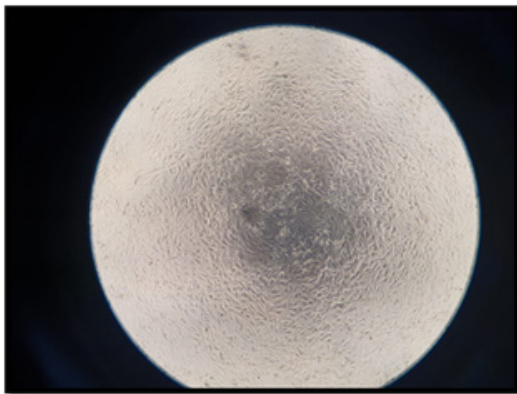
**Fig. 3.** Cellular viability percentage on REF cell line culture for five isolates (*Streptococcus uberis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterobacter cloacae complex*, *Escherichia coli*)

## DISCUSSION

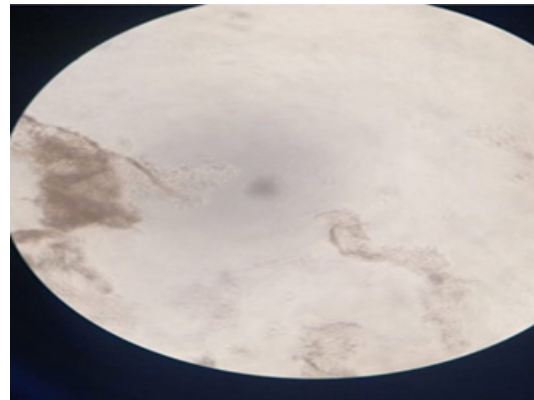
*Streptococcus uberis* was showed the cellular viability percentage on REF cell line culture (16.12%), Phenotypic studies the *Streptococcus uberis* have made it possible to identify and characterize potential virulence factors in *S. uberis* strains, including the capsule, plasminogen activating factor, uberis factor, M- and R-like proteins, neutrophilic toxin, hyaluronidase, and extracellular matrix binding proteins. However, it is understood that environmental cues may influence how bacteria express their virulence factors.<sup>1u</sup> Briefly, virulence factors includes: plasminogen activator proteins such as PauA and PauB and SK, resistance to phagocytosis presented by a hyaluronic acid capsule, CAMP factor, a surface dehydrogenase protein GapC, sortases, Opp proteins implicated in dynamic transport of solutes, lactoferrin binding proteins, and

adherence and invasion of epithelial cells mediated by *S.uberis* adhesion Molecule (SUAM).<sup>1v</sup> By acting on the proteins of the extracellular matrix, plasmin, which is created when plasminogen is activated, grants access to deep tissues. The first plasminogen activator identified in *S. uberis* strains that could activate bovine plasminogen was PauA activator, which had a molecular weight of about 30 KDa. *Escherichia coli* was showed the cellular viability percentage on REF cell line culture (17.61%). Cyclomodulins are toxins made by pathogenic *E. coli* strains, and they include cytotoxic necrotizing factor (CNF), cycle inhibiting factor (Cif), colibactin, and cytolethal distending toxins (CDTs). A toxin called colibactin is created by the enzyme polyketide synthetase (pks).<sup>1w</sup> Due to their capacity to affect cellular differentiation, apoptosis, and cell proliferation by interfering

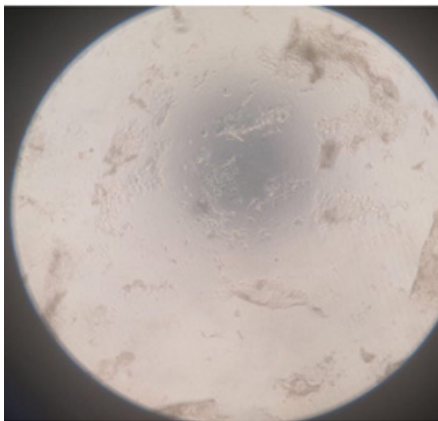
with the eukaryotic cell cycle and/or encouraging DNA damage, cyclomodulins are gaining more and more attention.<sup>1x</sup> *Enterococcus faecalis* was showed the cellular viability percentage on REF cell line culture (9.48%). It has been demonstrated that *E. faecalis* colonizes the digestive system by creating bacterial biofilms.<sup>1y</sup> Enhance the generation of ROS, which in turn induce colonic epithelial DNA double strand breaks aneuploidy and tetraploidy and extracellular superoxide, which stops the cell cycle,<sup>2p</sup> and chromosomal instability, may be linked to a local inflammatory response, alterations in the mucosa's nutrient compositions. Reactive oxygen species (ROS) produce by *E. faecalis* harm colon epithelial DNA and participate in the development of adenomatous polyp and.<sup>2p</sup> {<sup>21</sup> *Enterococcus faecium* was showed the cellular viability percentage on REF cell line



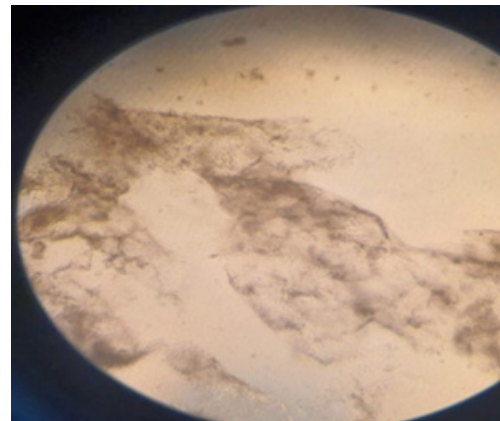
**Fig. 4.** Rat embryo fibroblast (REF) Cell-line (control state)



**Fig. 5.** *Streptococcus uberis* impact on REF cell- line



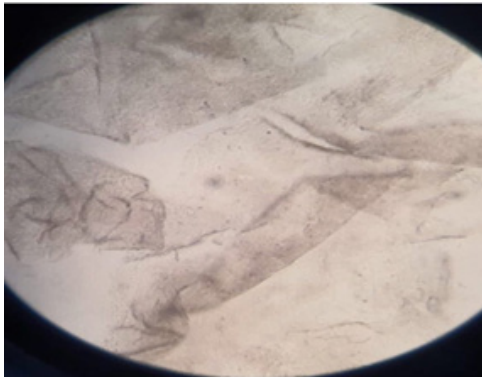
**Fig. 6.** *Enterobacter cloacae* impact On REF cell-line



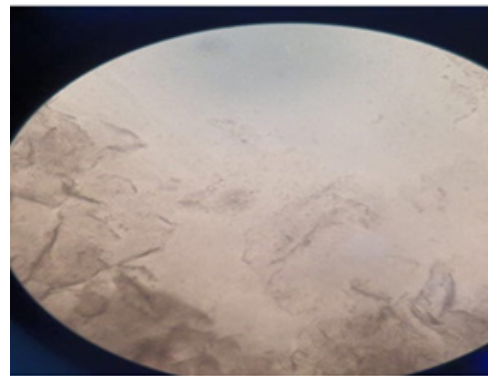
**Fig. 7.** *Enterococcus faecium* impact on REF cell-line

culture (16.39%). A study showed that hydrogen peroxide is produced by *E. faecium* at quantities that harm cells. Oxygen radicals' ability to disrupt membranes may make surrounding intestinal

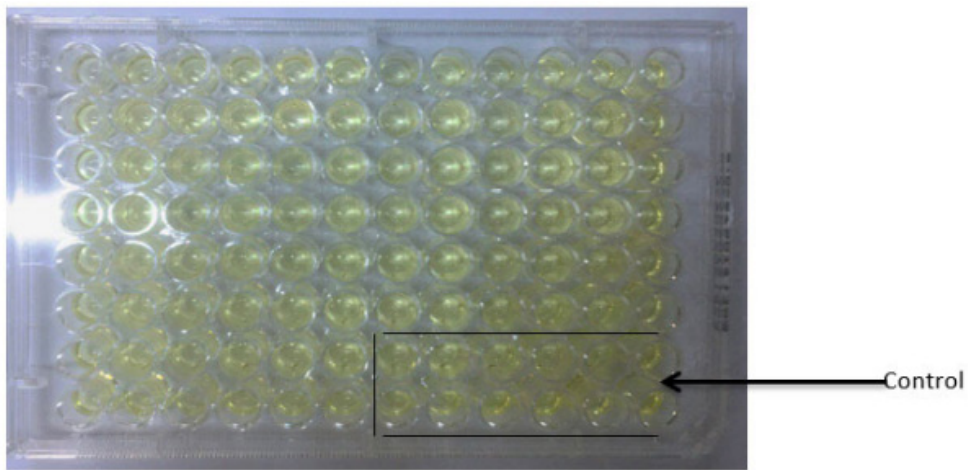
epithelial cells more susceptible to cellular harm, according to research on *E. faecium* transposon insertion mutants. Evidence suggests that the DNA of colonic epithelial cells is harmed by the



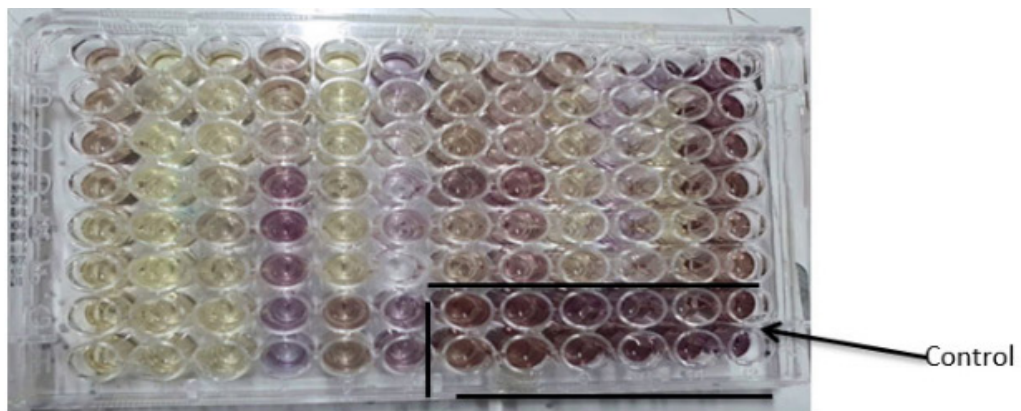
**Fig. 8.** *Enterococcus faecalis* impact On REF cell-line



**Fig. 9.** *Escherichia coli* impact on REF cell- line



**Fig. 10.** A microtiter- plate of REF cell line culture and five isolates after added MTT indicator before incubation



**Fig. 11.** Metabolism of MTT to a formazan salt by viable cells, MTT indicator after incubation

**Table 6.** Statistical analysis for five isolates (*Streptococcus uberis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterobacter cloacae* complex, *Escherichia coli*) comparable with control

	Paired Sample Test									
	Mean	Std Deviation	Std Error	Paired Difference Significance		t	dt	One-Sided p	Two-Sided p	
				Lower	Upper					
Pair1 Positive control- <i>Streptococcus uberis</i>	412667	008622	004978	391249	434084	82.903	2	<.001	<.001	
Pair2 Positive control- <i>Enterobacter cloacae</i>	417667	005859	003383	403111	432222	123.462	2	<.001	<.001	
Pair3 Positive control- <i>Enterococcus faecium</i>	411333	010116	005840	386204	436463	70.428	2	<.001	<.001	
Pair4 Positive control- <i>Enterococcus faecalis</i>	445333	020207	011667	395136	495531	38.171	2	<.001	<.001	
Pair5 Positive control- <i>Escherichia coli</i>	405333	012014	006936	375489	435177	58.437	2	<.001	<.001	



formation of extracellular superoxide and hydrogen peroxide.<sup>22</sup> *Enterobacter cloacae* was showed the cellular viability percentage on REF cell line culture (15.11%). *Enterobacter cloacae* its capacity the release of different cytotoxins including pore forming thiol- activated cytotoxins similar to the shiga like toxin a!,<sup>23</sup> hemolysins, and enterotoxins are crucial for its pathogenicity.<sup>24</sup> The colorimetric technique called the MTT assay is used to measure the metabolic activity of cells and to determine how many viable cells are present, as shown in Figure 10, 11.

Cell line culture are helpful model systems for researching typical cellular biochemical and physiological processes, and they can even be employed for diagnosis. The uniformity and reproducibility attained while employing cell culture systems is the key benefit. Cell culture essentially involves the multiplication and survival of cells in a lab setting. Cell growth is used in a variety of frequently occurring contexts, and Cell culture is usually thought of as a method by which cells are grown under controlled circumstances outside of a living organism (e.g., temperature, pH, nutrient, and waste levels),<sup>25</sup> the cells being investigated in the cell culture can be easily controlled when the traits and variables of cellular differentiation are known. A typical circumstance that is offered by the internal cellular program and external factors is cell differentiation.<sup>26</sup> In order to support the development of rat and human pluripotent stem cells, rat embryonic fibroblasts (REF) are used. REF not only provide a substrate for the growth of pluripotent stem cells, but they also secrete vital growth factors that help to preserve stem cell pluripotency. Early stages use REF that have been isolated from rat embryos,<sup>27</sup> REF must be irradiated or given mitomycin C to stop cell growth in order to function as feeder cells. Additionally, to prepare conditioned medium for feeder free pluripotent stem cell development, the treated cells can be employed. A fibroblast is the main functional cell in fibrous tissue. These cells are renowned for producing a lot of extracellular matrix (ECM) proteins, including collagen, glycosaminoglycans, and proteoglycans. They are irregularly shaped and adherent. By themselves, this elevates them to crucial status in numerous cell culture procedures, reasons using fibroblasts in cell culture because fibroblasts are simple to cultivate,

and fibroblasts foster an ideal environment for development. Fibroblasts produce circumstances that are favorable for other cell types to grow in addition to their own by changing the properties of the environment around them. Indeed, it has been demonstrated that growing fibroblasts releases substances into the medium for cell culture that encourage development,<sup>28</sup> and fibroblasts offer a model system for many illnesses and ailments it is known that fibroblasts act as agents of inflammation, disease related inflammation, and the evolution of cancer.<sup>29</sup> The MTT assay is used to determine the cell viability and measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials is done by counting viable cells after staining with a vital dye, yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole).<sup>31</sup> The MTT assay measures colorimetrically the metabolic activity of cells. Under specific circumstances, the activity of NAD (P) H-dependent cellular oxidoreductase enzymes may indicate the presence of live cells. These enzymes have the ability to decrease the tetrazolium dye. In the mitochondria of live cells, MTT, or 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, is converted to purple formazan, when the amount of purple formazan produced by cells treated with an agent is compared to the amount of formazan produced by untreated control cells, conversion can be directly related to the number of viable (living) cells because this reduction only occurs when mitochondrial reductase enzymes are active. By creating a dose response curve, it is possible to determine how effectively the substance kills cells. MTT solutions that have been dissolved in balanced salt solutions or tissue culture media are yellowish in hue and devoid of phenol red. Viable cells mitochondrial dehydrogenases split the tetrazolium ring, releasing purple MTT formazan crystals that are insoluble in water. The physiological condition of cells and variations in mitochondrial dehydrogenase activity in various cell types can influence the limitations of the MTT technique. MTT tests are typically carried out in the dark because the MTT reagent is light sensitive. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli,

growth factors, and other reagents that promote cell growth, cytotoxicity studies, and the derivation of cell growth curves.

### CONCLUSIONS

The cell line culture study revealed a reduction in the cellular growth viability on REF cell line culture as a result of supernatants activity extracted from (*Escherichia coli*, *Streptococcus uberis*, *Enterobacter cloacae* complex, *Enterococcus faecalis* and *Enterococcus faecium*), respectively. There are changes in morphology shape cells, Irregular shape disruptions and damage cells line REF and decrease in cells number because of the supernatant toxic effects for the isolated bacteria. The results showed the cellular viability percentage were for *Streptococcus uberis* (16.12%), *Enterococcus faecium* (16.39%), *Enterococcus faecalis* (9.48%), *Enterobacter cloacae* (15.11%) and *Escherichia coli* (17.61%), respectively. These results indicated the possible important role of these bacteria in developing the colon rectal cancer.

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#### Conflict of interest

Conflict of interest was collage of science, University of Baghdad, Baghdad, Iraq.

#### Funding of Interest

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