



Conventional and molecular approaches in bacterial contamination detection for meat samples

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Abstract: Since meat and its derivatives are the main sources of human deals, it must be free of contamination and hazard. This study was conducted to investigate the probability presents of bacterial contamination of several types of imported meat. All type of meat specimens show a rate of bacterial contamination. Conventional culture methods reveals that *Salmonella spp.* form the higher rate of isolated bacteria followed by Staphylococcus and Bacillus, while *Staphylococcus and Pseudomonas* form the predominant detected isolates by molecular assay using PCR techniques. Susceptibility of isolated bacteria to antibiotics reveals that Impenem and Nalidic acid are the more effective antibiotics against all types of bacteria. Detection of MIC against *Salmonella* isolates reveals also that Impenem is the most is the most effective with low concentration reach to 1.4 ug / ml.

Key words : Meat, Bacterial contamination, molecular assay, MIC. Impenem.

Introduction

Meat and is an essential source of good quality protein that provide us with the major amino acids for our daily requirement. Microbial source tracking (MST) methods allow the identification of the types of microbial contaminants, the extent of contamination, and the possible source of contamination (1). Bacteria have accounted for more than 70% of deaths associated with foodborne transmission (2). Microbial contamination include broad range of foods not only meat, Coliform group and *Escherichia coli* as fecal indicator contamination were implicated in 18 and 7% of Domiati cheese samples, and were not found in any Feta cheese and sterilized milk samples. *Staphylococcus aureus*, aerobic spore formers, yeasts and molds were detected in 4.5%, 40%, 22.5% and 4.5% of Domiati cheese samples respectively (3).

Molecular approaches are useful tools for the detection of fungi or bacteria even of their low quantity depending on nucleic acid sequence in addition to rapid molecular detection (4,5). Bacteria are one of the most agents for food poisoning. Occurrence of bacteria on food don't effect the taste or texture of food but it cause a big health problem through the causes of several disease according to bacterial types (6).

A number of studies have pointed outbreak of infections due to consumption of food contaminated and poor hygiene, however, few of these reports provide evidence of several outbreak caused by *Salmonella*, *Shigella*, *E. coli* and *Listeria* spp in different parts of the world (7). Many types of bacteria produced substances that had an effective virulanicity value. Exopoly saccharides (EPS) can be extracted from different type of bacteria and their content and antibacterial value varies with bacterial types, it was found that extract was higher in *Bacillus subtilis* in comparison with *Pseudomonas aeruginosa* (8).

Meat contamination occurs through poor handling and storage practices. Many types of bacteria found on meat some of them are pathogenic and form a major cause of food poisoning and other diseases such as *Campylobacter*, *Salmonella*, *E.coli*, *Bacillus* and other types (4).

Infectious bacteria produce miserable toxins and enzymes like *Escherichia coli* O157:H7. It is a zoonotic foodborne pathogen of major importance that produced Shiga toxin. The diseases associated with *E. coli* O157:H7 infection are hemorrhagic colitis (HC) and hemolytic uremic syndrome (9).

The *Clostridium* genus forms other bacteria types that cause meat and food contamination, *Clostridium perfringens* is an important pathogen of human gastrointestinal tract diseases such as food poisoning, antibiotic-associated diarrhea, and sporadic diarrhea as well as nosocomial diarrheal disease outbreaks and can cause morbidity if contamination of raw meat and poultry occurs, if these products are properly handled and prepared, particularly in restaurants and catering facilities.(10).

Food-producing animals, including cattle, chickens and turkeys are recognised as reservoirs of enteric bacteria, such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Clostridium perfringens*, *Listeria* spp., and *Yersinia* spp. (2,11) .

Microbial studies on imported meat available in Iraqi markets were limited, so the present study was carried out to searching for the presence of bacterial contaminants on different type of imported meat using conventional and molecular methods.

Materials and Methods

Samples collection

Fifty specimens of different types of meat (AlMurad, Al Kaffel, Al Huda, Al Hasnawi, Jeckor, fresh sheep meat and fresh beef meat) were taken in this study. Two small pieces of each specimen were added to separated plain tube contain brain heart infusion broth in addition to swab samples that taken from meat surface then brought to laboratory .

All samples were incubated at 37°C for 24-48 hours, swabs of each sample were cultured on different types of solid media including nutrient agar, MacConkey agar, EMB agar, Manitol salt agar and blood agar. Colonies were identified morphologically and biochemically(12).

Antibiotic susceptibility test

Different types of antibiotic disk were used to investigate the sensitivity and resistance of isolated bacteria to Cephalixin, Imipenem, Ampicilin, Nadlox acid and Cefotaxim using Kirby and Bauer disk diffusion method, the CLSI, (13) were dependent in results interpretation.

Molecular assay

Small pieces of each meat specimen type were cultured for 24 hrs. at 37°C in brain Swabs of selected drugs were used for bacterial DNA extraction using promega DNA EXTRACTION KIT, the concentration of DNA were estimated by nanodropspectronic.

DNA for each sample were amplified by polymerase chain reaction (PCR) using set of specific primers listed in table 1, the DNA amplification was done by using mono and multiplex amplification process with a final product of 20 and 25 µl respectively. Agarose gel electrophoresis for the products were carried out and the bands of bacterial genes were detected by E-graph gel documentation(14).

Table 1 : Primers used in bacterial diagnosis

Reference	Size	Sequence (5-3)	Primer	Bacteria
15	370bp	GGC CGT GTT GAA CGT GGT CAA ATC A TIA CCA TTT CAG TAC CTT CTG GTA A	TstaG422 Tstag765	<i>Staphylococcus spp.</i>
16	884bp	CCGATACGCTGCCAATCAGT ACGCAGACCGTAAGGGCCAGAT	Ec1 Ec2	<i>Escherechia coli</i>
17	480bp	TATCCTCTCTATATGCACAG CTGTAGTGGAAGCTGTTATA	LT3 LT4	
18	100bp	GTG AAA TTA TCG CCA CGT TCG GGC AA TCATCG CAC CGT CAAAGG AAC C	InvA	<i>Salmonella spp.</i>

Results

Cultural investigations on all samples of imported meat show occurrence of bacterial contamination including different types of bacteria .*Salmonella Spp.* (11 isolates) Form the most predominant followed by *Staphylococcus and Bacillus spp.* (10 isolates , while ,*proteus spp.* represent the lower isolates (table,2)

Table 2. Distribution of bacterial isolates on meat specimens

%	No. of Isolates	Meat types						Bacterial types
		Mumtaz	Jekoor	Al-Hasnawi	Al-Kafeal	Al-Murad	Al-Huda	
20.83%	10	1	2	2	2	2	1	<i>Staphylococcus spp.</i>
6.25%	3		1		1		1	<i>Proteus spp.</i>
20.83%	10	2	3	1	1	2	1	<i>Bacillus spp.</i>
16.66%	8	1	1	2	1	1	2	<i>Pseudomonas spp</i>
22.91%	11	1	3	2	1	2	2	<i>Salmonella spp.</i>
12.5%	6	2	1	-	-	1	2	<i>Escherechia coli</i>
	48	7	11	7	6	8	9	Total

Susceptibility of bacteria isolated from different meat sources to five types of antibiotics reveals that resistance or sensitivity of isolates were differs with the differences of bacteria and antibiotics types. Impenem represented the most effected antibiotics against the tested bacteria. Furthermore, *Escherichia coli and Proteus spp.* Show more sensitivity to most antibiotics (table 3).

Table 3.Suceptibility of bacterial isolates to some antibiotics .

Cefotaxime 5ug	Nadlixic acid 30 ug	Ampicillin 30 ug	Impenem 10 ug	Cephalexin 30ug	Type of Bacteria
I	I	R	s	I	<i>Staphylococcus spp.</i>
S	S	R	S	R	<i>Proteus spp.</i>
R	I	R	I	R	<i>Bacillus spp.</i>
R	S	R	S	I	<i>Pseudomonas spp</i>
I	I	R	S	S	<i>Salmonella spp.</i>
S	S	R	S	I	<i>Escherechia coli</i>

The eleven isolates of Salmonella, the predominant bacterial isolates, were selected for detection of the minimum inhibitory concentration (MIC) towards three types of antibiotics. Results confirmed that the lowest concentrations of MIC appeared with impenem when compared with other types of antibiotics (table 4).

Table 4. Minimum inhibitory concentration (MIC) of *Salmonella* isolates to β Lactam antibiotics.

AMP(\geq 32ug/ml)	CTX(\geq 64ug/ml)	IMP(\geq 4ug/ml)	<i>Salmonella</i> isolates No.
3.6	3.4	1	Isolate 1
8.4	2.2	0.5	Isolate 2
5.2	6.4	1.2	Isolate 3
4.6	4.8	2.6	Isolate 4
10.6	3.2	1.2	Isolate 5
6.8	1.4	0.5	Isolate 6
8.2	6.4	4.3	Isolate 7
12.8	8.2	1.6	Isolate 8
4.4	0.8	0.4	Isolate 9
12.8	3.8	0.8	Isolate 10
8.6	2.6	2.2	Isolate 11
		1.4 \pm	Mean \pm SD

Molecular tools were used for further detection of bacterial contamination in meat specimens. Monoplex and multiplex polymerase chain reaction assay proved appearance of different type of bacteria with variation in numbers of isolates. *Staphylococcus* spp. and *Salmonella* spp. form the predominant bacterial occurrence in all type of meat that reach to 18 isolate for each, whereas, *Proteus* spp. appearance was rare (table 5, figure 1 and 2).

Table 5. genetic diagnosis of bacterial contamination of different type of meat

No. of bacterial isolates						No of contaminated specimens	No. of specimens	Types of meat
<i>Escherechia coli</i>	<i>Salmonella</i> spp.	<i>Pseudomonas</i> spp	<i>Bacillus</i> spp.	<i>Proteus</i> spp.	<i>Staphylococcus</i> spp.			
2	1	4	1	-	4	12	16	Al-Huda
3	2	4	2	1	3	14	20	Al-Murad
4	1	3	3	-	4	15	20	Al-Kafeal
3	2	3	2	-	2	10	14	Al-Hasnawi
2	2	2	2	-	2	9	10	Jekoor
2	1	2	2	-	3	9	10	Mumtaz
16	9	18	12	1	18	69	90	total

Detection of bacterial contamination of meat specimens by molecular assay give areal proof for meat contamination, using of universal bacterial primers pointed out of various bands represent the appearance of different bacterial isolates (Figure 1, 2).

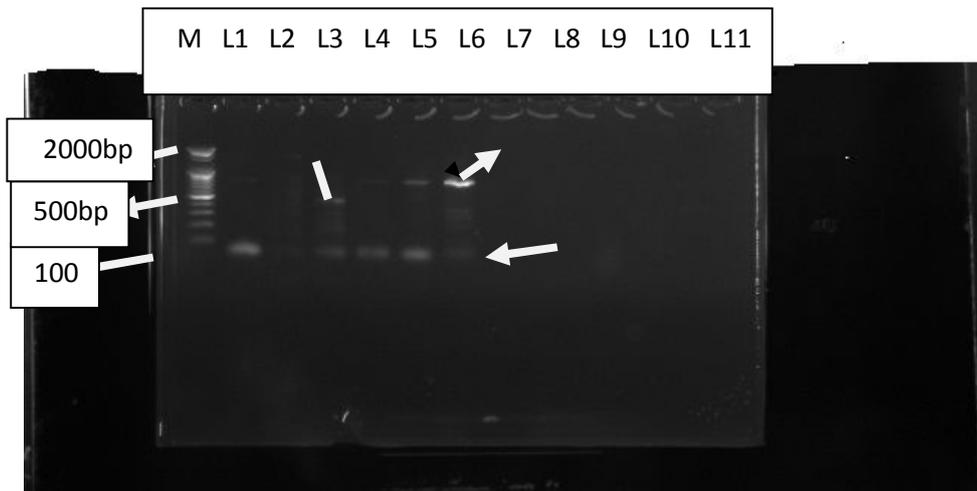


Figure 1. Gel electrophoresis of extracted DNA using multiplex PCR assay, different bands refer to different type of bacterial contaminants (100,370 and 884bp) .

Discussion

All type of human uptake foods must avoided of microbial contamination, meat is one of the most food that uptake worldwide because of their essential nutrient materials. Contamination of food with Pathogenic microorganisms cause illness, toxic and complications that leads some time to death (19).

Results of the present study pointed that all types of studied meats were contaminating with one or more bacterial type, although some of them are not harmful. The occurrence of bacteria on meat due to their compositions that make it as a good media for growth and multiplying of all type of microorganisms, meat is not only sensitive to microbes but it involved in disease spreading (20).

Different type of bacteria were isolated and identified in this study, *Staphylococcus* and *Salmonella* form the predominant isolated bacteria. The rate of isolated bacteria from meat differs with the type of meat, place and storage method. In many countries, meat regarded as a great source of pathogenic bacteria that cause many diseases. Many types of bacteria, some of them are pathogenic such as *E. coli*, *Campylobacter* and *Salmonella*, proved as a contaminant agent for meat (21).

The present study revealed that some of bacterial isolated from imported meat are pathogenic for human such as most enteric bacteria and *Staphylococcus*. Ali *etal*, (22) found that 84% of their meat samples were found to be contaminated with bacterial species, including *Klebsiella*, *Enterobacter*, *Staphylococcus aureus* and *Bacillus subtilis*. Contamination of food by microbes can be inhibited by other microorganisms, Contamination by mould in bread can be inhibited by several microorganism, Lactic Acid Bacteria (LAB) has no degrading effect over the nature, taste and texture of bread, makes it suitable for the bio preservation of bread(23).

Isolated bacteria were tested for their sensitivity to set of antibiotics, results expressed variation in susceptibility of isolates to different antibiotics. Variation in bacterial susceptibility correlated with type of

bacteria and antibiotics in addition to highly ability of some bacteria to make alterations in certain genes responsible for antibiotic resistance(24).

Salmonella isolates were chose for detection of MIC against three antibiotics type, Impeneme expressed the lowest MIC for all isolates in compared with other types of antibiotics. Many types of antibiotics had a broad spectrum action to different types of microbes, although there is some specificity of antibiotics action according to their target sites on microbes (25).The antibiotic sensitivity test and MIC had a good value in detection of inhibitory effects, itwas observed that MIC Value of Azithromycin against *Proteus vulgaris*, *Enterococcus faecalis*, and *Enterobacter aerogenes* was 4µg/ml, 1.2µg/ml and 0.12µg/ml respectively(26).

Using of polymerase chain reaction assay in bacterial contaminant detection give a good tools in more accurate detection, the three gene primers used in this study reveals surly appearance *Salmonella*, *Staphylococcus* and *E.coli*. Several studies on microbial meat contamination pointed out variation in percentage of contaminants according to tolls that used in bacterial isolation and identification, most of these studies performed that PCR assay is the more accurate method in microbial detection (11, 22).

Conclusion:

Cultural and molecular assay reveals that *Salmonella* and *Staphylococcus* are the most bacterial contaminants for meat specimens, furthermore ,the susceptibility investigation showed that Impenem an Nalidic acid antibiotics are the more effective against these types of bacteria .

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