# Isolation and Identification of Some pathogenic bacteriafrom Traded Diets in Restaurants at EL- Taif City – Kingdom of Saudi Arabia

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**Abstract:** The main aim of this study was to isolate and identify of pathogenic bacteria from traded diets in restaurants at El – Taif City Kingdom of Saudi Arabia. The collected samples were fifty, they showed high variability and counts of aerobic bacteria e.g. fresh cut salad, processed meat and chicken and rice, no visible sign of defect was observed on them. Only three samples were scored considerable amounts of coliform bacteria (e.g. salad, shawrma and samposa). In conclusion, twenty bacterial strains (M01 –M20) were isolates under aerobic conditions from twelve traditional food products and characterized by morphology and some biochemical characteristics. All isolates were divided into three groups, bacilli, cocci, and short rods. The strains were further identified by partial 16SrRNA gene sequencing and phylogenetic analysis. Fifteen strains were identified by 97-100% identity including *Bacillus circulance* (4), *Bacillus subtilis* (4), *Staphylococcus aureus* (3), and *Echerichia coli* (4). Other isolates were identified by 85-92% identity, therefore may be considered as new species and named: *Bacillus sp*. (M07, M09, M10) and *Staphylococcus sp*. (M12,M13). These results indicate the importance of strict hygiene during handling practices in order to avoid contamination of the food products.
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## 1. Introduction

Food-borne illnesses are major international health problems and important causes of reduced economic growth (WHO, 2002). Food- and water-borne diarrheal diseases are the leading causes of illness and death in developing countries, killing an estimated 2.2 million people annually, most of them are children (Mensah *et al.,* 2002). Bacteria have accounted for more than 70% of deaths associated with foodborne transmission (Hughes *et al*., 2007). Food-borne illnesses associated with *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Escherichia coli 0157:H7 and Salmonella enteritidis* present a major public health concern throughout the world and from restaurant as a fast food (37.5%), (Isara*et al.,*2010). The presence and growth of microorganisms in food may be caused spoilage and reduction in quality and quantity (Soliman and Badeaa, 2002).

Bacillus species are Gram positive rod, grow under aerobic conditions and produce heat resistant spores. Species in the genus range from 36% for *Bacillus cereus* to over 60% for some thermophilic isolates (Priest, 1993). *Bacillus cereus* causes two types of food-borne diseases: a diarrheal syndrome caused by complex enterotoxins (diarrheal toxin) and emetic syndrome caused by emetic toxins. It has been frequently isolated from raw vegetables and sprouts (Harmon *et al.,* 1987; Kim *et al.,* 2004). *Bacillus cereus* occurred in 98% of test minced meat, 60% of sausage, 40% of rice grains, 44% Koshari or ice-cream and 36% of pasteurized milk samples investigated by Saleh *et al.* (1993).

*E. coli* is Gram - negative bacteria, short rod, grow under aerobic conditions and non spore forming. Outbreaks can infect thousands of people causing bloody diarrhea and hemolytic uremic syndrome (HUS) that can result in severe illness or even death (Chattaway*et al.,* 2011). Higher numbers of outbreaks have been attributed to the consumption of fresh leafy produce and monitor at quality of water and foods (Sospedra*et al.,* 2012). Other sources of contamination were feacal contamination, soil and irrigation water are more commonly encountered (Islam *et al.,* 2004 & Hutchison *et al.,* 2008). Among reported cases, raw vegetables have been identified as a source of enterotoxigenic*Escherichia coli,* which causes traveller'sdiarrhoea (Beuchat, 1996). More than 90% of raw meat and shellfish were contaminated with *E. coli*. The highest level of coliforms was found in bean sprouts and fresh-cut salad, with 50% of samples containing more than 5 log cfu/g (Seow*et al*., 2012).*Staphylococcus aureus, Bacillus cereus* also were isolated from fresh product (De Giusti*et al.,* 2010).

*Staphylococcus aureus*is a Gram-positive, catalase positive coccus, 0.5–1.5 µm in diameter that forms clusters of cells that appear as characteristic 'bunches of grapes' when viewed microscopically. The first link between *Staphylococcus aureus*and food poisoning has been ascribed to an outbreak associated with eating cheese. *Staphylococcus aureus also* has been spread a wide range of food vehicles including dairy product, cream-filled bakery, poultry and egg products as well as salads and canned mushrooms (Verdier*et al.,* 2009). *S. aureus* is also associated with enterotoxin-mediated food poisoning (Waters *et al.,* 2011&Spanu*et al.,* 2012). Consumption of foods contaminated with *S. aureus*can cause gastroenteritis, nausea, vomiting, diarrhea, and abdominal pain within 1-6 hrs post-consumption of contaminated foods (U.S. Food and Drug Administration, 1998). Meat was a substantial (11.2–25.0%) source of bacterial toxins produced such as *S. aureus, B. cereus* and *E. coli* (EFSA, 2007).The process of cooking should kill the bacteria but some bad practices of handling or storage can even increase the bacterial load of the initial product.

Recently, Kingdom of Saudi Arabia reported that Injury, poisoning and external causes group was the second most of common cause of death. The number of deaths due to this group was 8355 (18.05% of the total number of deaths) (Ministry of Health,2010).

Bacteriological method for detecting pathogens typically involves culturing the organism in selective media and identifying isolates according to their morphological, biochemical, and/or immunological characteristics. This method is sensitive and permits the specific detection of microorganism of interest in complex environments such as foods and certain clinical samples. However, the method is time consuming and usually requires 5-11 days (Kramer *et al.,* 2009).

Over the past 25 years, numerous rapid methods have been developed to reduce the assay time. To date, well studied rapid methods such as enzymelinked immune sorbent assay (ELISA) and polymerase chainreaction (PCR) have reduced the assay time to 10–24 h and 4–6 h,respectively, and have achieved detection limits varying from $10^1$to $10^6$cfu/ml (cfu = colony forming units). Recent years molecular methods are progressively used for identification of pathogenic bacteria caused food poising disease. Many of these methods are based on 16S ribosomal DNA sequences (16S rRNA gene) and develop either hybridization or PCR techniques (Janda and Abbott, 2007). The methods included 16S r RNA gene sequences can be utilized to place diagnostics into a phylogenetic structure and can be connected to databases providing several thousand sequences that increase day by day (Amann and Ludwig, 2000;Janda and Abbotte, 2007).

The advent of DNA amplification by the PCR method and its application has significantly improved the specificity, sensitivity and the time necessary for detection of microbial pathogens in the environment (Bej, 2003). PCR has also become a valuable tool for investigating food-borne outbreaks and identifying pathogens such as *Staphylococci, Salmonella and E.coli* (Riyaz*et al.*, 2004 &Iyer and Kumosani,2010). Masoud*et al.,* 2012 studied the bacterial communities in raw milk and in Danish raw milk cheeses using pyrosequencing of tagged amplicons of the V3 and V4 regions of the 16S rDNA and cDNA. Therefore, the objectives of the present study are to isolate and identify some types of bacterial food poisoning existing indiets at EL-taifrestaurants.

## 2- Material and Methods

### 2.1. Food samples

Twelve traditional food products were used in the present study as source for isolation of pathogenic bacteria. All samples (50) were collected from local restaurant in Taif City, Kingdom of Saudia Arabia. These samples were rice (5), meat (5), salad (11), chicken (7), Koshare (1), soup (5), bergar (1), pasta (2), samposa (5), grap paper (1), markok (3) and shawrma (4). Samples were immediately transferred under aseptic condition in ice box to the laboratory.

### 2.2. Isolation of bacterial strains

Serial dilutions (up to $10^6$) were done in peptone water.then about 15 mL of Nutrient or MacConky agar was poured. After incubation, individual colonies were selected according to their morphological differences such as color, shape and size then transferred into 10 mL sterile nutrient broth. According to the standard method of the microbiological examination, streak plate technique was applied in the present study to isolate and purify culture bacterial strains (ICMSF, 1996) in nutrient agar plates. Standard Biochemical tests were performed following standard procedures according to Sharp, (1979) & Cappuccino and Sherman, (1999)

### 2.3. Preliminary identification of isolates

The isolates were first confirmed to the genus level by colony and cell morphology, sporulation, activity of urease, oxidase and coagulase, vogusproskaure,, reaction of citrate and methyl red, nitrate reduction, casein and starch hydrolysis, indole and Gram reaction and acid formation (Cowan and Steel, 1970).

### 2.4. Partial sequencing of 16S rRNA gene

#### 2.4.1. DNA extraction from culturable bacteria

The genomic DNA of culturable bacterial isolates was extracted using QIAamp DNA Mini Kit (Qiagene) according to manufacture'sprotocol.

#### 2.4.2. DNA sequencing

The PCR-amplified 16S rDNA fragments were amplified using two universal primers; fD1 (5´ AGAGTTCCTGGCTCAG3´) and rP2 (5´ACGGCTCCTTACGACTT3´) (Weisburg*et al.*, 1991). The PCR product was purified using an DNA-purification kits as recommended by the manufacturer and then sequenced. PCR fragments were analyzed by cycle sequencing, using the BigDye terminator cycle sequencing kit (Applied Biosystems, U.K.). This sequence step was commercially carried out by Macrogen Inc., Seoul, South Korea, through 16S rDNA sequencing using universal primer, 518F (5' CCAGCAGCCGCGGTAATACG3') (Ahemad and Khan, 2010). The obtained partial nucleotide sequences of the Materials & Methods **35** 16SrRNA gene, were aligned using Clustal W from MEGA 4.0 software (Tamura *et al.*, 2007)and compared with the homologous sequences of the type strains, available in the GenBank database.

## 3. Results and Discussion

### 3. 1. Isolation bacteria from traditional food

Twelve traditional food products were used in the present study as source for isolation of pathogenic bacteria. All samples (50) were collected from local restaurant in Taif City, Saudi Arabia. Table (1) showed that there were a high variability in bacterial counts among all samples. Even on samples with particularly high number of bacteria e.g. fresh cut salad, processed meat and chicken and rice, no visible sign of defect was observed on them. Other food samples were obtained considerable amounts of bacteria. Among all twelve commodities, only three samples were scored considerable amounts of coliform bacteria (e.g. salad, shawrma and samposa).

The mean aerobic bacterial and coliform counts of fresh – cut salad obtained in this study were similar with that of a recent study conducted Food and Drug Administration in the united state. This study showed that the total plate counts ranged from 4 to 8.3 log cfu/g (Badosa*et al.,* 2008). Similar, study in Singapore was conducted by (Seow*et al.,* 2012). This result may be due to contaminated water and soil or improper handling, as well as culture and producing conditions (Abadias*et al.,* 2008). There was an especially problem when untreated manure is utilized as soil fertilizers in the fields (Aycicek*et al.,* 2006).

The positive contamination of previous food sample (meat and chicken) were in good agreement with the reports of (Isara*et al.,* 2010). Lotfi*et al*, (1988) reported that 75% of raw minced meat was contaminated with *Bacillus cereus*. The high incidence percentage may be attributed to the abundance of amino acids, vitamins and essential nutrients in meat. Fresh meats of beef, pork and chicken foods, have pH values within the growth range of most of the organisms (Jay,1986).

Examination of cooked rice in this study showed an unsatisfactory microbiological quality for aerobic bacteria analysis. However, all the rice samples studied exceeded the European maximum level of mesophilic aerobic counts (Sospedra*et al.,* 2012). Little *et al.,* (2002) also found high levels of aerobic colony counts in cooked rice. Some of raw vegetables can contribute the rice based dishes contamination to handling involved in preparation, the manufacturing processes and temperature control of the final products.

### 3.2. Preliminary identification

Thirty five of pure single colonies were preliminary characterized by some physiological and biochemical tests according to the criteria of Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 2000). Twenty isolates showed differences in their morphological and biochemical characters were selected for further work. These isolates were named from M1 to M20 and subjected for further molecular identification.

**Table1. Results of aerobic bacteria and Coliform counts in the analyzed samples.**

| Name of sample (NO.) | | Viable cell count CFU/g ($10^3$) | |
|---|---|---|---|
| | | Bacteria | Coliform |
| Rice | (5) | 75.6 | ND |
| Meat | (5) | 84.9 | ND |
| Salad | (11) | 96.8 | 4.4 |
| Chicken | (7) | 80.1 | ND |
| Koshare | (1) | 72.0 | ND |
| Soup | (5) | 33.2 | ND |
| Bergar | (1) | 42.1 | ND |
| Pasta | (2) | 61.2 | ND |
| Samposa | (5) | 25.5 | 3.3 |
| Grap paper | (1) | 62.1 | ND |

CFU: Cell Forming Units; ND not detected

**Table 2: Source of isolation, isolate numbers and some morphological and biochemical characters of the purified Bacilli.**

| Source of Isolation (No.) | Isolate No. | Cell shape | Gram Stain | Spore-forming | Aerobic growth | Indol | urease | Oxidase | Vogus proskaur | Citrate | Acid form | | Hydrolysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Xylose | Glucose | Casein starch |
| Rice (5) | M01 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| grap paper (1) | M07 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Selek meat (3) | M06 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Steam Meat (2) | M05 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Hot salad (4) | M09 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Samposa meat (3) | M08 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Chicken Kofta (4) | M11 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Chicken Kapap (3) | M10 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Tabola salad (1) | M03 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Soup (5) | M02 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |
| Kosheri (1) | M04 | Bacilli | + | + | + | - | - | + | + | - | - | + | + + |

**Table 3: Source of isolation, isolate numbers and some morphological and biochemical characters of the purified cocci and short rod.**

| Source of Isolation (No.) | Isolate No. | Cell shape | Gram Stain | Spore-forming | urease | Citrate utilize | Coagulase | Indol | Vogus proskaur | Methyl red | Oxidase | *Acid form | Acid form xylose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pasta (2) | M13 | Cocci cluster | + | - | - | ND | + | - | + | ND | - | + | - |
| Markok (3) | M16 | Cocci cluster | + | - | - | ND | + | - | + | ND | - | + | - |
| Shawrma (2) | M15 | Cocci cluster | + | - | - | ND | + | - | + | ND | - | + | - |
| Bergar (1) | M12 | Cocci cluster | + | - | - | ND | + | - | + | ND | - | + | - |
| Hot salad (2) | M14 | Cocci cluster | + | - | - | ND | + | - | + | ND | - | + | - |
| Capage salad (2) | M18 | Short rod | - | - | ND | - | ND | + | + | + | + | ND | ND |
| Green salad (2) | M17 | Short rod | - | - | ND | - | ND | + | + | + | + | ND | ND |
| Shawrma (2) | M20 | Short rod | - | - | ND | - | ND | + | + | + | + | ND | ND |
| Samposa (2) | M19 | Short rod | - | - | ND | - | ND | + | + | + | + | ND | ND |

ND: Not Detected; *Acid form (Manitol – Fractose – Sucrose

All 20 isolates were divided into three groups, Gram-positive (G+*ve*) bacilli, (G+*ve*) cocci and Gram- negative (G-*ve*) short rod. Results of the preliminary tests of the first group were summarized and presented in Table (2). This group contained eleven isolates, which were (G+*ve*) bacilli shape positive oxidase and VP, negative urease,indole and citrate. The preliminary tests of the second (G+*ve* cluster cocci) and third (G-*ve* short rod) groups were listed in Table (3). Only Short rod isolates were positive MR and negative Citrate, other isolates were not determined.G+*ve* bacteria were showed positive coagulase test, fermented all tested sugar and produced acid form except xylose. Other strains were not determined. Noticeably, salad was the most contaminated food which contained all cell shape (cocci, bacilli and short rod).

### 3.3. Molecular Identification

Twenty strains (M01-M20) were identified by determine and analysis of the partial sequence of 16S rRNA gene. The species were initially determined by the BLAST program on NCBI (http://www.ncbi.nim.nih.gov/) based on the 16S rRNA sequences of type strains. The identity and coverage Percentage were presented in Table 4. The isolate M18 showed 100% identity and coverage with type strain *Escherichia coli* strain mohi KC013977. The identity percentage of the other isolates were 99%, except the isolates, M07, which recorded identity percentage of 92% with *Bacillus subtilis* strain p29-D09 (JQ35773); M09, which recorded percentage of 85% with *Bacillus circulans*strain BP9_5B (JN644554); M10, which recorded percentage of 90% with *Bacillus subtilis* strain M50 (JX102496); M12, which recorded percentage of 87% with *Staphylococcus aureus* strain 518F (VITSV4), and M13, which recorded percentage of 92 % with *Staphylococcus aureus* strain ET-1 (JX163860). Therefore these isolates were considered as new species and named: *Bacillus sp.* Strain, M07; M09&M10 and *Staphylococcus sp.* Strain,M12 and M13. Similar cases were reported and discussed previously by Drancourt *et al*, (2000). They stated that 99% identity in 16S rRNA gene sequence was the criterion used to identify an isolate to the species

level. A 97 to 99% identity in 16S rRNA gene sequence was the criterion used to identify an organism at the genus level, and <97% identity in 16S rRNA gene sequence was the criterion used to define a potentially new bacterial species. The phylogenetic tree was constructed using *Lactobacillus paraplantarum*(NR- 025447) as out of group element. The resulted phylogenetic tree (Fig.1) was composed mainly of four clusters (Cluster I – IV)

Cluster: I composed of two sub-cluster, (A) that contained *Bacillussubtilis*strain (M05) and three *Bacillus sp* strains,(M07, M09 and M10) with the type strain jQ835773. (B) *Bacillus subtilis*group that included,(M02, M04 and M06 with the type strain, KC443103.

Cluster: II composed of one sub-cluster (c) contained *Bacillus* group that included four *Bacillus circulans* isolates M08, M01, M11 and M03, at high similarity (98-100%) with the reference strain, JN644554.

Cluster: III composed of one sub-cluster (D) that included three *Staphylococcsaures* (M14, M15, and M16), at high identity percentage (99%) with the reference strain HE579073 in addition to two strains, *Staphylococcs sp.* M12&M13 with 87-92% identity respectively.

Cluster: IV.This cluster contained four *Escherichiacoli* isolates; M17,M18, M19, and M20 that share high similarity (99-100%) with type strain KC013977 located in the same sub-cluster (E).

After the molecular identification, it can be stated that the isolates recovered from traded diets in restaurants of Taif-city are: *Bacillus Subtilis* (4 strains), *BacillusSP*. (3 strains), *Bacillus circulans* (4 strains), *Staphylococcus aureus* (3 strains), *StaphylococcusSP*. (2 strains), and *Escherichiacoli* (4 strains) Table 5.The Source of obtained isolates were differ from processed and unprocessed food table 5. *Bacillus circulans*isolated from rice, meat and chicken samples, while Ithas been isolated previously from different rice products (Fango*et al.,* 2010 & Kim *et al.,* 2013). In addition, Most of *Bacillus subtilis* strains were isolated from soup samples. *Staphylococcus aureus*was occurred in bergar and shawerma chicken. These results are in agreement with Previous studies indicated that, *Staphylococcus aureus*has been isolated from chicken sandwish and chocolate (Iayer and Kumosani, 2010). Half isolates of *Escherichiacoli* were isolated from unprocessedfood. Same finding has been previously demonstrated by(Yossa*et al.,* 2010). They reported that most of Consumption of refrigerated ready – to- eat, fresh cut fruits and vegetables, often eaten with minimal processing, were a potential source of *Escherichiacoli.* On the other hand, Sospedra*et al.* (2013) stated that *Escherichiacoli Staphylococcus aureus*were also found in several vegetable dishes, *E. coli* was detected in 6.6% and 0.7 % of lettuce samples. Salad ingredients were eaten fresh vegetables without cooking processes and lettuce was done of the most contaminated sample.

**Table 4.Identity and coverage percentage according to the obtained 16S rRNA sequence.**

| Isolate No. | Name and Accession No. of the most related strain in NCBI GeneBank | | % Identity | % Coverag e | Suggested Name of the isolates obtained in this work |
|---|---|---|---|---|---|
| M01 | *Bacillus circulans* strain BP9_5B | JN644554.1 | 99 | 100 | *Bacillus circulans s* M01 |
| M 02 | *Bacillus subtilis* strain VRC08 | JX082288 | 99 | 100 | *Bacillus subtilis* M02 |
| M03 | *Bacillus circulans* strain BP9_5B | JN644554 | 99 | 100 | *Bacillus circulans* M03 |
| M04 | *Bacillus subtilis* strain: GS1 | AB773829 | 100 | 99 | *Bacillus subtilis,* M04 |
| M05 | *Bacillus subtilis* strain Sua-BAC018 | EU870513 | 97 | 100 | *Bacillus subtilis* strain M05 |
| M06 | *Bacillus subtilis* strain BAB-2438 | KC443093 | 100 | 99 | *Bacillus subtilis* strain M06 |
| M07 | *Bacillus subtilis* strain p29_D09 | JQ835773 | 92 | 100 | *Bacillus sp.* M07 |
| M08 | *Bacillus circulans* strain BP9_5B | JN644554 | 99 | 99 | *Bacillus circulans*strain M08 |
| M09 | *Bacillus circulans*strain BP9_5B | JN644554 | 85 | 100 | *Bacillus sp.* M09 |
| M10 | *Bacillus subtilis* strain M50 | JX102497 | 90 | 100 | *Bacillus sp.*M10 |
| M11 | *Bacillus circulans* strain BP9_5B | JN644554 | 98 | 100 | *Bacillus circulans*strain M11 |
| M12 | *Staphylococcus aureus* strain 518F | VITSV4 | 87 | 58 | *Staphylococcus sp.* M12 |
| M13 | *Staphylococcus aureus* strain ET-1 | JX163860 | 92 | 99 | *Staphylococcus sp.* M13 |
| M14 | *Staphylococcus aureus subsp. aureus* ST228 | HE579073 | 99 | 99 | *Staphylococcus aureus subsp. aureus* M14 |
| M15 | *Staphylococcus aureus subsp. aureus* ST228 | HE579073 | 99 | 99 | *Staphylococcus aureus subsp. aureus* M15 |
| M16 | *Staphylococcus aureus subsp. aureus* ST228 | HE579073 | 99 | 100 | *Staphylococcus aureus subsp. aureus* M16 |
| M17 | *Escherichia coli* strain moh1 | KC013977 | 99 | 98 | *Escherichia coli* M17 |
| M18 | *Escherichia coli* strain moh1 | KC013977.1 | 100 | 100 | *Escherichia coli* strain M18 |
| M19 | *Escherichia coli* strain moh1 | KC013977 | 99 | 100 | *Escherichia coli* M19 |
| M20 | *Escherichia coli* strain moh1 | KC013977 | 99 | 100 | *Escherichia coli* M20 |

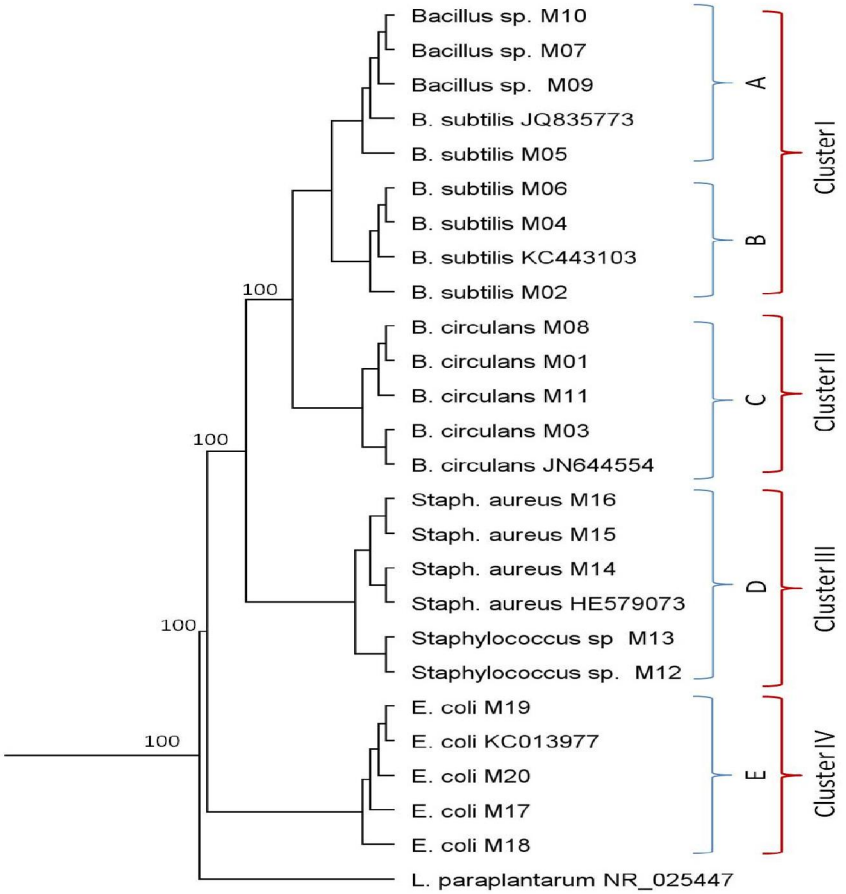


**Fig 1. Neighbor- joining phylogenetic tree based on 16s rRNA sequence using *Lactobacillus paraplantarum (NR-025447.)* as out of group. The tree showing the phylogenetic placement of the strains (M01 – M20) Isolated from traded diets In restaurants at EL- Taif city.**

**Table 5.Sources of isolation and suggested name of bacterial strains given according to the partial sequencing of 16S rRNA gene.**

| Strain name given after partial 16SSource of isolation (No.)rRNA sequencing | | | |
|---|---|---|---|
| *Bacillus circulans* | M01 | Rice | (5) |
| *Bacillus circulans* | M03 | Tabola salad | (1) |
| *Bacillus circulans* | M08 | Samposa meat | (3) |
| *Bacillus circulans* | M11 | Chickens kofta | (4) |
| *Bacillus subtilis* | M02 | Soup | (5) |
| *Bacillus subtilis* | M04 | Koshari | (1) |
| *Bacillus subtilis* | M05 | Steam meat | (2) |
| *Bacillus subtilis* | M06 | Selek meat | (3) |
| *Bacillus sp.* | M07 | Grap paper | (1) |
| *Bacillus sp.* | M09 | Hot salad | (4) |
| *Bacillus sp.* | M10 | Chicken kappa | (3) |
| *Staphylococcus sp.* | M12 | Berger | (1) |
| *Staphylococcus sp.* | M13 | Pasta | (2) |
| *Staphylococcus aureus subsp. aureus* | M14 | Hot Salad | (2) |
| *Staphylococcus aureus subsp. aureus* | M15 | Shawarma | (2) |
| *Staphylococcus aureus subsp. aureus* | M16 | Markok | (3) |
| *Escherichia coli* | M17 | Green salad | (2) |
| *Escherichia coli* | M18 | Capage salad | (2) |
| *Escherichia coli* | M19 | Samposa meat | (2) |
| *Escherichia coli* | M20 | Shawarma | (2) |

**References**


1. Ahemad, M. and Khan.M.(2010). Phosphate solubilizing and plant-growth-promoting *Pseudomonas aeruginosa*PS1 improves green gram performance in quizalafop-p-ethyl and clodinafop amended soil. Arch. Environ. Contam. Toxicol.58,361-372.
2. Amann R. and Ludwig W. (2000). Ribosomal RNA-targeted nucleic acidprobes for studies in microbial ecology. FEMS Micro..Rev 24, 555 – 565
3. Aycicek H., Oguz U. and Karci K. (2006). Determinationof total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey Inter. J. Hyg.and Environ. Health.209, 197–201.
4. Bej A. K. (2003). Molecular based methods for the detection of microbial pathogens in the environment. J. Micro. Methods53,139-140.
5. Beuchat L. (1996). Pathogenic microorganisms associated with fresh product.J. Food Prot. 59,204-216.
6. Cappuccino J.G. and Sherman N. (1999). Microbiology: A LaboratoryManual,. Addison Wesley Longman, Inc Harlow.4, 199–204
7. Chattaway M., Dallman T., Okeke I. and Wain J. (2011). Enteroaggregative*E. coli O104* from an outbreak of HUS in Germany 2011, could it happen again? J. Infect. Dev. Ctries. 5,425-436.
8. Cowan S.F and Steel K.J.(1970). Manual for the Identification of the MedicalMicroorganism. Cambridge: Cambridge University Press.1, 7-122.
9. De Giusti, M., C. Aurigemma L. , Marinelli D., Tufi D, De Medici S., Di-Pasquale C. De Vito and Boccia A.(2010). The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy. J. Appl. Micro. 1996-1006.
10. Drancourt M., C. Bollet A. Carlioz R. Martelin J. P. Gayral and D. Raoult (2000). 16S ribosomal sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J. Clin. Micro. 38,3623-3630
11. EFSA (2007). Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on monitoring of verotoxigenic*Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. The EFSA J. 579, 1-61
12. Fangio M.F., Roura S.I.and Fritz R.(2010). Isolation and identification of *Bacillus spp.* and related genera from different starchy foods.J. Food Sci.75, 218-21.
13. Harmon S., Kautter D. and Solomon H. (1987). *Bacillus cereus* contamination of seeds and vegetable sprouts grown in a home sprouting kit. J. Food Prot. 50, 62-75.
14. Holt J., Krieg N., Sneath P., StanleyJ. and Williams S. (2000) Aerobic Microaerophilic, Motile, Helical/Vibrioid Gram-negative Bacteria. In: Bergey's Manual of Determinative Bacteriology. Williams & Wilkins.9,41-47
15. Hughes C., Gillespie I.A., O'Brien S.J. and The Breakdowns in Food Safety Group,(2007). Foodborne transmission of infectious intestinal disease in England and Wales, 1992-2003 Food Control. 18, 766-772.
16. Hutchison M. L., Avery S. M. and Monaghan J. M. (2008). The air-borne distribution of zoonotic agents from livestock waste spreading and microbiological risk to fresh produce from contaminated irrigation sources. J. Appl. Micro. 105, 848-857
17. ICMSF International Commission on Microbiological Specification for Foods). (1996). Microorganisms in foods, Characteristics of microbial pathogens. Blackie Academic & Professional.5, 513 -515
18. Isara A., Isah E., Lofor P. and Ejide C. (2010). Food contamination in fast food restaurants in Benin City, Edo State, Nigeria: Implications for food hygiene and safety. Public Health. 124, 467-471.
19. Islam M., Jahiruddin M. and Islam S.(2004). Assessment of arsenic in the water-soil- plant systems in gangetic flood plains of Bangladesh. Asian J. Plant Sci. 3, 489-493.
20. Janda J. and Abbott S. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J. Clin Micro. 45, 2761–2764.
21. Jay J. M. (1986). Modern food microbiology (3rd ed.). Wayne, UK: Van Nostr and Reinhold.1, 214, 432.
22. Kim J., Marshall M.R. and Wei C.-I., (1995) Antibacterial activity of some essential oil components against five foodborne pathogens.J. of Agric. and Food Chemistry. 43,2839-2845.
23. Kim J.G., LuoY. and Gross K.C.(2004). Effect of package film on the quality of fresh-cut salad savoy. Post harvest Biol. Technol. 32, 99-107
24. Kramer M., Obermajer N., Matijasic B.B., Rogelj I. and Kmetec V.(2009). Quantification of live and dead probiotic bacteria in lyophilised product by real-time PCR and by flow cytometry. Appl. Genetics and Molecular Biotechnol. 84, 1137-1147.
25. Little C.L., Barnes J. and Mitchell R.T..(2002). Microbiological quality of take-away cooked rice and chicken sandwiches: effectiveness of food hygiene training of the management.

Communicable disease and public health/PHLS. 5, 289-298
26. Lotfi A.,Youssef H., El Khateib T., Seddik I., El TimawyA.and Ali, G.(1988). Incidence of *Bacillus cereus* in meat products.Assiut Veterinary Medical J. 20, 110-114.
27. Iyer A. and Kumosani T.(2010).PCR based Detection of Food Borne Pathogens World Academy of Science, Eng. and Technol. 44,699-701.
28. Masoud W., Vogensen F. K., Lillevang S., Abu Al-Soud W., Sørensen S. J. and Jakobsen M. (2012). The fate of indigenous microbiota, starter cultures, *Escherichia coli, Listeria innocua*and *Staphylococcus aureus*in Danish raw milk and cheeses determined by pyrosequencing and quantitative real time (qRT)-PCR. Inter. J. Food Micro.153,192-202
29. Mensah P., Yeboah-Manu D., Owusu-Darku K. and Ablordey A. (2002). Street Food in Accra, Ghana: how safe are they? Bulletin of the World Health Organization. 80, 546-556
30. Ministry of Health. (2010). Health Statistical Year Book. 1,196-199.Saudi Arabia.
31. Priest F.G. (1993).Systematics and ecology of *Bacillus*. In: *Bacillus subtilis* and other Gram-positive bacteria - Biochemistry, physiology, and molecular genetics. American Society for Micro.1, 3-16.
32. Riyaz S., Verma V. and Qazi G. N. (2004). Rapid detection of *Salmonella* by polymerase chain reaction. Mol. Cell Probes.18, 333-339
33. Saleh Y., EL –Fouly M., Khalil M. and ABO-State M.(1993). Incidence And Characterization Of *Bacillus Cereus*Islolated From Egypt Food. Qatar Univ. Sci.J. 13,75-80
34. Seow J., Ágoston R., Phua L. and Yuk H. (2012). Microbiological quality of fresh vegetables and fruits sold in Singapore. Food Control. 25, 39-44
35. Sharpe M.E. (1979). Identification of the lactic acid bacteria. Identification method for Microbiologists. Society for Appl. Bacteriology Technical Series1,220 -223
36. Soliman K. M. and Badeaa R. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol. 40, 1669-1675.
37. Sospedra J., Mañes J. and SorianoM. (2013). Report of toxic shock syndrome toxin 1 (TSST-1) from *Staphylococcus aureus* isolated in food handlers and surfaces fromfood service establishments. Ecotoxicology and Environmental Safety. 80, 288-290.
38. Spanu V., Spanu C., Virdis S., Cossu F., Scarano C. and De Santis E.P.(2012) Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. Int. J. Food Micro. 153,53-7.
39. Tamura K., Dudley J., NeiM.and Kumar S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version.
40. U. S. Food And Drug Administration.(1998) Department of Health and Human Services. Secondary Direct Food Additive For Human Consumption. 21 CFR. 173,1-300
41. Verdier I., Michel V., Delbes C. and Montel M.C. (2009). Do milking practices influence the bacterial diversity of raw milk.Food Micro. 26, 305-310.
42. Waters A.E., Contente C. T., Buchhagen J., Liu C.M., Watson L., Pearce K., Foster J.T., Bowers J.M., Driebe E.M., Engelthaler D.M., Keim P.S. and Price L.B.(2011). Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clinical Infectious Dis. 52, 1227–1230
43. WHO (World Health Organization.)(2002) Methods for foodborne disease surveillance in selected sites - report of a WHO consultation 18-21 March 2002, Leipzig, Germany., WHO/CDS/CRS/EPH/2002.22 (Geneva, Switzerland).
44. Yosaa N., Patel J., Miller P & Martin Y. (2010). Antimicrobial activity of essential oils against *E. coli*. In organic soil. J. Food Control.21, 1458-1465.

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