Isolation and Identification of some pathogenic Bacterial Species Contaminated from Meats in Butchers Shops and Kebab Restaurants in AL-Kut city

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Abstract:
This study was conducted for detection of pathogenic bacterial species that contaminate meat in the butchers shops and kebab restaurants in AL-Kut city. Ten samples from 10 butcher shops and 10 restaurants were collected. These samples were suspended in sterile normal saline in order to using for isolation and identification of pathogenic bacteria. The cultural properties and biochemical tests results revealed the bacterial isolates return to two bacterial species: Escherichia coli (40%) and Staphylococcus aureus (29%) in butchers shops, in front of E.coli (19%), S.aureus (28%) and Klebsiella sp. (9%) in restaurants. The antibiotics susceptibility pattern results showed all of these isolates were resist to most traditional antibiotics but in different ratios.

Introduction:
Food is a chemically complex matrix, and predicting whether, or how fast, microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods; the most

important are water availability, pH, and temperature (ICMSF, 1996).

Food-borne diseases are an important cause of morbidity and mortality worldwide. It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead et al., 1999). People's
increased traveling and free movement of foodstuffs has increased the risk of contracting food poisonings (Shahram et al., 2012).

Contaminated raw or undercooked red meats are particularly important in transmitting these food borne pathogens (Meng and Doyle, 1998). Escherichia coli are considered the most commensally living microorganism in the alimentary tract of nearly all domestic and wild animals as well as human. Enteropathogenic E. coli organisms usually lead to severe diarrhea in infants and it may also be the causal organisms in appendicular abscess, peritonitis and cholecystitis (Mackie and McCartney, 1989).

The Enterobacteriaceae group of bacteria is the most challenging bacterial contaminant to raw and processed meat products worldwide. E. coli, Klebsiellas species are the most predominant species in all food poisoning cases associated with some meat products. Due to the rising incidence of food borne infections, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat products, it depends greatly on investigating the causative agents in food (meat products), eliminating them to ensure food safety and to protect public health from microbial contamination of food (Al-Mutairi, 2011). Staphylococcal food poisoning is overwhelmingly the most prevalent of food borne infections in the United States (Ash, 1997).

This study carried out to investigate the diseases that caused by food contamination by the studied pathogens and determination the most bacteria that contaminate the fast food.

Materials and Methods:
Collection of samples:
Ten fast food restaurant and ten meat butcher shops in Kut city were chosen randomly to be used in the present study. A total of 10 samples of fresh meat and 10 Kebab samples were obtained aseptically and transferred to the laboratory in sterile plastic bags kept in Ice-Box, according to (Cheesbrough, 1984).

In the laboratory, one gram of each sample was weighted out and homogenized into 9 ml of sterile distilled water. From the ten–fold dilutions of the homogenates: 0.1 ml of $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilutions were plated in culture on the Nutrient broth media, Nutrient Agar, Blood Agar and Macconky's Agar by pour method. The plates were then incubated at 37°C for 24-48 hours. After the end of incubation period, colonies were counted using colony counter. The count were expressed as colony forming units (CFU). Pure isolates of bacterial species were then stored at 4°C for using in identification examination.

Identification of bacterial species:
The growing colonies transferred to new specialized media for each bacteria to obtain for a pure culture. Again the isolated bacteria were cultured at a 37°C for 24h and staining procedure were applied by using Gram stain. The biochemical tests were conducted by API 20 Kit to identify the isolated bacterial species.

Antibiotics susceptibility test:
Kirby-Bauer disk diffusion method was used to determine the antibiotic-resistant characteristics of the isolated organisms (Bauer et al., 1996). Mueller-
samples from butcher shops were contaminated with two strains of bacteria (Table.1). Figures 1 and 2 represent the frequency of the pathogenic bacteria in studied Kebab and meat samples, respectively. As shown in these figures the positive species *S. aureus* was the more frequent pathogen in Kebab samples followed by *E. coli* and finally *Klebsiella sp.* While in meat samples *E. coli* came at the first then *S. aureus*.

The antibiotics susceptibility patterns of these bacterial isolates towards some traditional antibiotics were examined due to diameters of inhibition zones (Tables 2 and 3).

**Results:-**

The results of the present study showed that the studied foods of restaurants were contaminated with three strains of pathogenic bacteria, while the positive samples of meat samples from butcher shops were contaminated with two strains of bacteria (Table.1). Figures 1 and 2 represent the frequency of the pathogenic bacteria in studied Kebab and meat samples, respectively. As shown in these figures the positive species *S. aureus* was the more frequent pathogen in Kebab samples followed by *E. coli* and finally *Klebsiella sp.* While in meat samples *E. coli* came at the first then *S. aureus*.

The antibiotics susceptibility patterns of these bacterial isolates towards some traditional antibiotics were examined due to diameters of inhibition zones (Tables 2 and 3).

**Table 1:** Detection of bacterial isolates from meat sample and kebab sample.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Gram stain</th>
<th>Meat samples</th>
<th>Kebab samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>40%</td>
<td>28%</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
<td>29%</td>
<td>19%</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>-</td>
<td>-</td>
<td>9%</td>
</tr>
</tbody>
</table>

**Figure 1:** The percent of pathogenic bacteria isolates from Kebab samples from fast food restaurant.
Figure 2: The percent of pathogenic bacteria isolates from meat samples from butcher shops.

Table 2: Mean of inhibition zone diameters (mm) of bacterial isolates of Kebab samples.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>Klebsiella sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincomycin</td>
<td>3±0.02</td>
<td>12±0.1</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>-</td>
<td>7±0.02</td>
<td>-</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>5±0.02</td>
<td>8±0.04</td>
<td>6±0.01</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>13±0.01</td>
<td>12±0.03</td>
<td>5±0.02</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>-</td>
<td>5±0.01</td>
<td>12±0.03</td>
</tr>
<tr>
<td>Cefixime</td>
<td>5±0.02</td>
<td>6±0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Means of inhibition zone diameters (mm) of bacterial isolates of meat samples.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S. aureus</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincomycin</td>
<td>-</td>
<td>10±0.03</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>-</td>
<td>6±0.02</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>6±0.01</td>
<td>7±0.02</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>12±0.02</td>
<td>13±0.02</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>-</td>
<td>6±0.04</td>
</tr>
<tr>
<td>Cefixime</td>
<td>4±0.02</td>
<td>5±0.03</td>
</tr>
</tbody>
</table>

(-) mean resistance
meat products are considered as an excellent source of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron. The source of infection is not determined in the majority of food born disease outbreaks. Currently the most important pathogens associated with meat products are Escherichia coli (Borch et al., 1996).

The present study demonstrated that meat samples from butchers shops in Kut City, were heavily contaminated with E. coli (40%). High contamination level of Coliforms in examined meat products may indicate unsanitary conditions of raw meat production from which produced. They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and workers hands. Also, during evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses. Undercooked meat products have caused many food poisoning incidents associated with E. coli which is present in the faeces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process.

In the current study, we evaluated the prevalence of S. aureus in meat; the distinct S. aureus populations on each product type suggest that meat animals are the predominant source of contamination. While a portion of the S. aureus isolates may have been the result of human contamination, a uniform pattern of human-associated strains was not observed. This result is agreement with other reports that S. aureus frequently are present in low numbers on raw meat surface occurs infrequently (Saadiah and Hassanein, 2010).

Biofilm forming bacteria is usually resistant to a wide range of antibiotics to find the prevalence of drug resistance bacteria; assays for susceptibility profiles and biofilm formation were performed. Resistance of bacterial isolates to available antibiotics and the biofilm formation ability of these isolates was commonly observed. The problem may be attributed to a number of possible sources, including the natural resistance of species to certain antibiotics possible transfer of antibiotic resistance among species.

The present study demonstrated that Fast foods samples from restaurants in Kut City, were heavily contaminated with S. aureus (28%). This represent a high level of contamination indicates a potential breakdown of hygiene at various stages of the food processing, S. aureus is most likely transmitted by hand of food workers. Others also reported the Staphylococcus aureus as a source of food contamination (Kalantari et al., 2012).

E. coli was the second most frequent food borne pathogen which was isolated from Fast foods samples (19%). Food or water or with the individuals handling the infant's child and unwashed vegetables or undercooked meat, by infected food workers via fecal-oral route. This result is agreement with other reports. It was suggested that it could increase the

Discussion:

Meat and meat products are considered as an excellent source of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron. The source of infection is not determined in the majority of food born disease outbreaks. Currently the most important pathogens associated with meat products are Escherichia coli (Borch et al., 1996).

The present study demonstrated that meat samples from butchers shops in Kut City, were heavily contaminated with E. coli (40%). High contamination level of Coliforms in examined meat products may indicate unsanitary conditions of raw meat production from which produced. They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and workers hands. Also, during evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses. Undercooked meat products have caused many food poisoning incidents associated with E. coli which is present in the faeces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process. This result was remarkably different from those previously reported by (El-Gohary, 1993)(78%) and (Vazgecer et al., 2004) (31%).

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**Recommendations:**
1- The use of modern techniques for the diagnosis isolated bacteria such as ELISA and PCR.
2- Study of pathological and histological effects of isolated bacteria in laboratory animals.

**REFERENCES:**

The incidence of enteric pathogens (Angelillo *et al.*, 2000). The bacterial isolate *Klebsiella* spp was the third most frequent food borne pathogen which was isolated from Fast foods samples (9%). This is due to the rapid proliferation of such pathogen in meat product regardless their count is high or low. *Klebsiellaspp* was isolated in different rates of incidence. As well as have shown higher incidence of *Klebsiellaspp* in meat product (Ammar, 2005).

The microbial resistance of *S.aureus* isolated from fast food samples showed a high resistance rate to commonly used [Lincomycin, Erythromycin and amoxicillin] which is clinically important and mayindicate inappropriate use of antimicrobials in Iraq. While, *E. coli* isolates showed low resistance to amoxicillin which is in agreement with the results of other investigators in other parts of the world (Cook *et al.*, 2009).

*Klebsiellaspp* was relatively resistant to commonly used antimicrobials. Our data are in agreement with several studies in other parts of the world showing an increasing portion of resistant isolates of *Klebsiella spp*. The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades. This increase is attributed to the selection pressure created by using antimicrobials in food-producing animals, in addition to the unregulated use of antibiotics by humans in developing countries (Allison and Gilbert, 1995).

**Conclusions:**
The highest rate of isolation of *S.aureus* in kebab samples while the highest rate of isolation *E.coli* in meat samples.

ICMSF, (1996). International commission on microbiological specifications for foods. Microorganisms in foods. 5 microbiological specifications of