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Bacterial Succession in Ten Diesel Polluted Soil in Gwagwalada, Abuja, Nigeria.

Joy Chinyere Ogbu [1], Akin-Osanaiye, B.C [2] and Reward Ebele Okonkwo [2,]

[1] Department of Microbiology, University of Abuja, Nigeria. ; chiyere.ogbu1@gmail.com
[2] Department of Microbiology, University of Abuja, Nigeria. ; chiyere.ogbu1@gmail.com
* Correspondence: chiyere.ogbu1@gmail.com; Tel.: (+234-8169655516)

Abstract: Bacterial Succession in ten Diesel polluted soil was carried out. Ten different Diesel polluted soil within Gwagwalada were selected and soil samples were collected from each site. Streaking method involving the use of serial dilutions was employed for the isolation of the bacteria. The number of viable bacterial count were determined. The bacterial species isolated were *Salmonella sp*., *Shigella sp*., *Escherichia coli* and *Klebsiella sp*. On the whole the data suggests that of the isolates gotten, gram negative bacteria are most adapted to conditions present in soils polluted with Diesel and hence could be exploited.

Keywords: Bacteria ; Diesel polluted soil ; Succession

1. Introduction

Diesel is a complex mixture and a common pollutant [1]. It consists mainly of aliphatic hydrocarbons ranging from C9 to C23 and a number of aromatic compounds. The environmental impacts of diesel when spilled include reduction of plant growth through direct toxic effects on the plants, reduced germination, and unsatisfactory soil conditions due to insufficient aeration of the pore space between the soil particles [1]. Thus, the biological functioning of microbial communities, which are central to the biogeochemical cycling of ecosystem, is truncated. This consequently affects the productivity of such ecosystems [1,2]. One of the most common contaminants in the environment is crude oil and its derivatives and due to their wide spread occurrence, they pose severe risks to human health and water bodies (surface as well as ground), and so, intense remediation practices are required at the contaminated sites [3]. Globally, there are rising concerns about the risks of environmental contamination from the exploration, transport and storage of petroleum. In Nigeria, crude oil production capacity increased to about 2.8 million barrels per day. Nigeria ranks as one of the Africa's largest producers of oil and the sixth largest oil.

Petroleum hydrocarbons represent the largest group of organic pollutants [4,5]. They are highly resistant to biodegradation, capable of accumulating in plants as well as in human and animal bodies [6,7], and exhibit carcinogenic and neurotoxic properties [8,7].The growing pollution of the natural environment urges the search for effective remediation methods. One of these is phytoremediation, which makes use and require a variety of analyses, while little is known about the impact of petroleum products on the biodiversity in agricultural ecosystems. Establishing links between microbiological diversity and soil functions is not an easy task [12,13]. Changes in the stability of soils and their ecological processes are affected by chemical pollutants [6,9,10,14]

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Processing and distribution of petroleum hydrocarbons as well as the use of petroleum products lead to contamination of soil [15]. Changes in soil properties due to contamination with petroleum- derived substances can lead to water and oxygen deficits as well as shortage of available forms of nitrogen and phosphorus [16].Hydrocarbon spill is a serious threat to the ecology [17]. According to [18], hydrocarbon contains substances that are toxic to the flora and fauna found in the ecosystem. Diesel contains low molecular weight compounds and high proportion of saturated hydrocarbons that are usually more toxic than long chained hydrocarbons. Diesel pollution is on the increase in Nigeria, as well as other developing countries [19].

Soil contamination with petroleum compounds reduces the growth of plants, among others, by the inhibition of germination and growth, photosynthesis, and respiration processes. The anatomical changes of roots, deformation of cells, reduction of the amount of root hair, vascular obstruction, and oil accumulation in tissues and their dehydration were observed [22,23]. The presence of petroleum hydrocarbons in the soil also influences the number and activity of the microorganisms colonising it, wherein the reaction of microorganisms depends on the type of contamination, although it mostly depends on its concentration in the environment [24]. Petroleum hydrocarbon contamination changes the carbon/nitrogen ratio. The presence of carbon promotes the growth and development of many microorganisms, although the lack of C:N balance may lead to the immobilisation of nitrogen by microbial biomass, making it unavailable to plants [25].

In spite of the increasing number diesel polluted soil in Gwagwalada environment, we are not aware of any study that has attempted to carryout the bacterial succession in ten diesel polluted soil here in Gwagwalada. The present study was therefore undertaken with a view to carryout the Bacterial Succession in soil samples polluted with diesel.

2. Materials and Methods

The study sites were 10 different diesel polluted soils situated at different locations in the town. The locations include May May Avenue diesel polluted soil, Abdul Shop diesel polluted soil, Tunde workshop diesel polluted soil, olushola ogbe diesel polluted soil, Ngige workshop close to God is able pharmacy, Al-Mahbub Agro machinery Agwandodo diesel polluted soil, Yellow generator workshop diesel polluted soil, diesel polluted soil opposite Jeparo hotel, Mustapha and Sanusi workshop diesel polluted soil and opposite Emma filling station diesel polluted soil around May May Avenue in Gwagwalada, Abuja. The sites polluted with diesel had a characteristic black color. Some of the surfaces were hard and others were Sandy and oily. Soil samples were collected at each site and transferred directly into sample bottles, they were then carefully transferred to the University of Abuja microbiology laboratory for analysis.

The culture media used for the isolation bacteria from diesel polluted soil was Nutrient Agar medium. The media was prepared according to manufacturer's instructions (28g of Agar was dissolved in 1000ml of water). 1ml of the soil sample was dissolved into 9ml of distilled water, agitating vigorously and making serial dilutions up to 10^5

A loopful of Dilutions 10^2 were streaked unto the solid nutrient agar plates. The inoculated plates were incubated at 37°C for 48 hours and subsequently monitored for growth. The colonies of the isolates were counted using a colony counter. Isolated colonies were further purified by sub-culturing and identified using bio-chemical tests.

Each isolate was examined for its size, shape, color, optical property, elevation and margin. Biochemical tests which were carried out include Gram reaction, Citrate, Urease, Spore characteristics, Starch Hydrolysis, Catalase, Oxidase, Indole and motility tests.

Catalase Test: Test is used to check microorganisms that produces the catalase enzyme. Catalase enzyme produced by these bacteria will neutralize the hydrogen peroxide and bubbles will be produced that are indicative of positive test. Mostly, catalase enzyme is produced by obligate aerobes and facultative anaerobic bacteria. The test is performed by tube or slide method by mixing the colony of bacteria with few

drops of 3% H2O2 on slide or to the test tube and looking for bubble formation within 10 seconds [26].

Oxidase Test: Oxidase test is helpful in the identification of microorganisms having ability to produce cytochrome oxidase enzyme. The test helps to differentiate oxidase positive Pseudomonacea and negative Enterobacteriacea families. Cytochrome oxidase based on the principle of transfer of electrons from donor (Electron transport chain) to final acceptor (oxygen) and reduction will takes place in the form of water. Cytochrome oxidase will oxidize the electron donor and the color will change to dark purple. This test is performed by impregnation of 1 percent tetra-methyl-p-phenylenediamine dihydrochloride acting as artificial electron donor into a filter paper and dried. The bacterial colonies are smeared on paper strip and check for color change within 10 sec [27].

Indole Test: Following test is helpful in the identification of to bacteria having the ability to produce tryptophanase enzyme. This enzyme will convert tryptophane amino acid into indole gas. Thus gas can be checked by adding different reagents such as Ehrlich's reagent or Kovac's reagent. Kovac's indicators contain paradimethyl amino benzaldehyde in isoamyl alcohol and conc HCl while Ehrlich's contain ethanol instead of isoamyl alcohol. Indole gas reacts with the reagent and the red color rosindole dye will form which indicates positive test [28].

Urease Test: Urease test helps in the identification of microorganisms having ability to produce urease enzyme. This enzyme belong to the amidohydrolases and phosphodoesterases superfamilies. Urease causes the hydrolysis of urea into NH3 and carbon dioxide. The ammonia formation will change the pH of the medium to alkaline and color will also change to pink at pH 8.1 indicating positive results. This test is used to identify the Helicobacter pylori that is urease positive. The test is performed by placing mucosa of infected stomach or colonies of bacteria in urea broth. A change in color within 30 minute indicate positive test [29].

Starch Hydrolysis Test: This test is helpful in the identification of microorganisms that can produce the alpha amylase and oligo-1, 6- glucosidase that causes the hydrolysis of starch. Often used to differentiate Clostridium and Bacillus. Starch is a large molecule and cannot cross the bacterial cell wall to be used as carbon source. To convert starch into small molecules release enzymes that hydrolyze the starch into glucose that can enter into metabolic pathways and used as energy source. To check this iodine is added to the agar medium that will turn to dark brown color due to hydrolysis of starch indicating positive results [30].

Motility Test: Following incubation for 18-24 hrs at 37°C, the colony in tube was observed for the presence of motile organisms. Production of cherry red reagent layer after introduction of Kovac's reagent in MIU medium indicates Indole positive reaction [32 - 33].

Citrate Utilization Test: Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrate permease. Simmons citrate agar slants of 2 ml in each vial was prepared by autoclaving at 15 psi 121°C. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the vials by means of a streak inoculation method with an inoculating needle and the vials were incubated for 48 hours at 37°C. Following incubation, citrate positive culture was recognized by the presence of growth on the surface of the slant of Simmons citrate agar and deep Prussian blue coloration of the medium [31 - 32].

Gram staining: Gram staining was performed for all isolated colonies according to the standard procedure. A smear of bacterial cells was prepared on a clean glass slide by a gentle heat fixation. The heat fixed smear was flooded with crystal violet solution for one minute. Smear was washed with water followed by adding mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and rinsed with water. Finally safranin was used as counter stains for 60-80 sec and washed with water. Cells were then

examined under microscope. S. aureus ATCC 25923 and E. coli ATCC 25922 were used as gram positive and gram negative control, respective

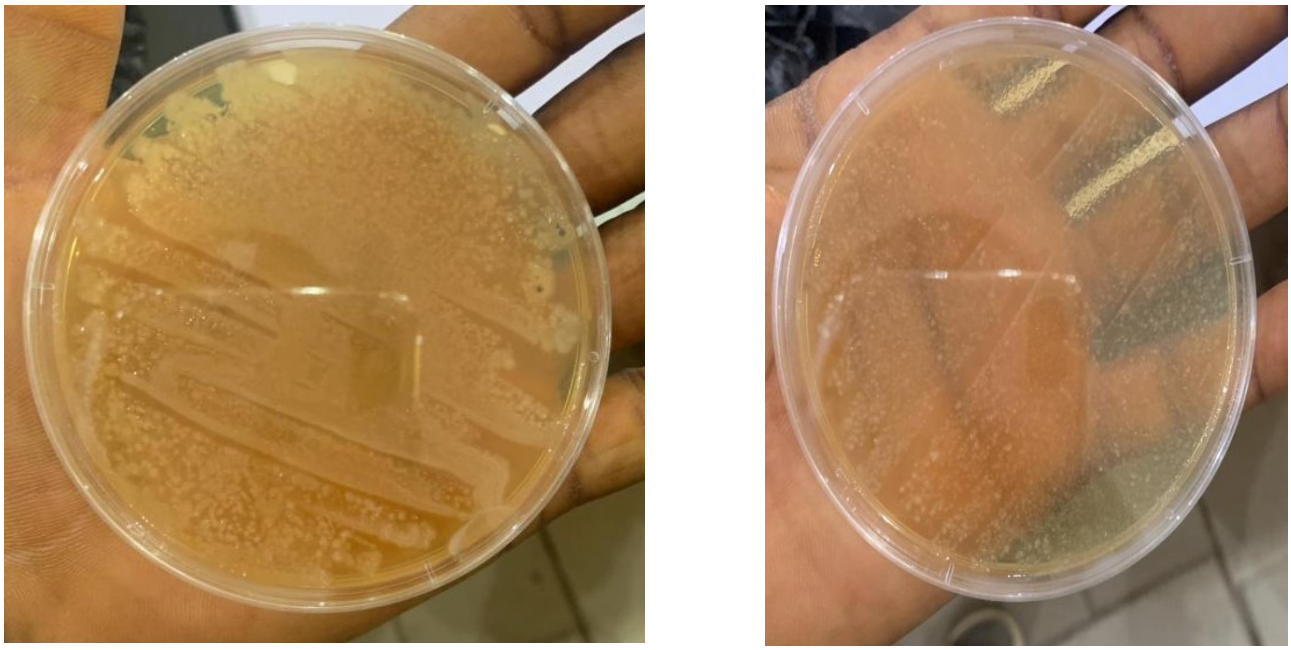
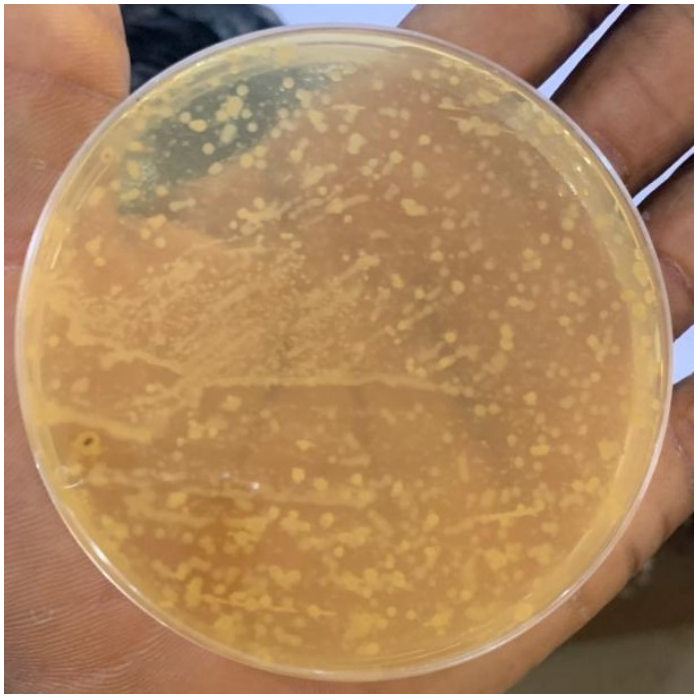


Figure 1. Samples from different diesel polluted soil

3. Results

Plate 1-10 shows the bacteria isolated from the ten different diesel polluted soil samples. Table 1 shows the morphological characteristics of the bacteria isolated from soil samples. Table



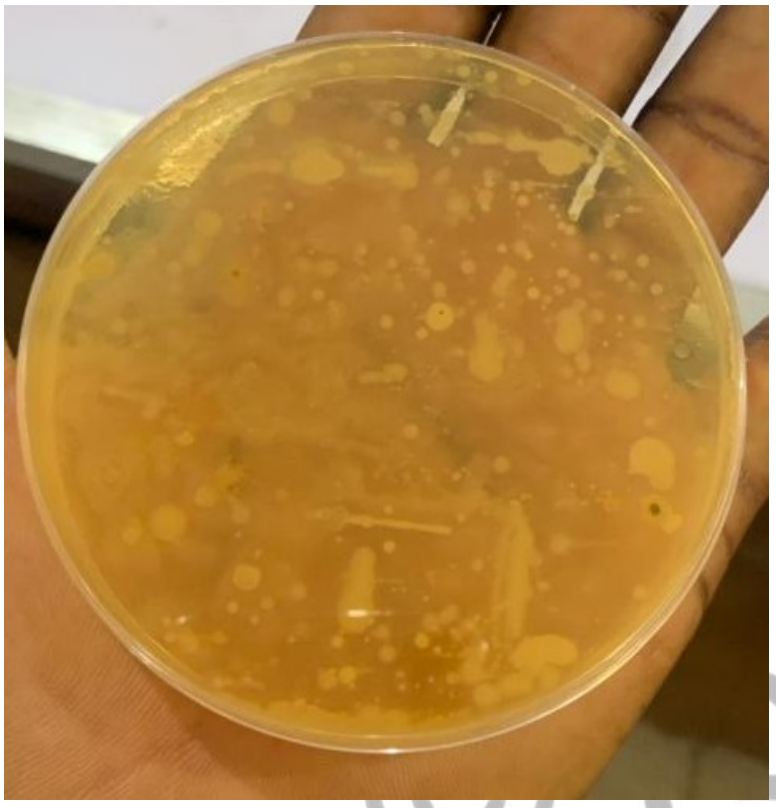
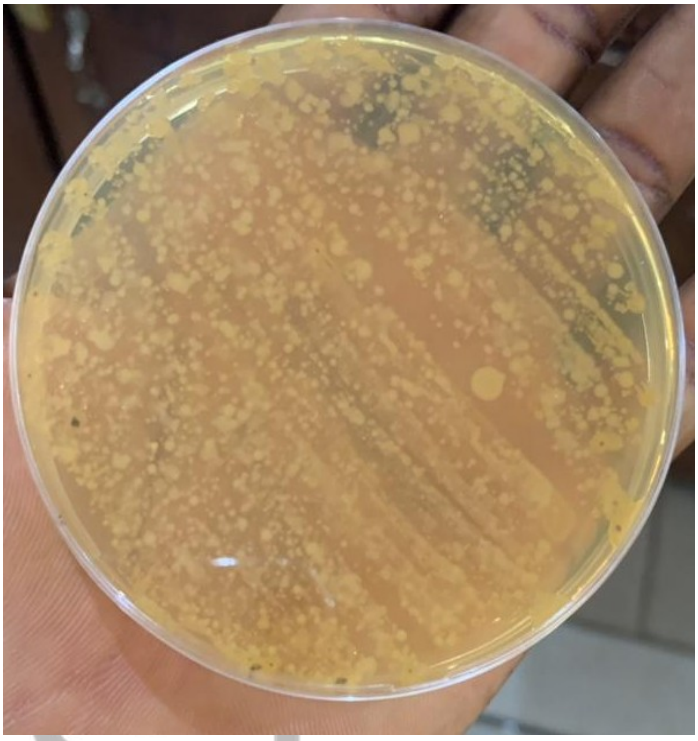
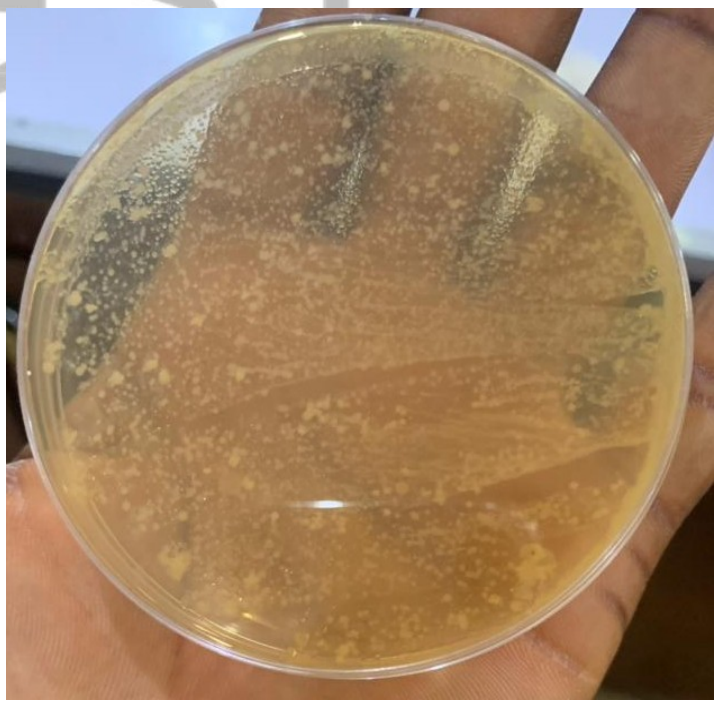


Plate 1-10. Bacteria isolated from ten different diesel polluted soil





Sample	No of Colony	Size	Shape	Colour	Optical property	Elevation	Margin
A	90	Small	Circular	White	Opaque	Flat	Entire
	231	Tiny	Circular	Colorless	Translucent	Flat	Entire
B	2	Medium	Circular	Yellow	Opaque	Raised	Entire
C	5	Small	Circular	White	Opaque	Flat	Entire
	82	Tiny	Circular	Colorless	Translucent	Flat	Entire
D	207	Small	Circular	Colorless	Translucent	Convex	Entire
	18	Medium	Circular	White	Opaque	Convex	Entire
E	48	Tiny	Circular	Milk	Opaque	Flat	Entire
	32	Tiny	Circular	Cream	Opaque	Flat	Entire
F	108	Medium	Circular	Yellow	Opaque	Flat	Entire
	114	Small	Circular	Cream	Translucent	Flat	Entire
G	10	Large	Irregular	Cream	Opaque	Flat	Entire
	46	Small	Circular	Yellow	Opaque	Flat	Entire
H	98	Tiny	Circular	Cream	Opaque	Flat	Entire
	18	Medium	Circular	Yellow	Opaque	Flat	Entire
I	52	Small	Circular	Yellow	Opaque	Flat	Entire
	116	Tiny	Circular	Colorless	Translucent	Flat	Entire
J	79	Small	Circular	Colorless	Translucent	Convex	Entire
	63	Medium	Circular	White	Opaque	Convex	Entire

Table 1. Morphological characteristics of bacterial isolates

Sample	No of Colony CFU/M	Gram Rxn	Citrate	Urease	Spore Xteristic	Starch Hydrolys	Catalase	Oxidase	Indole	Motility	Probable Organism
A	90	-	-	-	-		+	-		-	Shigella spp
	231	-	-	-	-		+	-		-	Shigella spp
B	2	-	-	-	-		+	-		-	Shigella spp
C	5	-	-	-	-	-	+	+	+	+	E. coli
	82	-	-	-	-	-	+	+	+	+	*E. coli*
D	207	-	-	-	-		+	-	-	+	Salmonella spp
	18	-	-	-	-		+	-	-	+	Salmonella spp
E	48	-	-	-	-	-	+	+	+	+	E. coli
	32	-	-	-	-	-	+	+	+	+	E. coli
F	108	-	-	-	-		+	-	-	+	Salmonella spp
	114	-	-	-	-		+	-	-	+	Salmonella spp
G	10	-	-	-	-		+	-	-	+	Salmonella spp
	46	-	-	-	-		+	-	-	+	Salmonella spp
H	98	-	-	-	-		+	-	-	+	Salmonella spp
	18	-	-	-	-		+	-	-	+	Salmonella spp
I	52	-	+	+	-	-	+	-	-	-	*Klebsiella spp*
	116	-	-	-	-	-	+	+	+	-	*Klebsiella spp*
J	79	-	-	-	-		+	-	-	+	Salmonella spp
	63	-	-	-	-		+	-	-	+	Salmonella spp

Table 2. Biochemical characteristics of bacterial isolates

4. Discussion

The bacteria species isolated included *Shigella spp, Escherichia coli, Salmonella spp* and *Klebsiella spp*. The isolation of *Shigella spp, Escherichia coli, Salmonella spp* and *Klebsiella spp* from the dump sites is an indication that the predominant bacteria in dumpsite soil polluted with diesel in Gwagwalada are Gram negative bacteria.

Klebsiella pneumoniae is a Gram-negative non-motile and encapsulated bacteriumfound in environmental conditions as diverse as soil, plant leaves, mammalian intestines,and waste waters [34,35]. It is an opportunistic pathogen that is able to colonize the mucosal epithelium of the gut and nasopharynx and to disseminate into the deep

tissuesand bloodstreams of susceptible patients, causing severe infections such as pneumonia, meningitis, endophthalmitis, pyogenic liver abscesses, and bacteremia [36–39]. The ability of this bacterium to form a biofilm on invasive medical devices leads to subsequenthealth care associated infections, particularly in the urinary and pulmonary tracts [37].K. pneumoniae infections are difficult to treat, particularly because of the pathogen's high endogenous antibiotic resistance. For example, K. pneumoniae is intrinsically resistant toampicillin, owing to the presence of β-lactamase (SHV-1) encoding genes in its chromosomal genome [40].

Shigelosis is an acute gastroenteritis caused by Shigella specis, including Shigella dysenteriae, Shigella flexneri,Shigella boydii and Shigella sonnei. It is one of the most common causes of morbidity and mortality in children with diarrhea in developing countries. Worldwide, approximately 165 million cases of shigellosis occurs and 1,100,000 death are caused by the disease per year, which two-third of the patients are children under 5 years of age .Epidemic usually occur in area with crowding and poor sanitary conditions ,where transmission from person to person common, or when food or water are contaminated by organism (41,42).

Escherichia coli are gram-negative bacilli of the family Enterobacteriaceae. They are facultative anaerobes and nonsporulating. Escherichia coli strains with the K1 capsular polysaccharide antigencause approximately 40% of cases of septicemia and 80% of cases of meningitis. Different strains of Escherichia coli are associated with a number of distinctive diarrheal illnesses. Among these are the enterotoxigenic Escherichia coli (ETEC), enteroinvasive Escherichia coli (EIEC), and Shiga toxin–producing Escherichia coli (STEC). Of the STEC,E coli O157:H7 is the prototypic strain. Each class of Escherichia coli has distinct somatic (O)and flagellar (H) antigens and specific virulence characteristics [43].

Salmonella infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries throughthe costs associated with surveillance, prevention and treatment of disease [4].

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