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# Isolation and Identification of Bacteria from Processed *Vernonia Amygdalina* Leaves sold in Some Markets around Kaduna Metropolis

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**Abstract**

***Vernonia amygdalina* is a member of the Asteraceae family, it is a highly appreciable vegetable in west and central Africa consumed in various dishes. It is one of the varieties of vegetables sold in several markets in kaduna metropolis, this study was carried out to isolate and identify bacteria associated with washed *Vernonia amygdalina* sold in Sabo, Maraban-rido and Ungwan-rimi markets. A total of 30 samples were analysed (10 from each market) yielding 49 bacteria isolates which were identified using gram staining and a total of 8 biochemical tests (Catalase, Indole, Oxidase, Methyl red, Voges-Proskauer, Urease and Triple sugar iron agar tests). The result obtained from this study has indicated the bacteria isolated from this vegetables belong to seven different genera; *Staphyloccocus* spp. (36%) was the most frequently isolated followed by *Escherichia coli* (27%), *Klebsiella* spp. (13%), *Salmonella* spp. (11%), *Bacillus* spp. (9%), *Shigella* spp. (2%) and *Proteus* spp. (2%). This isolates have been at one point linked to illness and diseases associated with food.**

**Keywords:** Bacteria and *Vernonia amygdalina*

## INTRODUCTION

*Vernonia amygdalina* is a member of the Asteraceae family. It is a small shrub that grows in tropical Africa, some may be up to 10m tall. It is commonly called bitter leaf because of its bitter taste which is caused by sesquiterpene lactones (e.gvernodalin, vernolepin and vernomydin) and steroid glucoside (vernonioside). Some of these compounds have significant anti-parasitic activity.(Ijeh *et. al.,* 2011).

The occurrence of bacterial populations on vegetables is recognized as a source of potential health hazard to man and his animals. This is due to their production of bacteriotoxins compounds which are capable of inducing several critical clinical symptoms in man following ingestion or inhalation; even though they differ in their degree and manner of toxicity. The contamination of fruits and vegetables by bacteria could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation. (Afolabi*et. al.* 2014)

Human activities contribute greatly to post harvest contamination either through mishandling such as improper harvest handling or lack of proper storage measures and personal hygiene This study therefore aimed at investigating the prevalence and occurrence of different bacterial and fungal species on the bitterleaf sold in Sabo, Maraban-rido and Ungwan-rimi markets from Kaduna state, Northern Nigeria; with the view of identifying the different species of bacteria and their prevalence. The results of this research is also intended to be used in suggesting possible ways of minimizing or avoiding possible health problem associated with these bacterial species.

## MATERIALS AND METHODS

Fifteen samples of the processed *Vernonia amygdalina* were collected from each of the three markets (Sabo, Marabanrido, and Kabala market). They were labeled and packaged separately in very neat polyethylene bags and brought to the department of microbiology Kaduna state university for physical and microbiological analysis.

Media was prepared by weighing approximate amount (28g) of the powder and dissolved in a litre of distilled water in a conical flask. The flasks was then plugged with cotton wool wrapped with aluminum foil and sealed firmly with masking tape. The media is then homogenized by boiling before sterilizing in the autoclave at $121^0$C for 15minutes.The sterile media is allowed to cool to about $45^0$C before being poured into sterile petri-dishes and allowed to set.Suspension was prepared by using motar and pestle to pound a small portion of the *Vernonia* and adding 2mls of sterile distilled water to make a suspension. Inoculating loop was sterilized first with ethanol and then flamed until red hot in spirit lamp. The sterilized loop was the dipped in the suspension and then streaked across the nutrient agar plate in 4 planes. Pure isolates were obtained by selecting discrete colonies and having them sub cultured onto Petri-dishes containing freshly prepared NA media. The bacterial isolates were streaked out onto NA plates.

The pure bacterial isolates were stained according to Gram's techniques as described by Baker (1967). They were identified using both macroscopic, microscopic characters and biochemical tests. The frequency of occurrence of each isolate was calculated using the formulabelow (Gonzalez, 2011);

$$\text{Frequency}(\%) = \frac{\text{number of specific isolate}}{\text{Number of Total isolate}} \times \frac{100}{1}$$

## RESULT

A total of 49 bacterial isolates were gotten from the three markets andeach isolate was identified using morphological characteristics, gram staining reaction and biochemical tests result

A gram positive bacilli with flattened whitish colonial morphology was isolated from sabo and marabanrido markets samples, this isolate gave a positive result to catalase oxidase and Voges-Proskauer tests and was negative to indole, methyl red and urease test yielding no gas with the triple sugar test. This bacteria was identified as a *Bacillus* sp. as shown in table 1 and 2 and plate1.

A gram positive cocci which whitish raised colonies on plate was isolated from both sabo, marabanrido and u/rimi markets, this isolate was positive to catalase, oxidase, methyl red and urease test and gave a negative result for indole and Voges-Proskauer test. This isolate also did not produce H2S gas with the triple sugar test. This Bacteria was identified as a *Staphylococcus* sp. as shown in table 1, 2, 3 and plate 3.

A gram negative bacilli with milky raised morphology on plate was isolated from both sabo, marabanrido and u/rimi markets, this isolate was positive catalase, indole and methyl red test and gave a negative result to oxidase, Voges-Proskauer and urease test giving off gas with the triple sugar test. This isolate was identified as *Escherichia coli*as shown in table 1, 2, 3 amd plate 4.

A gram negative bacilli with whitsh raised morphology on plate was isolated from both sabo, marabanrido and u/rimi markets,, this isolate was positive to catalase, methyl red and urease tests and negative to indole, oxidase and Voges-Proskauer tests giving off H2S gas with the triple sugar test. This isolate was identified as a *Klebsiella* sp. as shown in table 1, 2, 3 and plate 2.

A gram negative bacilli from plate SC1b with flattened distinct colonial morphology was isolated only from sabo market This isolate was positive to catalase, indole, methyl red and urease tests and negative to oxidase and Voges-Proskauer tests yielding both H2S and gas. This isolate was identified as a *Proteus* sp. as shown in table 1.

A gram negative bacilli with a whitish flattened colonial morphology was isolated from marabanrido and u/rimi markets, this isolate was positive to catalase and methyl red test and negative to indole, oxidase, Voges-Proskauer and urease test. This isolate also produced H2S and gas with the triple sugar test. This isolate was identified as a *Salmonella* sp. as shown in table 2 and 3.

A gram negative bacilli with with raised and milky distinct colony morphology was isolated only from marabanrido market, this isolate was positive for catalase and indole test and was negative for oxidase, methyl red, Voges-Proskauer and urease test and yielded neither gas nor H2S with triple sugar test. This isolate was identified as a *Shigella* sp. as shown in table 2 and plate 1.

The result obtained from this study has indicated the bacteria isolated from these vegetables belong to seven different genera (Table 4.4). *Staphyloccocus* sp. (36%) was the most frequently isolated followed by *Escherichia coli* (27%), *Klebsiella* spp. (13%), *Salmonella* sp. (11%), *Bacillus* sp. (9%), *Shigella* sp. (2%) and *Proteus* sp. (2%) as shown in table 4 and figure 1.

**Table 1.** showing identification and characterization of isolates from Sabo market

| Sample | culture morphology | Gram staining | Catalase | Indole | Oxidase | Methyl red | V.P | Urease | T.S.I | | | | Suspected pathogens |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | B | S | H2S | gas | |
| SA1 | Flattened whitish colonies | bacilli + | + | - | + | - | + | - | Y | R | - | - | *Bacillus* sp. |
| | a)Flat, whitish colonies. | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| SA2 | | | | | | | | | | | | | |
| | b)Fibroid whitish colonies | Bacilli + | + | - | + | - | + | - | Y | R | - | - | *Bacillus* spp. |
| | c) whitish raised colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| SA3 | a)Whitish distinct raised colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| | b)milky raised fibroid colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| | c)distinct raised pinkish colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| SB1 | a)whitish raised colonies | Bacilli - | + | - | - | + | - | + | Y | Y | - | + | *Klebsiella* sp. |
| | b) Flattened white colonies. | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| SB2 | a) yellowish raised colonies | Cocci + | + | - | - | - | + | - | Y | R | - | - | *Staphyloccoccus* sp. |
| | b)whitish flattened colonies | Bacilli - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| | c)milky raised colonies | bacilli - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| SB3 | a)flattened transluscent colonies | Cocci + | + | - | + | + | - | + | Y | Y | + | - | *Staphyloccoccus* sp. |
| | b)milky fibroid raised colonies | Cocci + | + | - | + | + | - | + | Y | Y | + | - | *Staphyloccoccus* sp. |
| | c)whitish raised colonies | Cocci + | + | - | + | + | - | + | Y | Y | + | - | *Staphyloccoccus* sp. |
| SC1 | a)whitish raised colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| | b)flattened distinct colonies | Bacilli - | + | + | - | + | - | + | R | Y | + | + | *Proteus* sp. |
| SC2 | a)Flattened white colonies | Bacilli - | + | + | - | + | - | + | Y | Y | + | - | *Escherichia coli* |
| | b)witish raised colonies | Bacilli - | + | - | - | + | - | + | Y | Y | - | + | *Klebsiella* sp. |
| SC3 | NO GROWTH | | | | | | | | | | | | |
| S4 | a)Flattened milky colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| | b)whitish raised colonies s | Bacilli - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |

Key;
S = sabo market
A, B, and C = the three different locations in Sabo market
1, 2, and 3 = samples bought from each location
S4 represent only one sample bought from fourth location
a, b and c = pure cultures obtained from each sample.
B = butt, S= slant, $H_2S$= Hydrogen sulphide, Y=yellow, R=red, -=Negative, +=Positive

**Table 2.** showing identification and characterization of isolates from Maraban-rido market

| Sample | Culture morphology | Gram staining | Catalase | Indole | Oxidase | Methyl red | V.P | Urease | T.S.I | | | | Suspected pathogens |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | B | S | H2S | gas | |
| MA1 | Flattened whitish with distinct colonies | Bacilli - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| MA2 | Flat, whitish raised colonies | Baccili - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| MA3 | Whitish distinct raised colonies | Cocci - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| MB1 | a) Flattened white colonies. | Bacilli - | + | - | - | + | - | + | Y | Y | - | + | *Klebsiella* sp. |
| | b)milky raised colonies | Cocci + | + | - | + | - | + | - | Y | R | - | - | *Staphyloccoccus* sp. |
| MB2cx | a) Flattened white colonies. | Bacilli - | + | - | - | + | - | - | Y | Y | + | + | *Salmonella* sp. |
| | b)milky raised colonies | Bacilli + | + | + | + | - | + | - | Y | R | - | - | *Bacillus* sp. |
| MB3 | No growth | | | | | | | | | | | | |
| MC1 | a)yellowish distinct and raised colonies. | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| | b)flattened spread out white colonies | Cocci + | + | - | - | - | + | + | Y | R | - | - | *Staphyloccoccus* sp. |
| | c)raised milky colonies | Bacilli - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| MC2 | Flattened white colonies | Bacilli - | + | - | - | + | - | - | Y | Y | + | + | *Salmonella* sp. |
| MC3 | a)flattened white colonies. | Bacilli + | + | + | + | - | + | - | Y | R | - | - | *Bacillus* sp. |
| | b)raised and milky distinct colonies | Bacilli - | + | + | - | - | - | - | R | Y | - | - | *Shigella* sp. |
| M4 | Flattened milky colonies | Bacilli - | + | - | - | + | - | - | Y | Y | + | + | *Salmonella* sp. |

Key;
M = Maraban-rido market
A, B, and C = the three different locations in Maraban-rido market
M4 represent only one sample bought from fourth location
1, 2, and 3 = samples bought from each location
a, b and c = pure cultures obtained from each sample.
B = butt, S= slant, $H_2S$= Hydrogen sulphide Y=yellow, R=red -=Negative, +=Positive

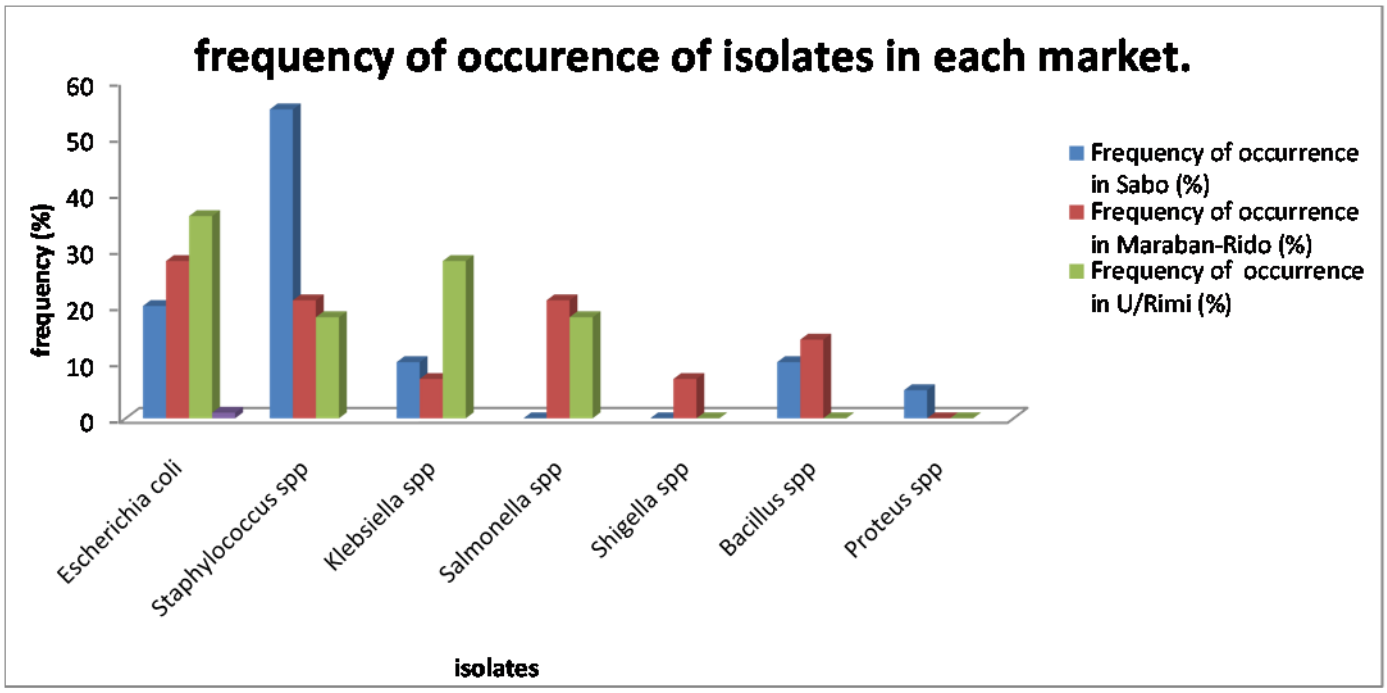
**Table 3**. showing identification and characterization of isolates from Ugwan-rimi market

| Sample | Subculture morphology | Gram staining | Catalase | Indole | Oxidase | Methyl red | V.P | Urease | T.S.I | | | | Suspected pathogens |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | B | S | H2S | gas | |
| UA1 | Flattened milky colonies | Bacilli - | + | - | - | + | - | + | Y | Y | - | + | *klebsiella* sp. |
| UA2 | a)whitish raised colonies | Bacilli - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| | b)Flattened distinct colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| UA3 | Flattened distinct colonies | Cocci - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| UB1 | Flattened mlky colonies | Bacilli - | + | - | - | + | - | + | Y | Y | - | + | *Klebsiella* sp. |
| UB2 | Flattened distinct colonies | Bacilli - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| UB3 | Flattened distinct colonies | Cocci - | + | + | - | + | - | - | Y | Y | + | - | *Escherichia coli* |
| UC1 | Whitish raised colonies | Bacilli - | + | - | - | + | - | + | Y | Y | - | + | *Klebsiella* sp. |
| UC2 | Flattened whitish colonies | Cocci + | + | - | + | + | - | + | Y | R | - | - | *Staphyloccoccus* sp. |
| UC3 | Spread flattened milky colonies | Bacilli - | + | - | - | + | - | - | Y | Y | + | + | *Salmonella* sp. |
| U4 | Flattened milky colonies | Bacilli - | + | - | - | + | - | - | Y | Y | + | + | *Salmonella* sp |

Key;
U = Ungwan-rimi market
A, B, and C = the three different locations in Ungwan-rimi market
U4 represent only one sample bought from fourth location
1, 2, and 3 = samples bought from each location
a, b and c = pure cultures obtained from each sample.
B = butt, S= slant, $H_2S$= Hydrogen sulphide Y=yellow, R=red -=Negative, +=Positive

**Table 4.** Frequency of distribution of different isolates from the three markets

| Isolates | Frequency of occurrence in Sabo (%) | Frequency of occurrence in Maraban-rido (%) | Frequency of occurrence in U/Rimi (%) | Total frequency of occurrence (%) |
|---|---|---|---|---|
| *Escherichiacoli* | 20 | 28 | 36 | 27 |
| *Staphylococcus*sp. | 55 | 21 | 18 | 36 |
| *Klebsiella* sp. | 10 | 7 | 28 | 13 |
| *Salmonella* sp. | 0 | 21 | 18 | 11 |
| *Shigella* sp. | 0 | 7 | 0 | 2 |
| *Bacillus* sp. | 10 | 14 | 0 | 9 |
| *Proteus* sp. | 5 | 0 | 0 | 2 |




**DISCUSSION**

Previously, there was no available information on the bacterial quality and prevalence on processed *Vernonia amygdalina* sold around these markets in Kaduna metropolis. This study has provided the baseline information on the occurrence of bacteria in the processed *Vernonia amygdalina* present to the consumers from these public markets. Since this vegetable is not easily contaminated by these microbes when in the field before being washed due to the concentration of bitter principles, the contamination at the market may be as a result of reduction of the concentration of the bitter principles in the bitter leaf which creates a suitable environment for the growth of these bacteria species, it may also be due to several mishandling processes or inadequate sanitary processes while processing.

Previous studies showed that *E.coli, salmonella and staphylococcus species* are frequently occurring organisms isolated from most vegetables. This also agrees with other studies such as that carried by Arafat and Mohammed (2013) on Tomato (*Lycopersicum esculentum*), green pepper (*Capsicum annum*) and Mloukhia (*Corchorus olitoris*).The presence of bacteria is a potential indicator of hazard to the consumer. Among the bacteria genera isolated in the study include *Escherichia coli* and *Staphylococcus* sp. which were predominant in the *Vernonia* samples. These bacteria have been known to be the leading cause of several ailments such as Diarrhoea, and may produce several toxins such as enterotoxins that can cause food poisoning (Zell *et al.,* 2008; Even *et al.,* 2010) making them pathogens of public health concern.

*E.coli* is a fecal organism which forms part of the normal intestinal flora of the digestive system in humans and a wide variety of animals and is commonly found in animal manures. (Dsmarchelier and Fegan, 2003). Its presence in the processed *Vernonia amygdalina*indicates the possibility that fecal contamination is occurring at some point during the processing and other pathogenic microorganism associated with fecal contamination may be present. Presence gram negative rods of *Salmonella* sp. and *Proteus* sp. is also a direct consequence of feacal contamination as a result of possible unhygienic handling or the contamination of the vegetable itself during processing or directly from source and this may have adverse effect on the health on the consumers (okonkwo *et. al*., 2008).

*Bacillus* sp. are part of the natural flora and are among the most common vegetable spoilage bacteria (Vanderzant and Spittstoesser, 1992) and the presence of *Bacillus* in vegetables may be said to be due to environmental factors. The survival of the *Bacillus* sp. depends on several factors such as nature of the organism, resistance to a new physical environment and the ability to form spores (Godon, 1997).

The dominance of Staphylococcus among the bacterial genera identified from the *Vernonia amygdalina* was an indication of poor hygienic practices by both the processors and the sellers.

This result is also consistent with other studies which showed that the contamination with these microorganisms could be as a result of discharge into the atmosphere through sneezing, coughing, handling or even the manner at which the

vegetables are sold continually predisposed them to contamination, more importantly, bacteria on the produce may multiply over time depending on the storage conditions especially those that are psychotropic (Montville and Matthews, 2008; Abadias and Oliveira, 2008).
The presence of other pathogenic and opportunistic bacteria such as *Klebsiella* sp.*Shigella* sp.*Bacillus* sp. and *Proteus*sp. in some of the *Vernonia* samples further highlights the need to safeguard the health of the consumers by proper washing and cooking of this vegetable before consumption.

**Recommendations**

Strict hygienic measures should be observed by *Vernonia amygdalina* processors to ensure that they do not serve as source of contamination The consumers of this vegetable should properly wash the vegetable and cook thoroughly before consumption Storage and preservation of the vegetable should be given a greater attention especially improved facilities at domestic level under controlled conditions and workshops specifically aimed addressing the control of human infection associated with consumption of contaminated vegetables should be organized.

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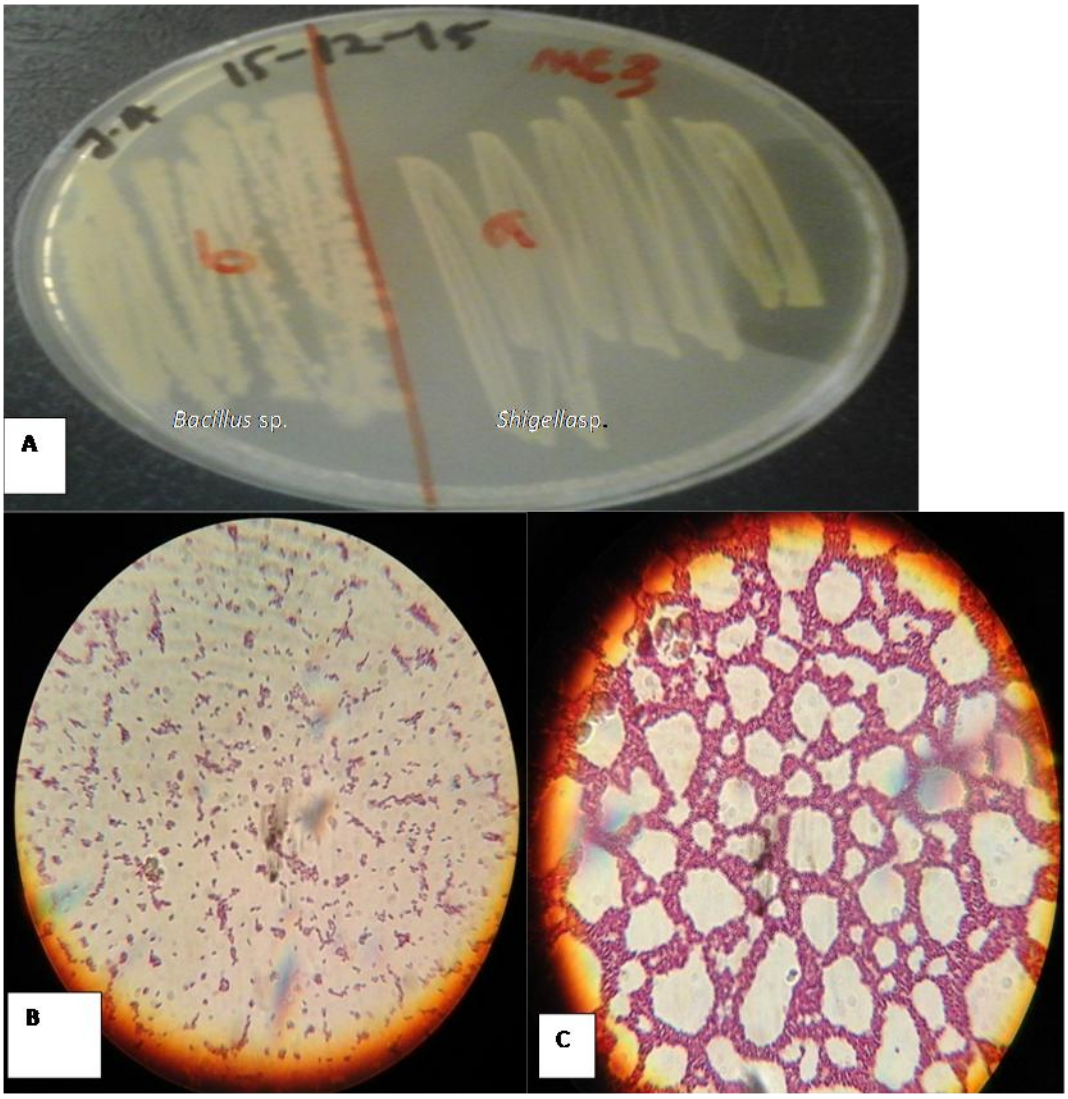


**Plate 1:A** shows Macroscoipic appearance of *Bacillus* sp. and *Shigella*sp. B shows microscopic character of *Bacillus* sp.and C shows microscopic character of *Shigella* sp.

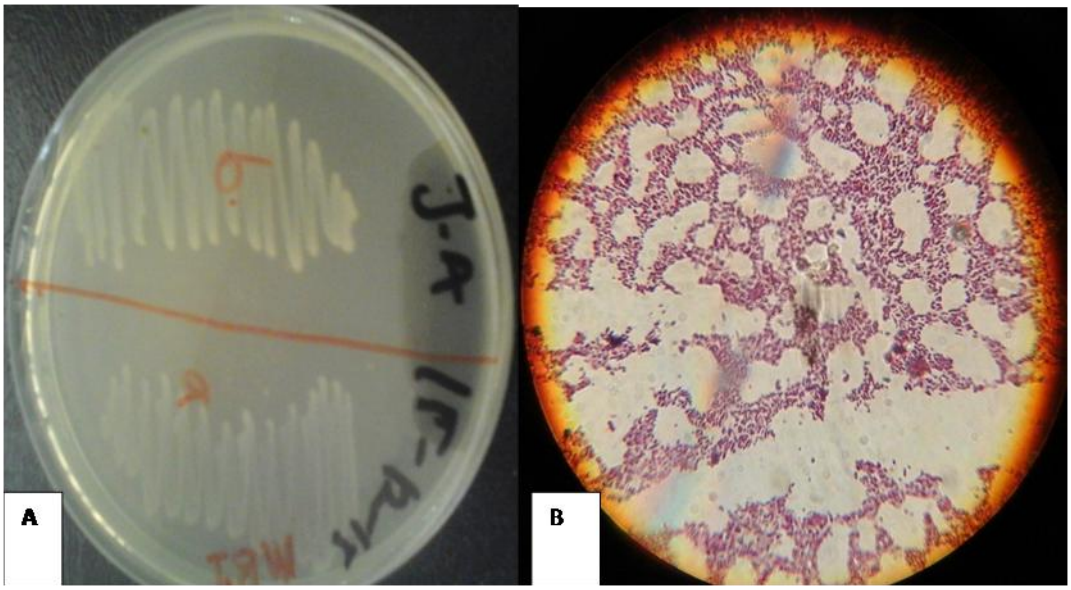


**Plate 2: A** shows macroscopic appearance of *Klebsiella* sp. B shows microscopic appearance of *Klebsiella* sp. on plate.

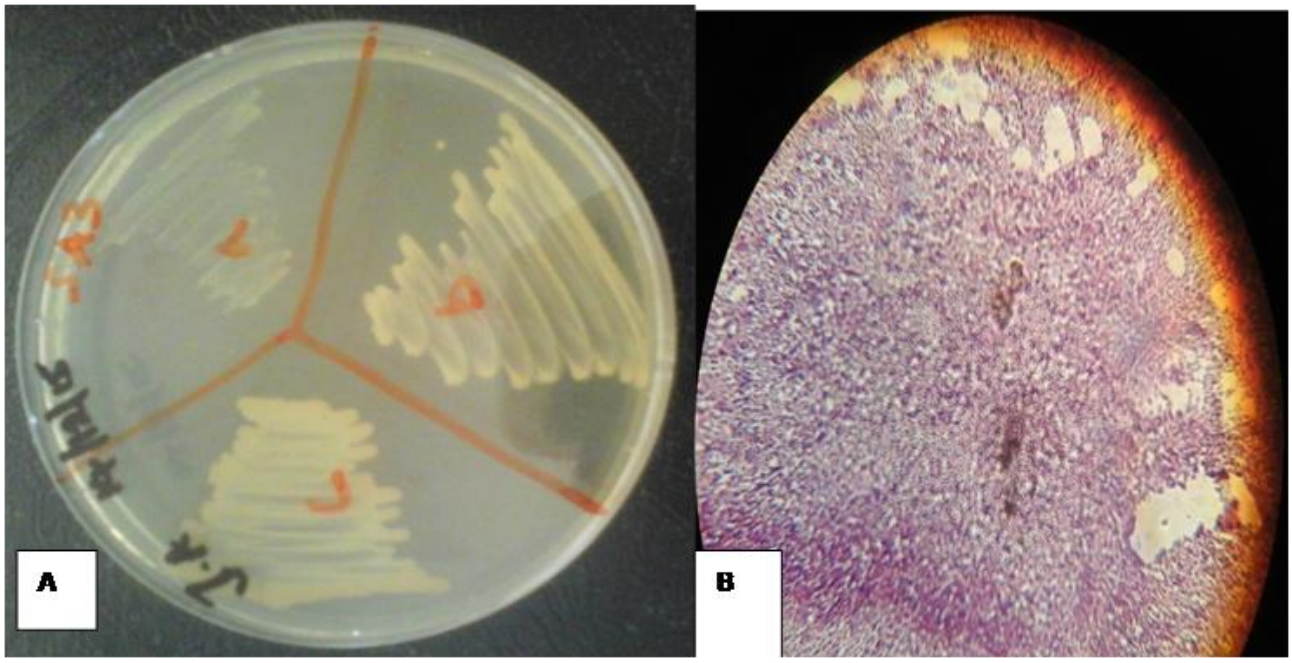


**Plate 3: A** shows macroscopic appearance of *Staphylococcus* sp. B shows microscopic character of *Staphylococcus* sp

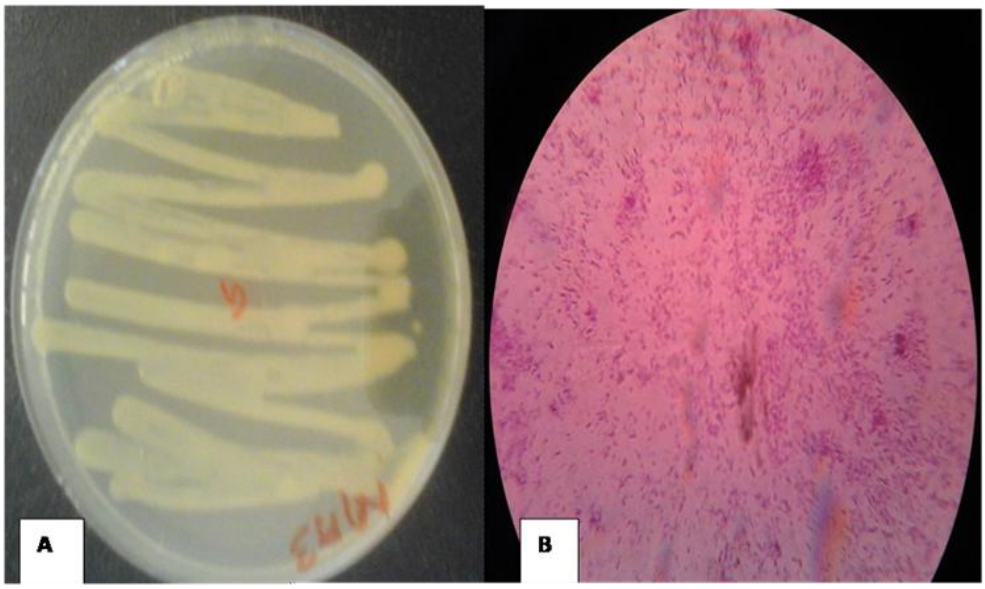


**Plate 4: A** shows macroscopic appearance of *E.coli.*B shows microscopic appearance of *E. coli*