MOLECULAR IDENTIFICATION AND ANTIBIOMGRAM OF Enterococcus spp. ISOLATED ON ENTEROCOCCUS SELECTIVE DIFFERENTIAL (ESD) MEDIA FROM MEAT, MEAT PRODUCTS AND SEAFOOD IN LIBYA

Hesham T. Naas1, Zaid Almajdoubi1, Aboubaker M. Garbaj1, Salah M. Azwai2, Fatim T. Gammoudi2, Said K. Abolghair2, Ashraf A. Moawad3, Ilaria Barbieri3, Hanan L. Eshamah1 and Ibrahim M. Eldaghyayes1,2

Address(es):
1Department of Food Hygiene and Control, Faculty of Veterinary Medicine, University of Tripoli, P.O. Box 13662, Tripoli, Libya.
2Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, P.O. Box 13662, Tripoli, Libya.
3Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Suez Canal University, 41522 Ismailia, Egypt.
4Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt.
5Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna, Via Bianchi, 9 - 25124 Brescia, Italy.

*Corresponding author: ibrahim.eldaghyayes@vetmed.edu.ly


ABSTRACT

This study was conducted to investigate the presence of Enterococcus spp. in meat, meat products and seafood. A hundred and four samples were randomly collected from different geographic localities in Libya. The samples were subjected to microbiological analysis for enumeration and isolation of Enterococcus spp. by conventional cultural and molecular identification using PCR and partial sequencing of 16S rDNA techniques. Out of 104 samples, 73 (70.2%) isolates were found to be enterococci based on their cultural characteristics on ESD medium. However, out of 36 samples subjected to molecular identification, only six isolates were confirmed to be Enterococcus spp. using PCR and partial sequencing of 16S rRNA technique. All enterococci strains tested for their antibiotic sensitivity profiles showed high percentage of multi-resistance phenotype. These results can be used for further studies on enterococci as an emerging food borne pathogen and its role in human infection in Libya and would suggest that meat, meat products and seafood might play a role in the spreading of enterococci through the food chain with antimicrobial resistance characteristics.

Keywords: 16S rDNA, antibiogram, enterococci, food, Libya

INTRODUCTION

Enterococcus spp. is a genus of lactic acid bacteria of the phylum Firmicutes that possess Lancefield group D antigen as some of streptococci. Enterococci are Gram-positive cocci, often occur in pairs (diplococci) or short chains bacteria of the gastrointestinal tract of healthy human intestinal flora (Aarestrup et al., 2001). Enterococci are able to survive in extremes of temperature (5 to 60 °C), pH (4.6 to 9.9) and high sodium chloride (6.5% w/v) (Murray, 1990). They are capable of growth in the presence of bile salts (40% w/v) (Fisher and Phillips, 2009) and they commonly occur in foods, especially those of animal origin such as meat and milk (Giraffa, 2003).

Previously, all streptococci of fecal origin that produce group D antigen were considered as enterococci (Hartman et al., 2001). Molecular biology studies (including oligonucleotide cataloging of 16S rRNA, DNA-DNA and DNA-rDNA hybridization), combined with physiological studies showed more detailed classification (Schleifer and Kibbner-Bilz, 1987). Members of this genus are: E. avium, E. casseliflavus, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirse, E. malodoratus and E. mundtii (Hartman et al., 2001).

Enterococci are recognized as opportunistic human pathogens and lately have distinguished themselves as major nosocomial pathogens causing bacteremia, endocarditis, urinary tract, central nervous system, intra-abdominal and pelvic infections (Franz et al., 1999). In addition, enterococci can be also used as an enteric contamination indicator (Foulquie Moreno et al., 2006).

Enterococci are also known for their capability to exchange genetic information by conjugation (Dunny, 2007) and may spread antibiotic resistance genes among non-pathogenic organisms (Coconnelli et al., 2003; Fisher and Phillips, 2009). Thus, there is a concern about their presence in uncooked fermented meats because of the contribution they may have to the baseline level of antibiotic resistance in other genera and the potential for transfer of antibiotic resistant bacteria from the indigenous animal microflora to the human gastrointestinal tract (Mathur and Singh, 2005), also leading causes of highly antibiotic-resistant and hospital-acquired infection (Aarestrup et al., 2001). Enterococci are recognized as opportunistic human pathogens, and as indicator for fecal contamination. Due to lack of good hygienic practice in the Libyan slaughterhouses and meat retail markets, therefore, the objectives of this study were to evaluate the presence of enterococci in meat, meat products of different animal species and seafood from different Libyan localities and for their antibiotic resistance profiles.

MATERIAL AND METHODS

Collection and preparation of samples

A total of 104 samples (Table 1) included: raw meat samples (51), meat products (30) and seafood (23), were randomly collected from different cities in Libya (Tripoli, Regdalin, Janzour and Tobruk). The samples were packed in sterile plastic bags, stored in an insulated icebox and transferred as quickly as possible to Food Hygiene and Control Laboratory Department, Faculty of Veterinary Medicine, University of Tripoli. All samples were subjected to Enterococcus spp. microbiological enumeration and isolation techniques. Decimal dilutions, culturing and enumeration techniques were performed according to the methods described by the American Public Health Association (APHA) (Downes et al., 2001). Briefly, 25 g from each sample was aseptically transferred into a sterile stomacher bag (Seward Medicals, UK) and homogenized (Stomacher 400, SeaWorld Medicals, UK) with 225 mL of sterile peptone water 0.1% (w/v) (Park Scientific, UK) at 230 rpm for 2 min.
Enumeration and isolation of Enterococcus spp.

Enumeration and isolation of enterococci were performed using enterococci selective differential agar medium (ESD) (Efthymiou et al., 1974). ESD plates were seeded by surface spreading of 0.1 ml of appropriate tissue homogenate serial dilutions and then incubated at 37 °C for 24 h. ESD plates were examined for the presence of either magenta, round, 2-3 mm diameter colonies (E. faecalis), or white, round, 2-3 mm diameter colonies (E. faecium), or pink, round, 2-3 mm diameter colonies (E. intermedia). Isolates were identified to the species level by using API 20 Strept system (bioMérieux®, France).

Identification of enterococci by PCR and partial sequencing of 16S rDNA

DNA extraction of enterococci isolates was performed by GF DNA extraction kit (Cat. # GF # G5620, Vivantis, Malaysia) as described in a previous study (Awai et al., 2016). The 16S rDNA was amplified using the universal oligonucleotides primers forward: S-D-Bact-0341-h-S-17 5'- CCTACGGGNGGCWGCAG and Reverse: S-D-Bact-0785-a-A-21 5'-GACTACHVGGGTATCCTAACTC-3' (Herlemann et al., 2011).

Electrophoresis, gel extraction and DNA sequencing

The amplified 16S rDNA PCR fragment (464 bp) was excised from the gel and the DNA was purified using GF-1 Ambi Clean kit (Cat. # GF-GC-100, Vivantis, Malaysia) as described in previously (Awai et al., 2016). The purified 16S rDNA amplicons underwent cycle sequencing with Big Dye® Terminator v1.1 kit (AB Applied Bioscience, TECHNE, TC-512, USA) and were sequenced on four capillary ABI PRISM® 3130-Avant Genetic Analyzer at ZSLLER Istituto Zooprofilattico Sperimentale Della Lombardia e dell’Emilia Romagna, Brescia, Italy. Sequences were assembled and edited using the SeqMan module within Lasergene package (DNASTAR Inc., Madison, WI, USA). The obtained consensus sequences were subjected to BLAST search both at NCBI (http://www.ncbi.nlm.nih.gov/nuccore) and at 16S bacterial cultures Blast Server for the identification of prokaryotes (http://bioinfo.unice.fr/blast/).

Antibiogram of isolated strains

Inoculum Preparation

Upon confirmation by PCR and partial sequencing of 16S rDNA gene isolated strains of enterococci were preserved by freezing at -80 °C in vials containing Brain Heart Infusion broth (BHI, Difco, Michigan, USA) supplemented with 30% (v/v) glycerol. To propagate the culture, frozen vial was thawed at room temperature, and 0.5 ml of thawed culture was transferred to 5 ml of BHI broth and incubated for 24 h at 37 °C. The inoculum was prepared from the second transfer of that culture (0.5 ml) to another 5 ml of BHI broth and incubated for 16 – 18 h at 37 °C. After the overnight incubation Muller Hinton agar plates (Oxoid, Hampshire, UK) were surface swabbed, then the selected antibiotic discs were dispensed and lightly pressed onto the inoculated agar surface according to (Coyle, 2005) then incubated at 37 °C for 24 h.

Antibiotic assay

The selection of antibiotics was based on their common use in food animal practice and included: (oxytetracycin (30 µg), streptomycin (10 µg) and vancomycin (30 µg)). The antibiotic discs were purchased from Oxoid with the exception of the enrofloxacin (5 µg), amoxicillin (25 µg) obtained from Arcomex Arab (Medical Diagnostics Co., Amman, Jordan), while colistin (10 µg), doxycycline (30 µg), gentamycin (10 µg), erythromycin (10 µg), were obtained from Mast Diagnostics (Mast group ltd., Merseyside, UK). The clear zones around antibiotic discs that has no growth, referred to as the zone of inhibition, were measured and scored as sensitive, intermediate (reduced susceptibility) or resistant according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015).

RESULTS AND DISCUSSION

Isolation and enumeration of Enterococcus spp.

One hundred and four samples from various regions of Libya comprising raw meat (51), meat products of different species (30) and seafood (23) were tested for the presence of Enterococcus spp. by using ESD medium (Table 1). Enterococcus spp. were isolated from the samples of raw meat: beef 12/17 (70.5%), camel 13/22 (59%) and chicken meat 11/12 (91.6%) respectively, and from the samples of seafood: fish 5/13 (38.4%) and shrimp 3/6 (50%) respectively, with counts ranging from 1.7x10⁴ to 6.8x10⁶ CFU/g. The maximum mean count of enterococci was recorded in chicken burger 3.8x10⁰ CFU/g; while the minimum mean count was in shrimp 1.1x10⁰ CFU/g (Table 1). The occurrence of Enterococcus spp. was 87.5% (7/8) with counts ranging from 7.7x10⁴ to 6.8x10⁶ CFU/g. The maximum mean count of enterococci was recorded in chicken burger 3.8x10⁰ CFU/g; while the minimum mean count was in shrimp 1.1x10⁰ CFU/g (Table 1). Detection of enterococci in chicken burger was 100% with counts ranging from 7.7x10⁴ to 6.8x10⁶ CFU/g with a mean counts of 3.8x10⁴ CFU/g. While, in ground chicken the rate was 100% with counts ranging from 9x10³ to 8x10⁴ CFU/g and the mean counts was 4.5x10³ CFU/g.

Identification of enterococci spp. by PCR and sequencing of partial 16S rDNA gene

A total of 36 (16 raw meat samples and 20 meat products samples) randomly selected isolates (36 out of 73 isolates were found to be enterococci based on their cultural characteristics on ESD medium) were sent for partial sequencing of 16S rDNA (464 bp) of enterococci strains using the universal oligonucleotides primers (FOR: S-D-Bact-0341-h-S-17 and REV: S-D-Bact-0785-a-A-21) (Fig. 1). Only six isolates (16.6%) (Table 2) were identified as Enterococcus spp. These isolates of enterococci were all isolated from meat products (beef burger, beef kebab, ground chicken and chicken burger) (Table 3).

Table 1 Comparison between growth on ESD medium and partial sequencing of 16S rDNA technique for identification of Enterococcus spp.

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>No. of Samples</th>
<th>No. of Suspected Enterococcus spp. Growth on ESD (%)</th>
<th>Average Count (CFU/g) of Enterococcus spp. on ESD</th>
<th>No. of Sequenced Isolates</th>
<th>No. of Positive Enterococcus spp. by 16S rDNA Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>17</td>
<td>12 (70.5)</td>
<td>2.2×10⁴</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Chicken meat</td>
<td>22</td>
<td>13 (59)</td>
<td>1.6×10⁴</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Chicken</td>
<td>12</td>
<td>11 (91.6)</td>
<td>4×10⁴</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Clam</td>
<td>4</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>13</td>
<td>5 (38.4)</td>
<td>4.4×10³</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Shrimp</td>
<td>6</td>
<td>3 (50)</td>
<td>1.1×10⁴</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Meat products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken burger</td>
<td>8</td>
<td>8 (100)</td>
<td>3.8×10⁰</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Chicken kebab</td>
<td>2</td>
<td>2 (100)</td>
<td>9×10⁴</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Chicken sausage</td>
<td>2</td>
<td>2 (100)</td>
<td>9×10⁴</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Beef burger</td>
<td>8</td>
<td>7 (87.5)</td>
<td>7.6×10⁴</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Beef kebab</td>
<td>2</td>
<td>2 (100)</td>
<td>9×10⁴</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Beef sausage</td>
<td>2</td>
<td>2 (100)</td>
<td>8×10⁴</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Ground beef</td>
<td>2</td>
<td>2 (100)</td>
<td>9×10⁴</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Ground chicken</td>
<td>4</td>
<td>4 (100)</td>
<td>4.5×10³</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>73 (70.2)</td>
<td></td>
<td>36</td>
<td>6</td>
</tr>
</tbody>
</table>
Figure 1: Representative gel of partial amplification of 16S rDNA (464 bp) products of isolated Enterococci strains using the universal oligonucleotides primers. First and last lanes contain DNA marker (M).

Table 2: Conventional and molecular identification of suspected Enterococcus spp. in different meat products samples (CFU/g)

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Suspected Growth of Enterococcus spp. on ESD</th>
<th>No. of Suspected Isolates Growth on ESD</th>
<th>No. of Sequenced Isolates</th>
<th>No. of Positive Enterococcus spp. by 16S rDNA Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken burger</td>
<td>E. intermediate</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Chicken kebab</td>
<td>E. intermediate</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Chicken sausage</td>
<td>E. intermediate</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Beef burger</td>
<td>E. intermediate</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Beef kebab</td>
<td>E. intermediate</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Beef sausage</td>
<td>E. intermediate</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ground beef</td>
<td>E. intermediate</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ground chicken</td>
<td>E. intermediate</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>29</strong></td>
<td><strong>20</strong></td>
<td><strong>6</strong></td>
</tr>
</tbody>
</table>

Table 3: Identity of suspected isolate after sequencing by blast NCBI

<table>
<thead>
<tr>
<th>Blast NCBI</th>
<th>Identity (%)</th>
<th>Isolate Code</th>
<th>Suspected Isolate on ESD</th>
<th>Type of Sample</th>
<th>Storage Condition</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus durans</td>
<td>100</td>
<td>4203.1</td>
<td>Enterococci</td>
<td>Beef burger</td>
<td>Frozen</td>
<td>Suqaljuma, Tripoli</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>100</td>
<td>4209.2</td>
<td>Enterococci</td>
<td>Chicken burger</td>
<td>Frozen</td>
<td>Salaheldin Tripoli</td>
</tr>
<tr>
<td>Enterococcus durans</td>
<td>100</td>
<td>4210.1</td>
<td>Enterococci</td>
<td>Beef kebab</td>
<td>Chilled</td>
<td>Salaheldin Tripoli</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>100</td>
<td>6205</td>
<td>Ground enterococci</td>
<td>Ground chicken</td>
<td>Chilled</td>
<td>Suqaljuma, Tripoli</td>
</tr>
<tr>
<td>Enterococci faecium</td>
<td>100</td>
<td>7202.1</td>
<td>Enterococci</td>
<td>Chicken burger</td>
<td>Frozen</td>
<td>Abusetta, Tripoli</td>
</tr>
<tr>
<td>Enterococcus durans</td>
<td>100</td>
<td>7202.2</td>
<td>Enterococci</td>
<td>Chicken burger</td>
<td>Frozen</td>
<td>Abusetta, Tripoli</td>
</tr>
</tbody>
</table>

Antibiotics Resistant Phenotype

The results (Table 4) showed testing of the six confirmed enterococci isolates from meat products against nine antimicrobial agents (amoxicillin, colistin, doxycycline, enrofloxacin, erythromycin, gentamicin, oxytetracyclin, streptomycin and vancomycin). Antibiotic resistance profile showed that, E. durans found in beef burger and E. faecium found in chicken burger were resistant to five out of nine antibiotics (55.5%). Meanwhile, E. durans from beef kebab and E. faecalis from chicken burger both were resistant to seven out of nine antibiotics (77.7%). On the other hand E. faecalis from ground chicken showed resistance to eight out of nine (88.8%), lastly E. durans from chicken burger was resistant to six out of nine (66.6%). In conclusion, enterococci isolates exhibited resistance to at least five out of nine (55.5%) of the tested antibiotics. All six enterococci isolates (100%) were resistant to colistin. While five out of six tested isolates (83.3%) were resistant to amoxicillin, enrofloxacin, erythromycin and streptomycin. Resistance to oxytetracyclin and doxycycline was recorded among 66.6% of the isolates. However, only two isolates (33.3%) were resistant to gentamycin and vancomycin (Table 4).
Enterococci spp. are widely distributed in nature and are associated with the spoilage of meat and meat products (Hugas et al., 2003). Current study was conducted to isolate Enterococcus spp. from 104 samples of different meat, meat products of different animal species and seafood, collected from various geographical places in Libya. This study reported the presence of Enterococcus spp. in most meat and all local un-heated treated meat products samples except one sample of beef burger by conventional cultural method. Generally, the incidence of Enterococcus spp. all over the collected samples of raw meat was 70.5% (36/51) and seafood was 34% (8/23). However, the incidence rate of enterococci in meat products was 96.6% (29/30); this high incidence in meat products could be attributed to low hygienic practice and cross contamination during preparation of such products. The results showed that the contamination with Enterococcus spp. in meat of different animal species and meat products was higher than that in seafood. The higher values could be as a result of contamination from the processing area, equipment used, also the means of transportation which was used in bringing the produce to the market centers and the hygienic practice employed by meat sellers and butchers. The meat during its preparation remains in the ground for a long time which creates a good environment for microbial pathogens to proliferate on it. On the other hand, seafood were sold at the seafood market freshly with better hygienic conditions that reduce the possibility from being contaminated (Franza et al., 2003).

The occurrence of Enterococcus spp. in meat of different animal species and seafood (73) was in beef, camel, chicken, fish and shrimp 70.5%, 59%, 91.6%, 38.4 and 50% respectively, with counts ranging from 1.5x10^4 to 6.8x10^7 CFU/g (Table 1). The average counts of Enterococcus spp. in camel meat was 1.6x10^5 ±1.2x10^6 CFU/g. Hugas et al. (2003) reported that the numbers of viable count of enterococci in contaminated beef, poultry and pork are usually in the range of 10^2 – 10^3 CFU/g. Meanwhile, our study did not detect enterococci among four examined samples of clam (bivalve shellfish). In contrast to Montiel et al. (2013) who found enterococci in all samples of clam examined with their densities generally higher in clams than sediment and water. Our result could be due to Enterococcus spp. were removed from hard shell clams by depuration occurred at the fish market where the samples were collected (Love et al., 2010). On the other hand, 30 samples of meat products revealed an incidence of 100% Enterococcus spp., except in beef burger was 87.5% (Table 1). The mean counts of enterococci were 2.2x10^4 CFU/g in beef, 3.8x10^5 CFU/g in chicken burger, 9x10^5 CFU/g in chicken kebab and beef Kebab, chicken sausage and ground beef, 8x10^5 CFU/g in beef sausage, and 4.5x10^5 CFU/g in ground chicken. Our study revealed that, the highest enterococci count was in chicken burger 3.8x10^5 CFU/g, however, the lowest count 1.1x10^4 CFU/g was recorded in shrimp. The most common enterococci recorded in our investigation in meat products were E. durans, E. faecalis and E. faecium, while, (Jahan et al., 2013; Sadeghifard et al., 2015) reported E. faecalis as a predominant isolate in all meat samples. In agreement with our findings, Naus et al. (2009a,b) recorded high enumeration of enterococci in all tested samples that included beef burger and beef sausage at rate of 2x10^4 and 9x10^4 CFU/g respectively. As for molecular confirmation only six out of 36 randomly selected enterococci isolates were identified and confirmed by partial sequencing of 16S rDNA (8.2%) were confirmed as Enterococcus spp. in particular E. durans, E. faecalis and E. faecium (Table 3).

Enterococci raise major concern during the last decades, as they are becoming one of the most important nosocomial infections causing serious illnesses in human. The presence of Enterococcus spp. in foods may act as reservoir of antibiotic resistance genes (Valenzuela et al., 2009). The susceptibility of enterococci isolates to different antibiotics was tested (Table 4) and the highest incidence of resistance was recorded to colistin (100%), colistin is a last-resort antibiotic in both animals and humans, this antibiotic is used against particularly dangerous types of multi resistant bacteria that can withstand many other antibiotics. The existence of such isolates in the food chain of humans is of a great concern not only to public health but also because of the ease of resistance gene transfer to other bacteria. Lower resistance rates (83%) were recorded against erythromycin, amoxicillin, streptomycin and enrofloxacin while it was (66.6%) to oxytetracyclin and doxycyclin. Only 33.3% of the isolates were resistant to vancomycin and gentamicin, similar results were recorded by Jahan et al. (2013). Vancomycin resistant enterococci (VRE) are nosocomial pathogens that have been detected in environmental habitats including soil, water and wildlife faces. The spread of opportunistic pathogens harboring VR genes beyond hospitals into community is a potential threat to public health as vancomycin is used as last-resort against many infections. Most of the isolated enterococci strains were resistant to more than five antibiotics out of nine (55.5%). In the contrary to Fracalanza et al. (2007) who found overall percentages of antimicrobial resistant of isolates were: 31.2% to tetracycline, 23.8% to erythromycin, 11.3% to streptomycin, 4.3% to chloramphenicol, 3.9% to gentamicin, 1.4% to enrofloxacin and 0.4% to ampicillin. In another work, Khibi et al. (2013) studied enterococci strains isolated from meat samples that showed 14% resistance to streptomycin and 100% to streptomycin and tetracycline.

CONCLUSION

In conclusion, our findings demonstrated the presence of Enterococcus spp. in meat, meat products of different animal species and seafood. Vancomycin resistant enterococci were also isolated from local meat products sold in different cities in Libya. Moreover, conventional cultural methods on ESD medium were less significant than using the molecular techniques as partial sequencing of 16S rDNA techniques for identification of enterococci. Only six enterococci isolates cultured on ESD medium were confirmed to be Enterococcus spp. by PCR and partial sequencing of 16S rDNA. The occurrence of resistant strains of enterococci in food of animal origin should be considered as important threat to public health.

Acknowledgments: This study was part of a project titled “Genetic authentication of bacterial isolates from meat and milk products in Libya and establishing the Food-borne Libyan-type Bacterial Collection (FILBC)” that was supported by a grant provided by the Libyan Authority for Research, Science and Technology (LARST). Authors are grateful to Veronica Papini, a technician in Istituto Zoono profilattico Sperimentale della Lombardia e dell’Emilia Romagna, Brescia, Italy, who performed the sequencing of the partial 16S rDNA.

REFERENCES


