ORIGINAL ARTICLE

The Bacterial Load and Contamination Rate of Raw Meat in Butcher Shops in Different Areas of Karbala City

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ABSTRACT

This study was aimed at assessing the types of bacterial isolates and their antimicrobial agentsprofile identified from raw meat and meat products butcher shops indifferent areas of Karbala city,A total of 100 random samples from raw meat were purchased from different butcher shops in Karbala city between October 2019 to February 2020 for the isolation and identification of microbial pathogens, The antibiotic resistant characteristics of the isolated organisms was determined by using Kirby-Bauer disk diffusion method and Polymerase chain reaction assaywas used to confirmall bacterial isolates obtained from direct colony of 24 hour by using16S rRNA genes, the results showed *Escherichia coli* was most Gram-negative found on meat samples about(45 samples) with percentage 45% fromall samples**,** 30% from samples contaminated with Gram-positivebacteria (*Staphylococcus*) and 6% was recorded as Salmonella, on the other hand , The results showed that the Ciprofloxacin and Gentamicin were susceptible to 95% of all isolates**.**

Keywords: *Staphylococcus, Salmonella, E.coli*, PCR, raw meats

INTRODUCTION

Raw meat and meat products are considered as an excellent source of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron. [(1)]The microbiological quality of these items is determined by a variety of influences, including the nature of the raw materials used, the effectiveness of the cooking procedure, the cleanliness of the environment, and personal hygiene. When low-quality meat is used and/or the food is undercooked, complications may occur[2]. Intrinsic influences, such as moisture content, pH, nutritive value, and the absence of inhibitors or inhibitory substances to micro-organism growth, also influence microbial growth in both raw meat and meat products. Temperature, relative humidity, oxygen supply, and other extrinsic factors such as chemical and physical properties are also examples of extrinsic factors[3]. Deterioration of meat occurs immediately after slaughter. This deterioration is the result of microbial, chemical and physical mechanisms. Freshly slaughtered animals can harbor few bacteria. However, the surface of the meat is, in most cases, vulnerable to contaminants through slaughter, evisceration as well as other post-slaughter procedures.[(4)]Micro-organisms most commonly present in meat and meat products are*, Escherichia coli, Bacillus sp, Salmonella sp, Clostridium sp, Shigellasp, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas* and *Proteus* etc[5].

The bacterial community Enterobacteriaceae is the main difficult bacterial contaminant for raw and manufactured product of the meat. *Escherichia coli* are known to be the most commensally living microorganisms in the digestive tract[6]. Food-borne diseases prove to be one of the most significant public health conditions worldwide. While reports from various countries continue to show rises in foodborne disease occurrence. The involvement of pathogenic species (food-borne infections) or the presence of harmful substances could be the result of a disease induced by the ingestion of infected food[4]. *Staphylococcal* food poisoning is the most common foodborne infection. It is often known in new and ready-to-eat foods as one of the main foodborne pathogens and is responsible for numerous illnesses all over the world. Additional methods of processing meat combined with poor sanitation were undermined food quality[9]. These dynamics serve as potential sources of foodborne illness, which is a serious public health problem due to the increased level of antibiotic resistance, as well as ignorance and complacency of some butchers about the meat manipulation, so, this study aimed to determine the bacterial load and contamination rate of raw meat inbutcher shops indifferent areas of Karbala city

MATERIALS AND METHODS

Collection of samples: A total of 100 random samples from raw meat were purchased from different butcher shops in Karbala city between October 2019 to February 2020 for the isolation and identification of microbial pathogens. Swabbing in a template of 5 cm x 10 cm region of carcasses was done with sterile cotton tipped swabs and soaked in buffered peptone water. Swab for culture should be directly put in its cover and transformed in the laboratory within a half hour of taking. The swab was inoculated onto culture media and incubated for 24 hours at 37°C.

Identification of bacterial species: The growing colonies transferred to new specialized media such as Blood Agar, S.S agar, MacConkey's AgarandEosin Methylene Blue (EMB) agar for each bacteria to obtain a pure culture. Again the isolated bacteria were cultured at a 37˚C for 24h and staining procedure were applied by using Gram stain. Also the motility and other biochemical tests such as catalase, oxidase, and API 20 Kitwere used to identify the isolated bacterial species[10].

Antibiotics susceptibility test: The antibiotic resistant characteristics of the isolated organismswasdetermined by using Kirby-Bauer disk diffusion method.After sterilization by autoclaving at 121C for 15 minutes, Mueller Hinton agar

plates were prepared and poured into Petri dishes, then it used for testing the isolated microorganism for antibiotic susceptibility. The diameter of inhibition zones (mm) were recorded for all of the plates and then compared with the standard[11].

Bacterial DNA isolation and PCR amplification of rRNA gene: . PCR products of fragment of RNAr16s from all isolates obtained from direct colony of 24 hour by using16S rRNA genes , it wasamplified from purified genomic DNA by use the universal primer 8F(5'-AGAGTTTGATCCTGGCTCAG-3') and 805R (5'-GACTACCAGGGTATCTAATCC-3'). The primers react with highly conserved regions of the bacterial 16S rRNA gene to provide PCR products of approximately 800 bp.The genomic DNA of isolates was extracted by Genomic DNA extraction Mini kit (Real Genomics) that used as template for amplification of the 16S rRNA gene , by universal primers for 16S rRNA gene of bacteria. Amplifications were performed by thermal cycler (Biorad) and with a temperature profile standardized for 16S rRNA gene amplification. The PCR amplification was carried out in 0.2 ml PCR tubes with 20 µl reaction volume containing Taq DNA polymerase (5U/reaction), PCR buffer (10x) with MgCl2 (1.5 mM), primers (10 nmol/reaction), deoxynucleotide triphosphate(dNTPs) (0.5 mM) of Thermo Fisher Scientific and template DNA. 5 min at 94 °C this isInitial denaturation followed by 30 cycles of 1 min at 94 °C,also 48 °C for 1 min this is annealing temperature or called annealing step,while the elongation step occur at 72 °C for 1.5 min and a final extension step of 8 min at 72 °C followed by a 4 0C soak until recovery.

Agarose Gel Electrophoresis: Electrophoresis on 1 percent agarose (GeNei, Bangalore, India) in 1X TAE buffer usually contains ethidium bromide (10 g/ml) was used to examine PCR products, and images were captured using a Gel Documentation Unit (Syngene, UK). Electrophoresis of 100bp normal molecular weight markers was used to determine the size of the amplified materials (GeNei, Bangalore, India).

RESULTS AND DISCUSSION

The results of this study after doing culturing and identification showed an*Escherichia coli* is most Gram-negative found on meat samples about(45 samples) with percentage 45% fromall samples and this results agree with [8]and [12] as well as[13] were found the *Escherichia coli*as following 40%,50% and 31% respectively.

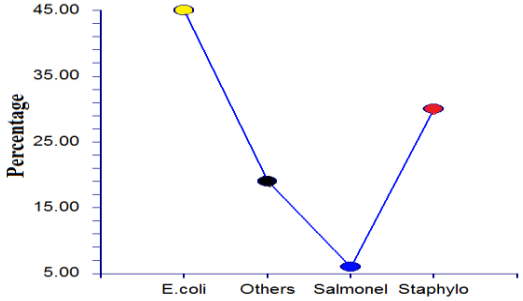
Also the results showed about (30 samples) with percentage 30% from samples contaminated with Gram-positivebacteria (*Staphylococcus*) these results agree with[14] and [1] werefound that*Staphylococcus* contamination about 29% and 28% ,respectively .Contamination with any of these microorganisms may take place throughout handling as well as preparation, or as a result of post-processing contamination, or they may be left unrefrigerated over many hours, and during that time *Staphylococcus* multiplies and occasionally produces enterotoxin.[15]

This studyshowed the *Salmonella* also found and occupied 5.83%, this organism that have several roles in contaminations and cases diseases, also it was agreement with[10,16] and[17].they are 19% (24 samples) of sampleswere non growth because several factors such asthe handling was improper, improper dilutions, defect in incubation conditions and other environmentalfactors that contributed in this case figure 1.

Table (1): The distribution of identified bacterial pathogenes from different region in karbala city.

Collection region	Number of samples with particular species			Total
	Escherichia coli	*Staphylococcus*	*Salmonella*	
City center	11	14	3	28
Husseiniya	10	1	2	13
Handia	14	11	0	25
Other	10	4	1	15

Figure 1: Percentage of Bacterial isolates from meat



16S rRNA is a valuable method to pathogen detection and identification. 16S rRNA gene sequences has been by far the more important housekeeping genetic indicator used to investigate bacterial phylogeny and categorization because these gene is present in almost all bacteria and its functioning has not changed over time. The use of this gene sequences allowed for the reclassification and renaming of several bacterial species, as well as the classification of uncultivable bacteria and it facilitated the classification of novel bacterial species and phylogenetic relationships[18].It's present in all prokaryotic cells and has both preserved and variable sequence parts that occurs at different levels, which is important for universal amplification[19].

Figure 2: Agarose gel electrophoresis of 16S rRNA gene in some bacterial isolates, represented aslane 1: M marker (1.5 K DNA Ladder), lane 2: Positive isolates (2,3, 5,6,7 and 8) and lane 3: negative isolates (1 and 4).

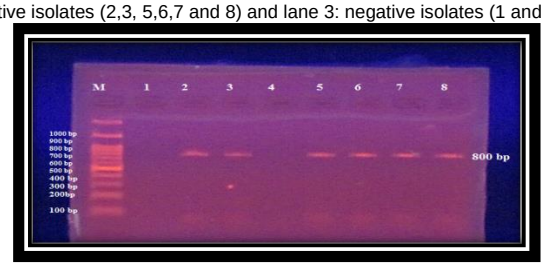


Table 2: Antimicrobial resistance percentage ofisolated microorganisms.

NO	Antibiotic	*Escherichia coli*	*Staphylococcus*	*Salmonella*
1	Tetracycline	**53%**	**65%**	**53%**
2	Gentamicin	**5%**	**2%**	**1%**
3	Ciprofloxacin	**3.5%**	**1%**	**2%**
4	Amoxicillin	**70%**	**31%**	**1%**
5	Erythromycin	**52%**	**100%**	**100%**
6	Cefixime	**69%**	**30%**	**98%**

Table 2 show the isolates from *Escherichia coli,Staphylococcus* and *Salmonella*were performed Antibiotic sensitivity tests against six Antibiotics are commonly used as shown in Table 2. The results showed that the Ciprofloxacin and Gentamicin were susceptible to 95% of the isolates as shown Table 2 and these results agree with[20] and[21].·Also the current study showed the erythromycin was resistance to 100% of *Staphylococcus* and *Salmonella* isolates while 50% to*Escherichia coli*isolates, all these results agree with[8]**.** They concluded from this study that in butcher shops, particularly Escherichia coli and Staphylococcus, remarkable microbial contamination showed that these pathogenic bacteria pose a significant potential risk to consumers (both direct and indirect). However, in meat samples, the appearance of these pathogenic microorganisms suggests low meat consistency and renders it a possible cause of foodborne infection. Therefore, raising awareness of meat safety and sanitation in both slaughterhouses and butcher shops also needs improved outreach campaigns that can efficiently explain the dangers involved with inadequate food processing, handling and storing practices to both customers and food vendors. In addition, more studies should be conducted in multiple study areas to isolate and classify the bacterial contaminants in meat.

REFERENCES

1. Eman M. Jarallah, SeaabImad Sahib & Khalid Yassen. Isolation and Identification of some pathogenic Bacterial Species Contaminated from Meats in Butchers Shops and Kebab Restaurants in ALKut city. Euphrates Journal of Agriculture Science-6 (4): 30-37 , (2014)
2. NematiM,Ghorbanpourh, razavieh s & Hoseini m. .Chemical composition and microbiological quality of the bonab kebabs sold in tabriz market. Journal of food safety 28 (2008).
3. Moshood A. Yusuf, Tengku Haziyamin Abdul Tengku Abdul Hamid & Ibrahim Hussain. Isolation and Identification of Bacteria Associated with Balangu Sold in Bauchi. Nigeria. IOSR Journal of Pharmacy. Vol. 2, Issue 6, Nov-Dec. 2012 PP. 38-48
4. Agbodaze d, nmai p, Robertson f, Yeboah-manu d, Owusu-darkok. & addo k. Microbiological quality of “khebab” consumed in the accra metropolis, ghana medical journal. 2005 volume 39, number 2
5. Jay J.M. Modern Food Microbiology. 6th Edition. Aspen Publishers Inc. Gaithersburg. Maryland, 2000, 60-89.
6. Al-Mutairi M.F. (2011). The Incidence of Enterobacteriaceae Causing Food Poisoning in Some Meat Products' Advance Journal of Food Science and Technology, 3(2): 116-121.
7. Saadia, M. & Hassanein, E. (2010). The Microbial Quality of Fast Food and Traditional Fast Food. Nature and Science, (10):8.
8. Kalantari S, Sepehri G, Bahrampour A & Sepehri E. (2012). Determination of bacterial contamination isolated from Sandwiches in Kerman City and their resistance to commonly used antimicrobials. Archives of Applied Science Research, 4 (2):1100-1105.
9. Diep B A, Gill S R, Chang R F, Phan TH, Chen J H & Davidson M G, (2006). Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant Staphylococcus aureus. Lancet 367, 731–739. doi: 10.1016/S0140 6736(06)68231-7.
10. Abebe Bersisa, Dereje Tulu, & Chaluma. Investigation of Bacteriological Quality of Meat from Abattoir and Butcher Shops in Bishoftu, Central Ethiopia. Negera International Journal of Microbiology. Volume 2019, Article ID 6416803, 8 pages
11. Clinical and Laboratory Standards Institute (CLSI). (2008). Performance standards for antimicrobial susceptibility testing. Approved standardM100-S17. Vol. 27, No. 1. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. Elmali M, Ulukanli Z, Tuzcu M, Yaman H, & Cavli P. Microbiological Quality Of Beef Doner Kebabs In Turkey. Archiv Für Lebensmittel hygiene · March 2005.
13. Gheorghe Ile, Alexandra Tabaran, Sorin Daniel Dan, Romolica Mihaiu, Oana Reget & Marian Mihaiu. Microbiological Assessment of Raw Meat Used in Fast-Food Products Sold for Public Consumption Bulletin Uasvm Veterinary Medicine 75(1)/2018
14. Senait G, Moorty PA. Isolation and identification of Staphylococcus species from ready-to-eat meat products in and around Debre-Zeit, Ethiopia. International Journal of Research. 2016 Apr 4;6.
15. ReyadR. Shawish & Naser A. Al-Humam. Contamination of beef products with staphylococcal classical enterotoxins in Egypt and Saudi Arab. GMS Hyg Infect Control. 2016; 11: Doc08.
16. Endale B & Hailay G. Assessment of bacteriological quality of meat contact surfaces in selected butcher shops of Mekelle city, Ethiopia. Journal of Environmental and Occupational Science, vol. 2, no. 2, pp. 61–66, 2013.
17. Hemmat M. Ibrahim, Mohamed A. Hassan, Reham A. Amin, Nesreen, Z. Eleiwa ,Samaa, S. Nadim. Conventional and modern identification techniques for identification of Salmonellae isolated from some meat products benha veterinary medical journal, vol. 29, no. 2:262-267, december, 2015
18. MignardS&FlandroisP. 16S rRNA sequencing in routine bacterial identification: a 30-month experiment. J Microbial. Methods. 2006; 67:574-581.
19. RamyaSrinivasan, UlasKaraoz, Marina Volegova, Joanna MacKichan, Midori Kato- Maeda, Steve Miller, RohanNadarajan, Eoin L. Brodie, Susan V. Lynch.Use of 16S rRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens. PLOS ONE | DOI:10.1371/ journal. pone, 2015.
20. María González-Gutiérrez, Camino García-Fernández , Carlos Alonso-Calleja , Rosa Capita . Microbial load and antibiotic resistance in raw beef preparations from northwest Spain.. *Food SciNutr.* 2020;8:777–785.
21. Wu S, Huang J, Wu Q, Zhang J, Zhang F, Yang X, Wu H, Zeng H, Chen M, Ding Y, Wang J. Staphylococcus aureus isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. Frontiers in microbiology. 2018 Nov 15;9:2767.