

Research article

Molecular detection of some bacteria from slaughtered sheep and cows in Basrah Province

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Abstract

The present study was undertaken to charge the microbial consignment of uncooked meat samples collected from the slaughtered animals of Basrah abattoir. In this, study fifty raw meat samples (25 meat Cow and 25 meat Sheep). In the present study, the pathogenic bacteria were identified as Enterococcus faecalis and Clostridium perfringens by 16S rDNA. This has a specific suggestion beginning the Public Health point of sight. The result designates considerable attendance of microbial contaminants in the meat where a shortage of correct hygiene in the slaughtered. The specimen (2) were diagnosed by amplifying universal primer of 16SrDNA. Sequence information is analyzed by (NCBI) program. Sequence results showed the dominance of Enterococcus faecalis and Clostridium perfringens.

Keywords: *Clostridium perfringens, Enterococcus faecalis, Meat, PCR.*

Introduction

The meat is the plump material of the body obtain by slaughter of healthy animals. It is main basis of minerals, vitamins and proteins to the numerous people of the world. As it is chief source of nutrients and due to constructive inherent factor, it is a perfect culture media for the enlargement of diverse types of microbes counting the fungi and bacteria. In carry out of slaughter, the most important sources of microbes are outside of the animal and the intestinal tract (1). Lately accepted "humane" method of slaughter-mechanical and electrical-have chemical, small consequence on contagion, but every technique is follow by stick and hemorrhage, which can bring in contagion (2). The microbiology of corpse meat is extremely needy on the circumstances below which animals are rear, slaughter and process. The level to which contagion happen and the work of art of the flowers reproduce the typical of cleanliness (3). The chief public health and financial difficulty is foodstuff

bear disease which enlarge worldwide mainly in individuals eat meal exterior their house ,due to unrestrained hygienic training of these type of the food ,food borne disease was describe according to World Health Organization (WHO), communicable or poisonous natural world of the disease occur from side to side use of dirty food (4). World inhabitants is rising extremely quick. The augment in inhabitants with resulting urgent insist for improved supplies of foodstuff has led to a constant look for work of fiction source of food and protein. It is, therefore, of vast significance to appear for option sources of foods for cookery to the stress in the years to approach as there are no added land masses to be exposed that would be obtainable for customary farming. Ruminants are almost certainly the most competent converters of plants into muscles since their digestive system be able to make use of cellulose and fibrous materials, the majority plentiful raw foodstuff material on the earth,

and still non-protein nitrogen. Ruminants change the materials into a well impartial source of protein and energy for human expenditure called Meat. Meat, an brilliant source of protein in human go on a diet is extremely vulnerable to microbial contaminations, which can reason its spoilage and food borne infections in human, resultant in financial and health dead (5). Food-borne pathogens are the most important reason of sickness and death in rising country estimate billions of dollars in medical mind and communal expenses (6). Food is careful the most significant power foundation for humans and animals. Meat might be simply impure by means of diverse pathogens if not grip suitably (7). Contamination of meat with the intestinal filling of slaughterhouse animals may serve up as a significant basis of this pathogen to foodstuff provide (8). Meat is significant in financial system and culture, even although its accumulation manufacture and use has been strong-minded to pretense risk for human health and the surroundings. As it is important source of nutrients and due to good turn clever inherent factor, it is an perfect culture media for the growth of diverse type of microbes counting the fungi and bacteria (9).

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 132

1. Sample collection

The samples collected from the slaughtered animals of Basrah abattoir. A total of 50 samples were collected (25 meat Cow and 25 meat Sheep). The sample were collected in sterile containers and immediately transported inside icebox to the laboratory for bacteriological analysis. All sample be treat aseptically, twenty-five grams of meat were weigh and aseptically incise into thin lesser piece through by means of sterilized table knife and then added to

225 ml of buffered peptone water. The inoculate medium were incubated at 37 °C for 24 hrs. (10). After enhancement of sample with buffered peptone water for 24 hrs. a loop-full of culture was streaked into plates of MacConkey and blood agar. The plate were aerobic and non-aerobic incubation at 37°C for 24 hrs. then was purify bacterial colony snap a dependency of every kind of diverse bacterial colony morphology shape, size and color.

2. Genetic Identification

1. DNA Extraction:

This process was complete by use commercially genomic DNA mini Kit (Geneaid, Korea).

2. Identification of bacteria by 16 Sequence ribosomal DNA (16 S rDNA)

A molecular identification of the microorganisms was perform by amplify the 16S rDNA gene by means of the universal bacterial oligonucleotide primers 27F and 1492R according to (11). Table (1).

Table(1):Universal bacterial oligonucleotide primers 27F

primer	TA	Length
27 Forward 5'- AGAGTTTGATCCTGGC-3'	60°C	16
1492 Reverse 5'- GGTTACCTTGTTACGACTT- '3	60°C	19

TA= Annealing temperature

Reagents:-

The reagents and their volume were used for PCR intensification are describe in the Table (2).

Table (2): Reagents and volume (50 µl) used in PCR amplification for 16S rDNA

Reagent	Volume per reaction
DNA template	4 µl
27 Forward primer	2 µl
1492 Reverse primer	2 µl
PCR PreMix	11 µl
Nuclease free water	31 µl
Total	50 µl

The PCR condition for amplification of 16S rDNA was described in Table (3).

Thermal cycling condition

Table (3): Program used amplification for 16S rDNA by PCR

product	Primer pair	Step	Temperature	Time	Number of cycle	Expected sizes
<i>16S rDNA gene</i>	27 F 1492 R	Initial denaturation	92°C	2 mint	1	1500 bp
		Denaturation	94°C	30 second	30	
		Annealing	51.8°C	45 sec.		
		Extension	72°C	1mint and 30s		
		Final extension	72°C	5 mint	1	
		cooling	4°C			

The product of PCR detected as those genomic bacteria electrophoresis with some changes as follows:

Reagents

Buffer 1X TBE

Ladder 1Kb DNA

Program

5µl of the PCR produce were laden the length of with 6µl of 1kb DNA ladder as a molecular indicator in 2.0 % molecular showing agarose gel. The gel ran at 60 V for 45 min and band pattern were examine below automatic UV trans-illuminator and

photographed using a camera. The band of 1500bp submit to 16S rDNA gene.

Identification of Bacterial

The 16S rDNA gene sequence was treat then analyze by Basic Local Alignment Search Tool 'BLAST' to search meant for homologous sequences in the National Center for Biotechnology in order file (NCBI) <http://www.blast.ncbi.nlm.nih.gov>. The bacterial sequence recognized by corresponding it with a sequence with the maximum individuality achieve from the GenBank database (12).

Results

In this study, different microorganisms were isolated. All suspected isolates were identified using phenotypic classification based on biochemical property of every isolate were additional established by oxidase, Gram's staining and catalase tests. Through these tests, the majority of the isolate show negative results for oxidase and positive result for catalase. Two isolates were performed Characteristics of bacteria Gram's stain shows single cocci and in chains and bacilli.

DNA Extraction of bacteria

On excellence assessment of DNA extracted from 50 bacterial isolates, a delegate picture of an agarose gel electrophoresis is exposed in Figure (1) which show bacterial DNA bands experiential below UV trans-illuminator.

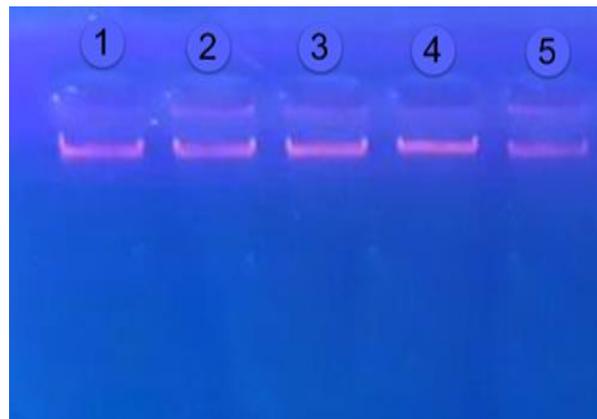


Figure (1): Agarose gel electrophoresis of Genomic bacterial DNA use a 0.8 % agarose gel contain ethidium bromide. Lane 1-5: bacterial

Amplification of bacterial isolates by 16S rDNA gene

The results of intensification of 16S rDNA gene as of 50 Bacterial isolate are shown in Figure (2).

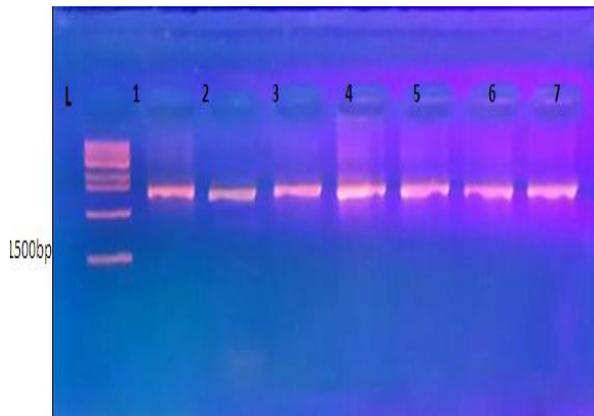


Figure (2): Amplification of 16S rDNA gene form bacterial isolates showed by agarose gel electrophoresis using a 2 % agarose gel containing ethidium bromide: Lane L: 1kb molecular weight DNA ladder, Lane 1-7: 16S rDNA gene bands of bacterial isolates.

The present study evaluated the microbial quality of raw meat sold in slaughtered animals of Basrah abattoir .All isolates were

examined for the specific amplification of 16S rDNA gene sequences PCR amplification of 16S rDNA using a set of primers: 27F and 1492R yielded a single amplification of~ 1500 bp for all the isolates see in figure (2). 16S rDNA fragment was amplified from total DNA of strains and partially sequenced. The sequences of isolates were checked and edited using BioEdit; the edited sequences were analyzed in BLAST online. According to alignment at NCBI, the BLAST results of the studied isolates (30, 48). The isolates were identified as strains belong to genus *Enterococcus faecalis* and *Clostridium perfringens*. After alignment with other 16S rDNA sequences in GenBank, it had a degree of similarity (100%).

Thirty- Enterococcus faecalis partial 16S rDNA gene, strain BGTRM10-13,1023BP-100%

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AAAGGTTACCTCACCGACTTCGGGTGTTACAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCGGGAAC
GTATTCACCGCGGGCGTGCTGATCCGCGATTACTAGCGATTCCGGCTTCATGCAGGCGAGTTGCAGCCTGCAATCC
GAAGTGAAGAGAAGCTTTAAGAGATTGTCATGACCTCGCGGTCTAGCGACTCGTTGTAATCCATTGTAGCAGCT
GTGTAGCCCAGGTCATAAGGGGCATGATGATTGACGTCACTCCACCTTCCTCCGGTTTGTCAACCGGCAGTCTC
GCTAGAGTGCCCACTAAATGATGGCACTAAACAATAAGGGTTGCGCTCGTTGCGGGACTTAACCAACATCTCA
CGACACGAGCTGACGACAACCATGCACCACCTGTCACTTTGTCCCGAAGGGAAAGCTCTATCTTAGAGTGGTC
AAAGGATGTCGAAGACCTGGTAAGGTTCTTCGCGTGTCTCGAATTAACCAACATGCTCCACCGCTTGTGCGGGCC
CCCGTCAATTCCCTTTGAGTTTCAACCTTGCCTGTACTCCACGGCGGAGTGCTTAATGCGTTTGTGTCAGCAC
TGAAGGGCGGAAACCTCCAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTG
CTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATAT
CTACGCATTTACCGCTACACATGGAATTCACCTCTCTCTTCTGCACTCAAGTCTCCAGTTTCCAATGACCCTC
CCCGTTGAGCCGGGGCTTTACATCAGACTTAAGAAACCCGCTCGCTTTACGCCAATAAATCCGGAC
AACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAATTAGCCGTGGCTTCTGTTAGATACCGTCAGG
GGACGTTCACTAAGCTCCTTGTCTTCTCTAACAACAGAGTTTT
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Forty eight- Clostridium perfringens strain FORC_003,1007BP-100%

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CGGTAGTCGAGCGATGAAGTTTCCTTCGGGAAACGGATTAGCGGGACGGGTGAGTAACACGTGGGTAACCTG
CCTCATAGAGTGGAAATAGCCTTCCGAAAGGAAGATTAATACCGCATAATGTTGAAAGATGGCATCATCATTCAAC
CAAAGGAGCAATCCGCTATGAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAACGGCCTACCAAGGCGA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCAACGCCGCGTGAGTGATGAAGGTTTTCCGGATC
GTAAAGCTCTGTCTTTGGGGAAGATAATGACGGTACCCAAGGAGGAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCGAGCGTTATCCGGATTAAGGGGCGTAAAGGGAGCGTAGGCGGATGATTAAGTGG
GATGTGAAATACCCGGGCTCAACTTGGGTGCTGCATTCCAACTGGTTATCTAGAGTGCAGGAGAGGAGAGTGG
AATTCTAGTGTAGCGGTGAAATGCGTAGAGATTAGGAAGAACCAGTGGCGAAGGCGACTCTCTGGACTGTA
ACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCTGGTAGTCCACGCCGTAACCGATGA
ATACTAGGTGTGGGGTTTCAACACCTCCGTGCCCGCTAACGCATTAAGTATTCCGCCTGGGGAGTACGGTC
GCAAGATTAACCTCAAAGGAATTGACGGGGACCCGCACAAGTAGCGGAGCATGTGGTTTAATTCGAAGCAACG
CGAAGAACCTTACCTACACTTGACATCCCTTGCACTTCTTAATCGAGGAAATCCCTTCGGGGACAAGGTGACA
GGTGGTGCATGTTGTCGTCAGCTCGTGTGATGTTGGGTTAA
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Discussion

The similarity of the present study with (13) which was isolated *Clostridium perfringens* from raw beef. This feature of the study propose the capability of *Enterococcus faecalis* to stop secondary contaminations frequently connected to *Cl.*

perfringens as explain by (14). As experiential in the route of this study, the method of slaughter of animals is accountable for the microorganism contamination (15). Traditional technique of butcher by means of table knife and hurtful

appearance appear additional able of report from diverse part of the world (16, 17). Food sickness cause by *C. perfringens* is amongst the extensive sickness resultant as of the expenditure of contaminated food, the vehicle of infection are naturally meat and poultry products. It has been resolutely recognized that an enterotoxin bent in the intestine next speculation of ingested vegetative cells is accountable for the ill health (18, 19). Meat substance irrespective of class may be contaminated with spore of *Clostridia* all through slaughter procedure and next conduct. Since *C. Perfringens* is a usual micro flora of the intestinal tract of animals, contamination of the corpse as of the intestinal filling as well as soil, dirt or

from personnel is nearly inevitable as support by (20, 21). Some enterotoxigenic *C. perfringens* strains were predictable to stand for less than 5% of the global *C. perfringens* isolates (22) secretarial for their unusual isolation and discovery in meat. Strains of *C. perfringens* that cause food poisoning occurs. The results were decided with (23). The answer of this study point to that an elevated proportion of meat samples can be predictable to be contaminated with *C. perfringens*. This information leads to the belief that the area in which meat and meat plates are ready might turn out to be likewise contaminated and therefore add to the option of after-cooking contagion.

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