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Research Article

Contamination of Agricultural Soils in Some Baghdad Areas with Antibiotics Resistant Pathogenic Fecal Bacteria

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Abstract:

Background: Early studies have shown that agricultural soil contains various types of microorganisms, especially bacteria, including coliform bacteria (Salmonella, Shigella, Klebsiella, *Escherichia coli*, and Enterobacter) with fecal Gram-positive bacteria like *Enterococcus faecalis*. Therefore, the current study aimed to investigate the contamination of Iraqi agricultural soils with pathogenic fecal bacteria (*Escherichia coli* and *Enterococcus faecalis*) and study the antibiotic sensitivity patterns of soil-isolated bacteria because it is a dangerous indicator when transmitted to humans.

Methods: Soil samples were collected from six locations (farms) in the capital, Baghdad, which were: AL-Jadria, AL-Latifia, Diyala River, AL-Jazera, and AL-Zafraniya (block 1 and block 2) during the study period from the end of November 2021 to August 2022; then were compared with the control samples (house garden). These bacteria were isolated by selective culture media and identified using the VITEK® 2 Compact system, and antibiotic sensitivity tests were carried out against 18 different antibiotics by the Kirby Power method. The t-test was used for the statistical analysis.

Results: The bacteriological study of agricultural soil showed the presence of fecal bacteria, and this is evidence of contamination of agricultural soil samples with these bacteria. The highest *E. coli* count was in the AL-Latifia farm (1. 48× 10^3), while the highest *E. faecalis* count was in the Diyala River farm (2.63 × 10^3). The antibiotic sensitivity profile illustrated that *E. coli* was resistant to ampicillin, ceftriaxone, cefoxitin, piperacillin, ceftazidime, and Teicoplanin but was sensitive to the rest of the antibiotics used, while *E. faecalis* was only resistant to levofloxacin and linezolid and highly sensitive to the other tested antibiotics.

Conclusion: The current study documented the presence of fecal coliform bacteria in studied soil samples, with markedly high resistance rates toward used antibiotics. These facts might be the result of irrigation with sewage water and the use of organic fertilizers.

Keywords: Agricultural soil; Antibiotic-resistant bacteria; *Enterococcus faecalis*; *Escherichia coli*; Fecal bacteria.

Introduction:

The soil contains pollution on the environment is the greatest problem. It is being contaminated in a wide range of ways. To maintain soil fertility and improve productivity, it is vital to prevent soil contamination (1). Usually, the majority of pollutants come from the production of something important and are released into the environment as trash, sewage, or accidentally; As a result, our soil, water, and other essential natural resources are being contaminated (2).

The basis of agriculture is soil, and soil is the basis for agricultural productivity, animal life, and the growth and life of plants (3). It is necessary for all crops grown produce food and for feeding animals. This natural resource is partially being lost due to increased pollution. However, the large amounts of man-made garbage, sewage, and other products from modern waste treatment plants, even polluted water, are also helping to or causing soil pollution (4). The health of all living organisms would be improved by taking rigorous control measures to preserve the fertility and productivity of the soil (5). The use of organic wastes as fertilizer on agricultural land, feces getting into irrigation water supplies, cattle, wild animals, and birds directly contaminating crops, and post-harvest problems including worker hygiene are just a few of the many problems sources (3).

*Escherichia coli a*re bacteria found in the intestines of people and animals and in the environment; they can also be found in food and untreated water, causing diarrhea and food poisoning (6). *E.* coli and other productive agricultural fecal contamination bacteria (FIB) can be employed as microbial surrogates for monitoring the quality of water as they serve as indicators of the occurrence of animal feces from warm-blooded animals. These bacterial

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species are native microorganisms that live in warm-blooded animals' intestines, and their presence in fresh and saltwater environments suggests the existence of pathogenic bacteria (7). There are four established indicators of faecal pollution: total coliform, fecal coliform, *E. coli*, and enterococcus. The test for total and fecal coliforms can also identify thermotolerant nonfecal coliform bacteria, therefore *E. coli* is thought to be a more reliable fecal indicator bacterium than total and fecal coliforms (1). Fecal coliforms (FC) could produce gas from lactose at 44.5 °C, which allows for their detection (8). A most typical FC is *Escherichia coli*, and although most of *the Escherichia coli (E. coli)* strains aren't really dangerous to humans, others, such as *E. coli O157:H7*, could (9).

Streptococcus faecalis (*Enterococcus faecalis*), *Streptococcus bovis*, *Streptococcus equinus*, and *Streptococcus avium* all are members of the subgroup classified as fecal streptococci species. (10, 11). These species can be used as indicator organisms, so they are prevalent in the digestive systems of warm-blooded animals like humans. Fecal streptococci species are recognized as indicator organisms because they can survive for a long period while not growing and reproducing in water and other environmental systems (12).

In a case study done by (13), Streptococci and other bacterial indicators were used to evaluate how well the soil groundwater treatment removed microbial contaminations (13). In a different study, lactobacilli, coliforms, streptococci, and other indicator bacteria were evaluated to see how anaerobic digestion impacted the indicator microorganisms in swine and dairy animal waste (14). In fact, for microorganisms in the soil to reach water, transport mechanisms should consider variables like variations in water flow and cell motility; as a result, the movement of the bacteria depends on the distance moved by the water, whether it be infiltration or surface runoff (4). Because of the contamination of agricultural soils by pollutants such as sewage, and human and animal waste, which contain pathogenic bacteria, which are considered a dangerous indicator when transmitted to humans, the current study aimed to isolate some pathogenic fecal bacteria (*Escherchia coli* and *Enterococcus faecalis*) from soil samples and study the pattern of antibiotic sensitivity of the isolated bacteria.

Materials and Methods

Collection of soil samples

This study was conducted in the capitol Baghdad, for six locations in the city of Baghdad, which include: (Al-Jadriya, Al-Jazera, Al-Latifya, Jsr-Dyala, Al-Zufrania sada1, Al-Zufrania block 1 and block 2) for the period from November 2021 until August 2022. These soil samples were collected from a depth of 5 cm using the square method in sterile bags, they were taken directly to the laboratory for bacteriological analysis and estimation of bacterial counts.

Counting of bacteria in soil samples

The dilution method was carried out by weighing 10 g of the studied soil sample, and 90 milliliters of distilled water (D.W.) were added to it and mixed in the blender for one minute on high speed, then several dilutions were made of it using sterile distilled water. Enumeration of bacteria was carried out by the pour plating technique according to (15). This was done by inoculating 1 ml tenfold serially diluted samples onto Nutrient Agar (aerobic bacteria), Eosin Methylene Blue Agar *(E. coli*), and Enterococcus Selective Agar (*E. faecalis*) with three replicates for each dilution to reduce errors that resulted from conducting the experiment. The inoculated plates were incubated at 37 ºC. for 24 hours, then the observed bacterial colonies were counted and expressed as colony-forming units per gram (CFU/gm.). The number of bacterial cells in the soil sample was determined from the equation (15):

No. of bacterial cells /1gm soil =No. of colonies × inverted dilution

Identification of bacteria

It was done by Vitek -2 kit (Bio mereux –France) by using Vitek-2 compact system for identification of isolated bacteria with cards of (GN: id- N291) for Gram-negative bacteria, and (GN: id- GPS67) for gram-positive bacteria.

Antibiotics sensitivity test

Antibiotic discs were used as kirby-bauer method for antibiotic sensitivity profile (16). They were: Amikacin (AMK -30 mg), Gentamicin (GA-10mg), Imipenem (IMP- 5mg), Trimethoprim /Sulfamethoxazole (SXT-25mg), Ampicillin (AMP-10mg), Tetracycline (TET-30mg), Ceftazidime (CAZ-30mg), Cefoxitin (FOX-30mg), Ceftriaxone (CRO-30mg), Levofloxacin (LEV-5mg), Tobramycin (TOB-30mg), Piperacillin (PIP-50mg), Tigecycline (TGC-15mg), Nitrofurantoin (F-100mg), Teicoplanien (TEC-30mg), Vancomycin (VA-30mg), Meropenem (MEM-30mg), Linezolid (LNZ-100mg). Muller-Hinton agar dishes were incubated at 37 ^{0}C for 24 hours, and then the comparison between inhibition zones with CLSI index to evaluate sensitivity and resistance towards used antibiotic discs (16).

Statistical analysis

The unpaired *t*-test was used for the statistical analysis, and normal tests were used. Analysis of variance (ANOVA) and Post Ho Caparisons were used to determine if there were significant differences in the concentration of microbial number in each test that was analyzed. A significant level of (0.05) was employed (If a P-value is less than 0.05, that means that the result is statistically significant. If a P-value is greater than 0.05, then the result is insignificant.).

Results

Isolation and identification of soil bacteria

Morphological characteristics and Gram-stain with some features of specific culture media were performed to identify isolated bacteria. The Gram-negative *E. coli* on Eosin methylene blue agar (EMB) appeared as large, blue-black colonies, often with a green metallic sheen, and were lactose fermenter on MacConkey agar, with no blood hemolysis on blood agar, while *E. faecalis* was isolated by Enterococcus selective agar and Bile Aesculin Azide Agar. To ensure bacterial identification, some biochemical tests were applied **(Table 1)** besides Vitek-2 cards of both Gram-positive and Gram-negative were also performed as 64 biochemical tests were highly confident and reached 95-99% of diagnosis probability.

Characteristics	Escherichia coli	Enterococcus faecalis
Gram staining	Negative	Positive
Shape (Cocci/Diplococci/Rods)	Rods	Cocci
Indole	Positive (+ve)	Negative (-ve)
Methyl Red (MR) test	Positive (+ve)	-
Voges Proskauer (VP)test	Negative (-ve)	Positive (+ve)
Citrate	Negative (-ve)	Negative (-ve)
Hemolysis (Alfa/Beta/Gamma)	Some Strains shows Hemolysis	Variable (Alfa or Beta)
Urease	Negative (-ve)	Negative (-ve)
Catalase	Positive (+ve)	Negative (-ve)
Oxidase	Negative (-ve)	Negative (-ve)
Nitrate reduction	Positive (+ve)	Positive (+ve)

Counting of soil bacteria according to the studied area

The viable count method as previously mentioned was achieved to count bacteria in the soil sample as well as in garden soil as the control sample. The counts are shown in Table (2).

Table (2): The average viable count of aerobic bacteria, *Escherichia coli*, and *Enterococcus faecalis.*

Location	* The average viable count (CFU/gm.)		
	Escherichia coli count	*Enterococcus faecalis count*	Total viable count (Aerobic bacteria)
Control (House garden)	1.78×102	1.94×102	9.11×105
AL-Jadria farm	2.91× 102	2.24 × 103	7.23× 106
AL-Latifia farm	1. 48× 103	1.76× 103	1.92 ×107
Diyala River farm	1.39 × 103	2.63 × 103	1.85 × 107
AL-Jazera farm	2.73 × 102	1.15 × 103	4.25 ×106
AL-Zafraniya farm 1	9.11 × 102	1.57 × 103	1.29 × 107
AL-Zafraniya farm 2	3.34 × 102	4.92 × 102	9.69 × 106

One way (ANOVA) analysis of variance showed significant differences with statistical significance, as the P value was < 0.05 in relation to the number of bacterial colonies between sites.

It is concluded that the soil samples which were taken from different regions of Baghdad contained different numbers of *E. coli* and *E. faecalis* that are excreted with human and animal feces, and the most contaminated sites were Al-Ltifia and Diyala river farm (1.48×10^3 and 1.39×10^3) CFU/gm. for *E. coli* respectively. For the *E .faecalis* the results were (1.76×10^3 and 2.63×10^3) CFU/gm. respectively.

Antibiotic sensitivity profile

The antibiotic sensitivity test (AST) conferred for all *E. coli* and *E. faecalis* obtained isolates (45 isolates) was determined by Kirby- Bauer method (the disc diffusion method) (16) using the above-mentioned commonly prescribed antibiotics namely, in accordance with the National Council for Clinical Laboratory Standards' recommendations for the Kirby-Bauer method and the Vitek-2 system verified the results. These 45 isolates were resistant to most of the antibiotics used in different proportions (Table 3), and among them, there were 11 (24.4%) isolates that are multidrug-resistant isolates (resistant to at least three of the antibiotic groups employed in the study), as shown in Tables (4 and 5).

Table(3): Antibiotic susceptibility pattern distribution of the 21 *E. coli* isolates and 24 of *E. faecalis* by location.

Location	*Escherichia coli*		*Enterococcus faecalis*	
	No. (%) of isolates	No. (%) of resistant isolates (1 to >3 antibiotic categories)	No. (%) of isolates	No. (%) of resistant isolates (1 to >3 antibiotic categories)
Control	2	0(0)	3	1(33.33)
1	2	1(50)	3	0(0)
2	4	2(50)	4	3(75)
3	4	1(25)	4	1(25)
4	3	0(0)	3	1(33.33)
5	3	1(33.33)	4	1(25)
6	3	0(0)	3	1(33.33)
Total	21	5(23.80)	24	8(33.33)

The results obtained by the Vitek-2 system of *E. coli* showed resistance to Levofloxacin, Tetracycline, Trimethoprim Sulfamethoxazole, Ampicillin/Sulbactam at percentage (95.2-100.0%), and the isolates were sensitive to Piperacillin / Tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Meropenem, Amikacin, Gentamicin, Tobramycin, Tigecycline, and Nitrofurantoin with a percentage (of 76.2-100.0%) as shown in Table (4). While the results showed that *E. faecalis* was resistant to Levofloxacin, Erythromycin, Tetracycline, and Tigecycline with a percentage (of 58.3-100.0%). The isolates were sensitive to Nitrofurantoin, Linezolid, Teicoplanin, and Vancomycin, and only ten isolates

were sensitive to Tigecycline with a percentage (of 41.6-100.0%) as shown in Table (5).

Table (4): Antibiotics susceptibility test for 21 Escherichia coli isolates by Vitek-2 system.

Antibiotics	*Escherichia coli*			P-value
	Resistance (%)	Intermediate (%)	Sensitive %	
Ampicillin/ Sulbactam	20 (95.2)	0 (0.0)	1 (4.7)	8×10^{-4} ***
Piperacillin/ Tazobactam	3 (14.2)	0 (0.0)	18 (85.7)	6×10^{-3} **
Cefazolin	3 (14.2)	0 (0.0)	18 (85.7)	6×10^{-3} **
Cefoxitin	1 (4.7)	0 (0.0)	20 (95.2)	8×10^{-4} ***
Ceftazidime	0 (0.0)	0 (0.0)	21 (100.0)	0.0000 ***
Ceftriaxone	2 (9.5)	0 (0.0)	19 (90.4)	5×10^{-3} **
Cefepime	5 (23.8)	0 (0.0)	16 (76.2)	4×10^{-2} *
Aztreonam	0 (0.0)	0 (0.0)	21 (100.0)	0.0000 ***
Meropenem	3 (14.2)	0 (0.0)	18 (85.7)	6×10^{-3} **
Amikacin	1 (4.7)	0 (0.0)	20 (95.2)	8×10^{-4} ***
Gentamicin	0 (0.0)	0 (0.0)	21 (100.0)	0.0000 ***
Tobramycin	0 (0.0)	0 (0.0)	21 (100.0)	0.0000 ***
Levofloxacin	21 (100.0)	0 (0.0)	0 (0.0)	0.0000 ***
Tetracycline	20 (95.2)	0 (0.0)	1 (4.7)	8×10^{-4} ***
Tigecycline	4 (19.0)	0 (0.0)	17 (80.9)	7×10^{-3} **
Nitrofurantoin	1 (4.7)	0 (0.0)	20 (0.0)	8×10^{-4} ***
Trimethoprim/ Sulfamethoxazole	20 (95.2)	0 (0.0)	1 (4.7)	8×10^{-4} ***

(P<0.05)*, (P<0.01)**, (P<0.001)***

Table (5): Antibiotics susceptibility test for 24 *Enterococcus faecalis* isolates by Vitek-2 system.

Antibiotics	*Enterococcus faecalis*			P-value
	Resistance (%)	Intermediate (%)	Sensitive %	
Levofloxacin	20 (83.3)	0 (0.0)	4 (16.6)	2×10^{-3} **
Erythromycin	24 (100.0)	0 (0.0)	0 (0.0)	5×10^{-4} ***
Linezolid	2 (8.3)	0 (0.0)	22 (91.6)	4×10^{-5} ***
Teicoplanin	4 (16.6)	0 (0.0)	20 (83.3)	2×10^{-3} **
Vancomycin	3 (12.5)	0 (0.0)	21 (87.5)	7×10^{-5} ***
Tetracycline	24 (100.0)	0 (0.0)	0 (0.0)	0.000 ***
Tigecycline	14 (58.3)	0 (0.0)	10 (41.6)	2×10^{-1}
Nitrofurantoin	0 (0.0)	0 (0.0)	24 (100.0)	0.000 ***

(P<0.05)*, (P<0.01)**, (P<0.001)***

The results showed that resistance levels of *E. coli* to levofloxacin were (100%), ampicillin/ sulbactam, tetracycline/trimethoprim, and sulfamethoxazole (95.2%), and are significantly lower than the level of sensitivity pattern, as shown in (Table 3).

Discussion

The soil samples that were taken from different regions of Baghdad contained different numbers of *E. coli* and *E. faecalis* that are excreted with human and animal feces. Possible explanations for the persistence of *E. coli* and *E. faecalis* from applications of manure were survival or that *E. coli* has naturalized and *E. faecalis* populations developed in the environment (7). Inputs of *E. coli* or *E. faecalis* from wildlife or household wastewater sources are two further explanations for this pattern's persistence(17). Significant inputs of the fecal organisms have been recorded in other places coming from wildlife agricultural research on water quality, it's also possible, but improbable, that the bacteria found in the groundwater system come from a domestic wastewater system, which could have been an influence, Hence, the farm where remains of cow and dog dung were discovered at the time of sampling may have been the source of this pollution (18).

The results showed markedly high resistance rates of fecal bacteria toward used antibiotics, similarly high levels of erythromycin and tetracycline resistance have been previously reported (19, 20). Hence, to ascertain the degrees of resistance to various antibiotics, it is crucial to perform specialized analyses of antibiotic resistance. It is not unexpected that we found that there are a few antibiotics with different levels of resistance, that were far less respectfully marked to the sensitivity pattern. Several studies have shown that adding animal manure to the soil enhances its store of antibiotic-resistant bacteria as demonstrated by (4). Multidrug resistance that is non-specific might originate from soil microorganisms. (17); which could be responsible for the increased levels of antibiotic-resistant bacteria to other antibiotics. This has a non-negligible impact on public health, given the many ways in which these bacteria can also infect humans and spread from the environment, especially in public green areas. Moreover, interesting with regard to antibiotic resistance, an infection sustained by this bacterium could be worrying (2).

Gram-positive bacteria may generate β -lactamases, an enzyme that breaks down antibiotics, or they may change the native penicillin-binding protein (PBP) genes to reduce the affinity and susceptibility of the penicillin-binding protein (PBP), which is their target site (21, 22). Overall, the majority of the pathogens on the WHO list are Gram-negative bacteria. Because of their specific structure, Gram-negative bacteria are more resistant than Gram-positive bacteria, and they are a major cause of disease and mortality worldwide (23, 24). Antibacterial drugs enzymes and non-enzymatic

processes are produced in GNB, where they may be acquired through the transfer of mobile genetic elements carrying resistance genes, similar plasmids that encode *β*-lactamases or a rise in inherent resistance based forward by chromosomal gene mutations (increasing the expression of target modifications, efflux pumps, permeability, or antibiotic-inactivating enzymes) (17, 25).

Conclusions

The current study documented the presence of fecal coliform bacteria in studied soil samples, with markedly high resistance rates toward used antibiotics. These facts might be linked to the irrigation method of the soil, the quality of the fertilizer, and the climatic conditions.

Recommendation

1- Sewage should not be used to irrigate crops which will contaminate the soil with pathogenic fecal bacteria that will be transmitted to plants and animals and then to humans through the food chain and should be treated if used.

2- Animal breeding fields and medical centers should be far from agricultural soil so that the soil is not contaminated with antibiotic-resistant pathogenic fecal bacteria, drugs, and other toxic substances.

3- Conducting studies similar to the current study on other sites in the city of Baghdad and other cities of Iraq, using indicators of a more general and comprehensive environment.

Authors' declaration:

We hereby confirm that all the Figures and Tables in the manuscript are ours.

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Authors' Contributions:

Study conception & design: (Fatima Khalid Dawood, Huda S.A. Al-Hayanni, and Maitham A. Sultan). Literature search: (Fatima Khalid Dawood, Huda S.A. Al-Hayanni, and Maitham A. Sultan). Data acquisition: (Fatima Khalid Dawood, Huda S.A. Al-Hayanni, and Maitham A. Sultan). Data analysis & interpretation: (Fatima Khalid Dawood, Huda S.A. Al-Hayanni, and Maitham A. Sultan). Manuscript preparation: (Fatima Khalid Dawood, Huda S.A. Al-Hayanni, and Maitham A. Sultan). Manuscript editing & review: (Fatima Khalid Dawood, Huda S.A. Al-Hayanni, and Maitham A. Sultan).

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تلوث الترب الزراعية في بعض مناطق بغداد بالبكتيريا البرازية الممرضة المقاومة للمضادات الحيوية

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خلفية البحث: أظهرت الدراسات المبكرة أن التربة الزراعية تحتوي على أنواع مختلفة من الكائنات الحية الدقيقة ، وخاصة البكتيريا بما في ذلك البكتيريا القولونية (السالمونيلا ، والشيغيلا ، والكليبسيلا ، والإشريكية القولونية ، والبكتيريا المعوية) مع البكتيريا البرازية الموجبة لصبغة غرام مثل *Enterococcus faecalis* هذه البكتيريا تعتبر مؤشر خطير عند انتقالها إلى الإنسان. لذلك هدفت الدراسة الحالية إلى التحري عن تلوث الترب الزراعية العراقية بالبكتيريا البرازية الممرضة (الإشريكية القولونية *Escherichia coli* و المكورة المعوية البرازية *Enterococcus faecalis*) ودراسة أنماط الحساسية للمضادات الحيوية للبكتيريا المعزولة من التربة.
الطرق: تم جمع عينات التربة من ستة مواقع (مزارع) في العاصمة بغداد ، وهي: الجادرية ، اللطيفية ، نهر ديالى ، الجزيرة ، والزعفرانية (قطاع 1 وقطاع 2) خلال فترة الدراسة من نهاية شهر تشرين الثاني لسنة 2021 إلى آب لسنة 2022؛ ثم تمت مقارنتها مع عينات التحكم (تربة حديقة المنزل). وقد تم عزل هذه البكتيريا عن طريق الاوساط الزرعية الاختيارية ، وشخصت بواسطة جهاز فايتك VITEK® 2 Compact system ، وأجريت عليها اختبارات الحساسية للمضادات الحيوية تجاه 18 مضاد حيوي مختلف بطريقة كيربي باور.
النتائج: أظهرت الدراسة البكتريولوجية للتربة الزراعية وجود بكتيريا برازيه وهذا دليل على تلوث عينات التربة الزراعية بهذه البكتيريا. وكان أعلى تعداد للإشريكية القولونية في مزرعة اللطيفية (1.48×10^3) ، بينما كان أعلى تعداد للإشريكية البرازية في مزرعة نهر ديالى (2.63×10^3). أوضحت نتائج الحساسية للمضادات الحيوية أن الإشريكية القولونية كانت مقاومة للأمبيسيلين ، سيفترياكسون ، سيفوكسيتين ، بيبيراسيلين ، سيفتازيديم ، وتيكوبلانين لكنها كانت حساسة لبقية المضادات الحيوية المستخدمة ، في حين أن بكتيريا *E. faecalis* كانت مقاومة فقط لليفوفلوكساسين والليزوليد وكانت حساسة لبقية المضادات الحيوية.
الاستنتاجات: الدراسة الحالية وثقت وجود البكتيريا القولونية البرازية في عينات التربة المدروسة ، مع معدلات مقاومة عالية بشكل ملحوظ تجاه المضادات الحيوية المستخدمة. قد تكون هذه الحقائق نتيجة الري بمياه الصرف الصحي واستخدام الأسمدة العضوية.
الكلمات المفتاحية: التربة الزراعية، البكتيريا المقاومة للمضادات الحيوية، المكورات المعوية البرازية، الإشريكية القولونية، البكتيريا البرازية.