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Characterization of Bacterial Communities in Palestinian Lamb Meat by Phenotyping and 16S rRNA Gene Sequence Analysis

توصيف المجتمعات البكتيرية في لحم الضأن الفلسطيني بواسطة التنميط الظاهري و تحليل جين S rRNA16

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Abstract: The main purpose of the present study was to isolate, identify and quantify bacteria in Palestinian fresh lamb meat. Phenotyping and 16S rRNA gene sequence analysis was used to identify bacteria present in lamb meat samples. Thirty-four bacterial isolates were obtained from 20 samples of fresh lamb meat collected from 4 meat shops in Tulkarem city in Palestine. Bacterial counts were in a range of 3 x 103 - 1.5 x 105 cfu / g with Staphylococcus aureus being the highest in numbers among other bacteria. Enterobacteriaceae and Staphylococ-caceae were the predominant bacterial families detected in fresh lamb meat samples. Two bacterial isolates, which were not identified by phenotyping, were identified by 16S rRNA gene sequence analysis. There was an agreement between phenotyping and 16S rRNA gene sequencing in identification of 19 bacterial isolates. On the other hand, a disagreement was observed between phenotyping and 16S rRNA gene sequencing in identification of the remaining bacterial isolates. Fresh lamb meat seems to be a good medium for growth of various bacterial species.

Keywords: Fresh lamb meat, 16S rRNA gene sequencing, Phenotyping, Staphylococcus aureus, Enterobacteriaceae, Staphylococ-caceae.

المستخلص: الهدف الرئيسي من هذه الدراسة هو عزل و تحديد و قياس البكتيريا في لحم الضأن الفلسطيني الطازج. تم استخدام التنميط الظاهري و التحليل الجيني S rRNA16لتحديد البكتيريا الموجودة في عينات لجم الضأن. تم الحصول على 34 عزلة بكتيريا من 20 عينة من لحم الضأن الطازج جمعت من 4 محلات لبيع اللحوم في مدينة طولكرم. تراوحت اعداد البكنيريا من 3000 الى 150000 خلية / غرام و كانت بكتيريا Staphylococcus aureus هي الاعلى من حيث العدد مقارنة بانواع البكتيريا الاخرى. كانت بكتيريا Enterobacteriacea و Stapylococcaceae هي البكتيريا السائدة الموجودة في لحم الضأن الطازج. تم التعرف على اثنتين من العزلات البكتيريا عن طريق التحليل الجيني و التي لم يتم التغرف اليها عن طريق التنميط الظاهري. كان هناك توافق بين النميط الظاهري و التحليل الجيني في تحديد انواع 19 عزلة بكتيرية. من ناحية اخرى، كان هناك اختلاف بين التنميط الطاهري و التحليل الجيني في تخيد انواع العزلات المتبقية. تبين من الدراسة ان لحم الضأن الطازج هو بيئة غذائية جيدة لنمو انواع كثيرة من البكتيريا.

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الكلمات المفتاحية: لحم الضأن الطازج، تحليل الجين S rRNA16، التنميط الظاهري، Enterobacteriaceae، Staphylococcus aureus.

INTRODUCTION:

Fresh red meats, especially lamb meat is considered a high-energy type of food with rich nutritional value, which makes it one of the main items in our meals (Jamilah, Abbas, and Rahman, 2008). Fresh lamb meat provides an important source of proteins and a large number of vitamins and minerals (Jamilah et al., 2008). Lamb meats, by their nature, are easily me-tabolized and therefore offer suitable substrates for the growth and metabolism of microorganisms (Thanigaivel and Anandhan, 2015). The microorganisms that eventually cause the spoilage of flesh foods are either present at the time of slaughter or introduced by workmen and their cutting tools, or by water and air in the dressing, cooling and cutting rooms (Newman, 2005).

The bacterial growth that causes fresh meat spoilage is influenced by intrinsic and extrinsic factors. Intrinsic factors include expression of the physical and chemical properties of the meats themselves (Bruckner, Albrecht, Petersen, and Kreyenschmidt, 2012). Intrinsic factors comprise water activity, the structure of the meats, the pH value, and the initial content of psychrotrophic bacteria present on the surface of the meat (Bruckner et al., 2012). Extrinsic factors include storage conditions i.e., storage tem-perature and availability of oxygen (Conforti, Statti, Uzunov, and Menichini, 2006). The most common bacteria in fresh meat include the genera of Aci-netobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus and Micrococcus, lactic acid bacteria and various genera of the Enterobacteriaceae family (Pennacchia, Ercolini, and Villani, 2011). Staphylococci, Corynebacterium, Streptococci, Micrococcus, Salmonella, Escherichia coli, and yeast have been isolated from fresh lamb meat (Mostafa et al., 2018). In another study, it was reported that the most common bacteria found in fresh meat were bacteria of the genera Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus and Micrococcus, lactic acid bacteria and various genera of the Enterobacteriaceae family (Pennacchia et al., 2011).

Lamb meat has a short shelf-life of about one day or less at ambient temperature (15-30°C), and a few days at refrigerating temperature (0-10°C) (Lucera, Costa, Conte, and Nobile, 2012). Identification of bacterial populations in fresh lamb meat can help in controlling meat spoilage and increase its shelf life. To our knowledge, no studies have been carried out to identify the bacterial content of fresh lamb meat in Palestine.

Determining the nucleotide sequence of a defined region of the chromosome is a precise method for the identification and typing of microorganisms (Malhotra, Sharma, Njk, Kumar, and Hans, 2014). The rRNA genes are necessary for the continued existence of all microorganisms and highly conserved in the bacterial kingdom (Yoon et al., 2017). Phenotypic identification of bacterial species using enzymes activity, or other protein production is usually difficult and not always reliable; due to the similarity and interference of these properties between members of bacterial families (Mezzatesta, Gona, and Stefani, 2012). The major advantage of the Analytical profile index 20E (API 20E) system is that it is a more

convenient, rapid and easy method to identify gram negative bacteria than the conventional tests (Juang and Morgan, 2001). Other chemicals like hydrogen peroxide (H2O2) as catalase reagent, and human plasma for coagulase reaction are mainly used to identify the gram-positive bacteria (Jahan, Rahman, Parvej, Ziqrul, and Chowdhury, 2015). In the present study, phenotyping of bacteria in fresh lamb meat was used as a preliminary identification, which was then confirmed by 16S rRNA gene sequence analysis of bacterial DNA.

The main objective of the present study was focused on screening of bacterial content of fresh lamb meat using phenotyping and 16S rRNA gene sequence analysis.

MATERIAL AND METHODS:

Samples of fresh lamb meat:

Twenty samples of 100 gm weight of fresh lamb meat, which were obtained after slaughtering in Palestine, were collected from 4 different butcher shops of the local market in Tulkarem city. Each sample was placed in a plastic sterile bottle and placed in a refrigerator bag. Samples were then transferred to the laboratory for microbiological analysis within one hour or refrigerated at 4°C and analyzed within 24 hours (Thanigaivel and Anandhan, 2015).

Samples processing and quantification of bacteria:

Samples were aseptically cut into thin smaller pieces of 10 gm each. Each piece was submerged in a sterile tube that contained 90 ml of sterile diluent saline peptone (SPO) [0.1 % bactopeptone (Difco, Detroit, MI, USA), 0.85 % (w/v) NaCl (Merck, Darmstadt, Germany), 0.03 % Na2H2PO4, 2H2O (Merck). The tubes were shaken vigorously by using the vortex (Thanigaivel and Anandhan, 2015). Ten-fold dilutions (10-1) were prepared for each sample and spread 1 µl on each type of culture media, which included blood agar, MacConkey agar, and chocolate agar. The cultured samples were incubated at 37°C for 24 h under aerobic or anaerobic conditions using candle jars, and the number of colony forming units (CFU) was recorded from a suitable dilution of each sample.

Isolation of bacteria:

Characterization and identification of the bacterial isolates were done by initial morphological examination of the colonies (macroscopically), which included colony appearance, size, elevation, form, edge, consistency, color, odor, opacity, hemolysis and pigmentation. A colony from each group of colonies that has the same properties was subcultured on its specific medium (Nagarajan, Wahab, and Alex, 2018).

Phenotyping of bacterial isolates:

The analytical profile index 20E kit (API 20E), (Biomerieux, 20 100, France) was used to provide a fast identification system for the gram negative Enterobacteriaceae and other non-fastidious gram-negative rods. Other chemicals were used to identify the gram-positive bacteria like hydrogen peroxide (H2O2) as catalase reagent, and human plasma for coagulase reaction (Jahan et al., 2015). These tests were done by emulsifying one colony of each gram-positive isolate with one drop of catalase reagent and monitoring the vigorous bub-bling occurring; to identify if an isolate is a Streptococcus or Staphylococcus

(Reiner, 2016). Further-more, another colony from each sample was mixed with one drop of human plasma, and the coagulation was monitored to identify isolates that are Staphylococcus aureus (coagulase +ve) or other bacterial species (coagulase -ve) (Varghese and Joy, 2014). Identification of coagulase negative Staphylococci like, Staphylococcus saprophyticus was performed by monitoring the presence or absence of hemolysis on the blood media (Martison, Fávero, Lia, Lourdes, and Souza, 2012). Novobiocin (5 µg) disc was used to check the resistance or susceptible of coagulase negative Staphylococcus bacteria on the Muller Hinton media (Pailhoriès et al., 2017). Resistance was defined as the presence of an inhibition halo ≤ 12 mm or the absence of a halo (Martison et al., 2012). The identification of S. saprophyticus was performed based on Novobiocin resistance and absence of hemolysis (Martison et al., 2012).

Sequence analysis of the 16S rRNA gene:

Extraction of deoxyribonucleic acid (DNA):

To extract DNA from bacterial isolates, 3 extraction protocols were used. In the first protocol, 2 colonies of an overnight bacterial culture were placed in an Eppendorf tube filled with 1ml of UltraPure DNase/RNase-Free Distilled Water and boiled for 10 minutes in a water bath, and then centrifuged for 5 minutes at 1,000 rpm (Dashti, Dashti, and Jadaon, 2014). In the second protocol, 2 colonies of an overnight bacterial culture were dissolved in 500 µl UltraPure DNase/RNase-Free Distilled Water and were placed in a Solo Microwave (MS23F301TAK, Malaysia) for 10 seconds, followed by centrifugation for 2 minutes at 1000 rpm (Dashti et al., 2014). In the third protocol, the heat shock procedure of Jose and Brahmadathan (2006) was used by suspending one colony of each bacterium in 50 µl of Ultrapure DNase/RNase-Free Distilled Water in a PCR tube and placed in a PCR machine (Smart Gradient PCR B960) that was adjusted to 94°C for 5 minutes, followed by cooling on ice for 3 minutes and centrifuged for 3 minutes at 1,000 rpm (Jose and Brahmadathan, 2006).

Polymerase chain reaction (PCR):

The Polymerase Chain Reaction-Ready™ (PCR-Ready™) High Specificity kit (Syntezza com-pany, PCR-S-192, Jerusaleml) was used to amplify DNA in PCR technique. According to the manufacturer instructions, a total volume of 25 µl of diluted primers (0.5 µM of each primer) and template DNA were added to the PCR Ready™ tubes. The reaction mixture was composed of 11 µl of forward primer U968-GC (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGA GAA GAA CCT TAC-3), 11 µl of reverse primer L1401 (5'-GCG TGT GTA CAA GAC CC-3'), and 3 µl of bacterial DNA. A total of 30 PCR cycles were per-formed in 0.2 ml tubes with a Fast Thermal Cycler (Applied Biosystems™ Veriti™96-Well machine) with the following temperature profiles: 94oC for 3 min followed by 94oC for 30 s, 56oC for 30 s and 68oC for 60 sec for 30 cycles; then the PCR products were terminated at 68oC for 10 min.

To check for PCR products, 5 µl of each PCR product were separated to 2 % agarose mixed with Gel Red TM Nucleic (cat. 41003, US). The gel was run at 100 Volt for 2 h in 0.5 X Tris-boric acid-EDTA (TBE) buffer (45 mM Tris-base, 89 mM boric acid, 2.5 mM EDTA pH 8.3). DNA molecular marker (1Kb DNA Ladder

RTU, Cat. DM010-R500, Gene DireX) was used as a standard. The gel was examined with a UV transilluminator and photographed.

Sample sequencing and database Search:

The PCR products were purified using Norgen PCR Purification Kit (cat. 14400, Biotek Corporation). According to the manufacturer's instructions, five volumes of Binding Buffer were added directly to the tube containing the PCR reaction (approximately 100 µl) and mixed well by Vortexing. Every spin column assembled with one of the provided collection tubes, and the samples were applied to the column and centrifuge for 1 minute at 8,000 rpm, the flowthrough was discarded and reassembled the spin column again with its collection tube. After that, 500 µl of Wash Solution was added to column and centrifuged for 1 minute at 10,000 rpm, the flowthrough was discarded and reassembled the spin column with its collection tube, the column was spindled for 2 minutes at 14,000 rpm; in order to thoroughly dry the column. Finally, the column assembled with one of the provided 1.7ml Elution tubes, 50 µl of Elution Buffer added directly to the center of the column bed, not onto the side of the column to obtain the best DNA recovery, the samples were stood at room temperature for 1 minute, centrifuged for 2 minutes at 14,000 rpm. The PCR purified products were sent to the Molecular Genetics Laboratory in Al-Istishari Arab Hospital in Ramallah, Palestine for sequencing. A database search was performed for the obtained sequences using the BLAST software (National Center for Biotechnology Information, Maryland, USA), and the identified sequences were deposited in the gene bank database. Accession numbers were obtained for all sequences.

RESULTS:

Identification of the bacterial isolates by phenotyping:

Of the 20 collected samples of lamb meat, a total of 34 bacterial isolates were obtained and identified. Thirty-two of the obtained isolates were identified by phenotyping (Table 1). The identified bacteria included 4 isolates of Staphylococcus aureus, 3 of Staphylococcus saprophyticus, 4 of Staphylococcaceae family, 1 of Klebsiella pneumoniae ssp. ozaenae, 5 Cedecea lapagei, 1 Enterobacter gergoviae, 3 Enterobacter cancerogenus, 1 Escherichia fergusonii, 2 Proteus vulgaris, 2 Klebsiella oxytoca, 2 Enterobacter cloacae, 1 Hafnia alvei, 1 Salmonella choleraesuis, 1 Klebsiella pneumoniae and 1 Pseudomonas fluorescens/putida. Four isolates of Staphylococcus were identified only at the genus level. Two-gram negative bacterial isolates were not identified using the biochemical tests (Table 1).

Sequence analysis of 16S rRNA gene:

Three extraction protocols of DNA were applied for all 34 isolates. DNAs of gram-positive bacteria were isolated successfully using the three extraction protocols, whereas some gram-negative bacterial DNAs were obtained by some protocols and failed in the others (Figure 1). With the exception of 4 isolates of Cedecea lapagei and one isolate of Klebsiella pneumoniae, all bacterial DNAs were successfully extracted using PCR-heat shock. Figure 1 shows the distribution of the extracted bacterial DNA through all protocols. It was possible to extract DNAs of 29 isolates using PCR-heat shock. DNAs from 17 isolates were extracted by Microwave irradiation. However, only DNAs from 11 isolates were obtained using the boiling protocol. Those differences in the obtained DNA among the bacterial species using the 3 protocols

might be due to the physiological characteristics of the bacteria species such as the constitution of the cell wall, the physiological state which the cell is in or cell concentration.

All the 34 sequences of 16S rRNA gene obtained from the DNA of the bacterial isolates were deposited in the Gene bank database. The identified isolates are shown in Table 1. The 2 isolates, which were not identified by phenotyping, were identified as Acinetobacter lwoffii in homologies of 99.80 % and 99.50 %. Gram-positive bacteria (4, 5, 14, and 30), which were only identified at the genus level by phenotyping, were identified by the 16S rRNA gene sequence analysis as S. edaphicus, S. haemolyticus, S. edaphicus, Macrococcus epidermidis, respectively. Furthermore, differences in the phenotyping and molecular identification of 9 isolates were observed (Table 1). Biochemical tests for bacterial isolates 7, 15, 16, 18, 20, 22, 28, 29, and 33 showed that they belong to K. pneumoniae ssp ozaenae, E. fergusonii, Cedecea lapagei, K. oxytoca, E. cloacae 1, Enterobacter cloacae 2, K. oxytoca 2, P. fluorescens/putida, and E. cancerogenus, respectively. On the other hand, sequencing of 16S rRNA gene for the same isolates showed that they belong to E.r cancerogenus, E.r tabaci, E. xiangfangensis, E. cancerogenus 2, E. hormaechei, E. hormaechei, E. hormaechei, P. helmanticensis, and Pluralibacter gergoviae, respectively (Table 1).

Quantification of bacteria in lamb meat samples:

Bacterial counts in fresh lamb meat samples were in a range of 3 x 103 - 1.5 x 105 cfu / g, with S. aureus being the highest in numbers among other bacteria (Table 2). Some bacteria like Staphylococcus spp., Cedecea lapagei and Enterobacter spp. were isolated from more than 2 meat samples. Other bacteria were isolated only from one or two meat samples, like P. vulgaris, A. lwoffii, S. enterica / choleraesuis, and Pseudomonas helmanticensis.

DISCUSSION:

In the current study, 34 bacteria were detected in fresh lamb meat samples (Table 1), which indicates that meat is a rich medium for growth of spoilage and pathogenic bacteria. Staphylococcus spp., Cedecea lapagei and Enterobacter spp. were the predominant bacteria in lamb meat samples. Enterobacter spp. and Pseudomonas spp. were found to be among the predominant bacteria in lamb meat (Wang et al., 2019). Ahmed and Sabiel. (2016) reported that the members of the family Enterobacteriaceae are usually associated with the contamination of meat products and their incidence in meat was considered as a public health problem. In the present work, S. aureus was the most frequent bacterium present in meat samples. Staphylococcus aureus has the ability to colonize raw meat and spread into meat products during the different processing stages of the meat supply chain (Velasco, Quezada-Aguiluz, and Bello-Toledo, 2019). The pathogenicity of S. aureus is due to its structure and secondary metabolites, among which are toxins that could cause staphylococcal diseases transmitted by contaminated meat (Velasco et al., 2019).

Table (1): Bacterial isolates from lamb meat samples identified by biochemical tests and 16S rRNA gene sequence analysis.

Isolates No.	Biochemical Identification	Sequencing of 16SrRNA gene	Homology %*	Gen Bank accession no.

1	Staphylococcus aureus 1	Staphylococcus aureus	99.40	MK695866
2	Staphylococcus aureus 2	Staphylococcus aureus	98.40	MK695940
3	Staphylococcus saprophyticus 1	Staphylococcus saprophyticus	99.40	MK695941
4	Staphylococcaceae, coagulase -ve	Staphylococcus edaphicus	100	MK695942
5	Staphylococcaceae, coagulase -ve	Staphylococcus haemolyticus	99.70	MK713339
6	Staphylococcus aureus 3	Staphylococcus aureus	98.90	MK713337
7	Klebsiella pneumoniae ssp ozaenae	Enterobacter cancerogenus	99.70	MK713337
8	Staphylococcus saprophyticus 2	Staphylococcus saprophyticus	100	MK713332
9	Staphylococcus saprophyticus 3	Staphylococcus saprophyticus	97.00	MK696049
10	Enterobacter gergoviae	Pluralibacter gergoviae	98.10	MK696050
11	Cedecea lapagei 1	Cedecea lapagei	99.70	MK696051
12	Enterobacter cancerogenus 1	Enterobacter cancerogenus	99.70	MK713323
13	Staphylococcus aureus 4	Staphylococcus aureus	99.50	MK713325
14	Staphylococcaceae, coagulase -ve	Staphylococcus edaphicus	99.80	MK713324
15	Escherichia fergusonii	Enterobacter tabaci	98.00	MK713331
16	Cedecea lapagei 2	Enterobacter xiangfangensis	99.70	MK713330
17	Proteus vulgaris 1	Proteus vulgaris	100	MK713329
18	Klebsiella oxytoca1	Enterobacter cancerogenus	99.70	MK713335
19	Not identified	Acinetobacter lwoffii	99.80	MK689408
20	Enterobacter cloacae 1	Enterobacter hormaechei	99.80	MK690048
21	Salmonella choleraesuis	Salmonella enterica/choleraesuis	98.80	MK690186
22	Enterobacter cloacae 2	Enterobacter hormaechei	99.80	MK690181
23	Cedecea lapagei 3	Cedecea lapagei	99.20	MK689855
24	Cedecea lapagei 4	Cedecea lapagei	98.80	MK713334
25	Hafnia alvei 1	Hafnia paralvei	99.80	MK684353
26	Enterobacter cancerogenus 2	Enterobacter cancerogenus	99.50	MK695980
27	Not identified	Acinetobacter lwoffii	99.50	MK713321
28	Klebsiella oxytoca2	Enterobacter hormaechei	99.80	MK704397
29	Pseudomonas fluorescens/putida	Pseudomonas helmanticensis	99.80	MK695699
30	Staphylococcaceae, coagulase -ve	Macrococcus epidermidis	99.80	MK695699
31	Proteus vulgaris 2	Proteus vulgaris	98.70	MK685208
32	Cedecea lapagei 5	Cedecea lapagei	98.90	MK713322
33	Enterobacter cancerogenus3	Pluralibacter gergoviae	99.70	MK684347
34	Klebsiella pneumoniae	Klebsiella pneumoniae	99.40	MK684237

* percentage of identical nucleotides of the closest relative found in the Genbank database

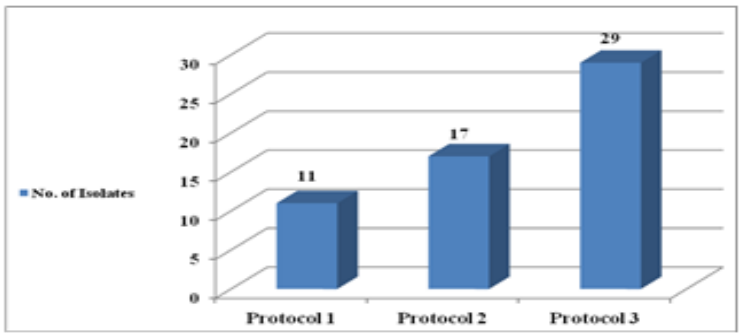

Figure (1): distribution of the bacterial isolates among the three protocols used to extract their DNAs. protocol 1: boiling method. protocol 2: microwave irradiation. protocol 3: PCR heat shock

Table 2. The number of colony forming unit (cfu / g) for bacterial isolates collected from lamb meat samples

Bacterial isolates	Number of lamb meat samples*	Average number of CFU / g
Staphylococcus aureus	4	1.5×10^5
Staphylococcus saprophyticus	3	2.5×10^4
Staphylococcus edaphicus	2	4×10^4
Enterobacter gergoviae1	2	2.1×10^4
Cedecea lapagei 1	4	5×10^4
Enterobacter cancerogenus	4	2.5×10^4
Enterobacter tabaci	1	6.5×10^4
Enterobacter hormaechei	1	7×10^4
Enterobacter cloacae	2	3×10^3
Salmonella choleraesuis	1	1.5×10^4
Hafnia alvei 1	1	3×10^3
Acinetobacter lwoffii	2	3×10^4
Pseudomonas helmanticensis	1	3×10^3
Proteus vulgaris	2	5×10^3
Klebsiella pneumoniae	1	3×10^3
Staphylococcus haemolyticus	1	4×10^4
Enterobacter xiangfangensis	1	5×10^4
Macrococcus epidermidis	1	5×10^4

*Number of lamb meat samples that contain the bacterial isolate

Bacterial counts were in a range 3 x 103 - 1.5 x 105 cfu / g (Table 2). In another study, Martineli et al. (2009) found that bacterial counts in lamb meat were in a range of 1.0 x 101 to 8.0 x 104 cfu /cm for mesophiles; 1.0 x 100 to 4.4 x 104 cfu /cm for psychrotrophic (Martineli et al., 2009). Contamination of raw meat with bacteria can occur during slaughtering, cutting, and storage, due to inadequate hygiene conditions. Good hygiene conditions might prevent growth or minimize microbial contamination in meat.

There was an agreement between the phenotyping and the 16S rRNA gene sequence analysis in identification of 19 bacteria out of the 34 bacterial isolates in lamb meat samples. However, 2 isolates, which were not identified by the phenotyping, were identified as Acinetobacter lwoffii by 16S rRNA gene sequence analysis (Table 1). Four isolates of Staphylococcus, which were only identified by phenotyping at the genus level were identified at the species level by 16S rRNA gene sequencing. Furthermore, there was a disagreement between phenotyping and sequencing of the 16S rRNA gene for 9 isolates of Enterobacteriaceae family at the genus and species levels. Characteristics of this family include being motile, catalase positive, oxidase negative, reduction of nitrate to nitrite; and acid production from glucose fermentation (Janda and Abbott, 2015). The biochemical and molecular studies on Enterobacter cloacae have shown genomic heterogeneity, comprising six species: E. cloacae, E. asburiae, E. hormaechei, E. kobei, E. ludwigii and E. nimipressuralis (Mezzatesta et al., 2012). Enterobacteriaceae family contains a large number of genera that are biochemically and genetically related; for this reason,

many additional morphological, biochemical, and physiological tests are always required (Juang and Morgan, 2001).

The highly conserved structure and sequence of the rRNA genes facilitate the use of PCR amplification and sequencing of those genes (Cody, Bennett, and Maiden, 2014). The advantage of 16S rRNA gene analysis is that it can be used for the identification of all bacteria (Patel et al., 2000). Biochemical tests might be used as a preliminary identification test, but the molecular methods are more accurate and should be used as confirmatory tests for hard to identify isolates (Moraes, Perin, Júnior, and Nero, 2013)

According to Moraes et al. (2013), 29 lactic acid bacteria (LAB) isolates were identified using Biolog, API50CHL, 16S rDNA sequencing, and species-specific PCR reactions. The different methods provided different patterns of genera and species identification for the LAB isolates; the identification results were compared, and it was concluded that the molecular analysis was the most reliable (Moraes et al., 2013). According to Juang and Morgan. (2001), API identification systems mostly can identify the gram-negative microorganisms in activated sludge only at the genus level, many additional morphological, biochemical, and physiological tests are always required for further identification.

CONCLUSION:

The results of the current study showed contamination of fresh lamb meat with various bacteria. Good hygiene condition during slaughtering, handling and storage of lamb meat can reduce microbial contamination. The sequencing of the 16S rRNA gene seems to be a good and accurate tool for identification of bacteria at the species level compared to phenotyping methods.

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