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Research article

Isolation and identification of some bacteria from imported meat (beef burger) by using vitek2 technique

Heifa'a J. Hassan Jenan Nadhim Sadeq Basima J. Mohamed
Muthanna Hadi Hussain

College of Veterinary Medicine, University of Al-Qadisiyah, Iraq
Corresponding Author Email: Haifa.hassan@qu.edu.iq

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Abstract

Iraqi's local markets contains a significant variety of imported meats from different sources, beef burger is one of them, the current study aimed to knowledge occurrence and prevalence of bacteria in meat of beef hamburger by using vitek 2 technique in Al-Diwaniyah city. Forty Samples of beef burger were chosen randomly from local markets, all the isolates submitted to culture on many of media agar like MacConkey, blood base agar, SS agar, manitol salt agar, Eosin-methylene blue agar, Chromagar Orientation, , and salmonella Chromagar, then the isolates tested by vitek 2 technique to confirm final diagnosis, the study was shown there are many of bacteria reside in beef hamburger at different percentage, E.coli (55%), Klebsiella pneumonia (38%), Klebsiella oxytoca (2%), Staphylococcus aureus (44%), Proteus mirabilis (13%), Salmonella typhimurium (18%), Enterococcus feacalis (8%) and serratia marcescens (3%). It could consideration meat of beef burger is an eligible nutrient to the multiplication of many of bacteria types that may be very dangerous if it transmitted to human.

Keywords: Bacteria, Beef burger, vitek2 technique.

Introduction

Burger is commonly cooked food in most worlds' countries, it starts for first time in early years of twenty century in Hamburg City in Germany, and it spread to other countries, it consists mainly of chicken meat or beef meat (1) (2). The Meat is the important compound in many foods meals, it consist from sufficient nutrient elements that help the bacteria to growth (3). The meat consist from several compound such as phosphorus, vitamins, fat, water, protein and iron the meat is main nutritive source of protein was eaten by humans. Most the meat has high water content that helps the bacteria to grow more activity (4). Contaminated hamburger is main sources of foodborne illness (5) and (6), Meat is the main edible part of domestic mammals for growth the bacteria (7). Food borne diseases occur due to be ingestion of contaminated food by toxins and bacteria; degree of disease severity (signs and symptoms) depend on amount of contaminated food by etiological agents of the disease (8). Food borne organisms responsible for as many diseases that happen each year and challenge safety of public health (9). Vitek2 is sensitive and accuracy laboratory assay, it used for detection the bacteria negative and positive bacteria, it characterized by the specificity in addition, sensitivity more than classical biochemical tests methods, this technique starts using in most microbiology laboratories (10). There are many studies worldwide have shown that germs can grow and multiplicated on the meat, such as

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Salmonella spp, Campylobacter spp , E. coli, beef meat possible carry it (2). Furthermore, *E. coli* can survive well in frozen and non-frozen Meat (11) and (12). Sources of contamination of the bacteria are may be occure from direct contact with the skin of the animal after slaughter, where there are other sources of bacteria are equipments that used with meat production, like the clothes, hands and the physical tools are considered important way to spread the bacteria (13). (14) Food contamination may be required the infection during the production, the processing, the distribution, the marketing, and handling or preparation, Contaminated food will be negative results on the public health. Also (15) found most bacteria that detected in the meat were *En. agglomeram, E coli, C. freundii*, and less commonly are of the genera *Proteus spp, Shigella sonnie, Klebsiella spp, S. aureus* and *E. coli*, all these are normal flora live on skin of human and animals, their presence in foods due to direct handling by a human, (16) found *Salmonella spp*, Campylobacter *spp*, and *E. coli* can colonize on the meat, it comes from the gastric and intestine of wild and domestic animals. There are many of the changes that occur in the contaminated hamburger bad odors, bad flavor, and discoloration of the meat, gas production and decrease in pH (acidosis) (17). The aims of the present study are identify of bacterial types that grow on the commercial hamburger (beef hamburger) and determination the percentage of each one by using modern assay called Vitek 2 technique.

Materials and Methods

Sample collection:

Forty imported meat samples (beef hamburger) were taken from the commercial markets in Al-Diwaniyah city , all samples were sent to the microbiology laboratory, (3) grams of each sample was taken and put in (5) ml of nutrient broth and incubated in the incubator at (37°C) for (18-24) hours, then using a sterile loop taken a broth and streaked on differential and selective media including MacConkey Agar, blood base agar, salmonella shigella agar, manitol salt agar, Eosin-methylene blue agar, Chromagar Orientation and salmonella Chromagar. Primary cultures were evaluated by visual examination of the morphology of the bacterial colonies and were subculture again and staining by Gram stain.

Biochemical Identification (Vitek 2 technique):

Biochemical and physiological characteristics are determined with indicator mediums. Commercially available miniaturized systems are now frequently used for this purpose (18). The card of VITEK 2 Compact consist from (64) wells, it contain s substrate of specific test. These Substrates responsable about many of biochemical activities such as enzyme hydrolysis, alkalization, acidification, and growth in the media that contained on inhibition compounds. The film is without cover on both sides of the card for allows for changing level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism- substrate admixtures. The entire cards have transfer tube used for inoculation. Each card have specific codes that contain all the information on product type, expiration date, lot number, and a unique identifier number that can be patched on the card (19). The procedure was done according to) 19). A sterile swab was used to transfer the colonies of a pure culture and to put it in (3.0) ml tube of sterile tube consist from (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a (12 x 75) mm polystyrene test tube. It was used DensiChek to estimation of turbidity to adjusted (0.5-0.6) Mf. Each card contains bactria suspensions for using an integrated vacuum. The tube put in a special cassette, then was transfer in the neighbouring slot while the transfer tube insert into the corresponding suspension tube. The filled cassette was placed into a vacuum chamber station. After the vacuum is applied and the air is reintroduced into the

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station, the organism suspension will be forced through the transfer tube into micro-channels that fill all the test wells (19). Using the Barcode reader, the cards were registered in the software part of the system to be correlated with readings of the optical system and the database of the software. Inoculated cards were passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. All card types are incubated online at 35.5 + 1.0ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time ,the information are taken at 15-minute intervals during the entire incubation period, Finally, a complete report containing the name of the unknown bacteria will be printed (19)

Results

Bacterial Isolation:

The first results were shown to isolation of bacteria on differential culture media (MacConkey agar) growth of 2 groups of bacteria Lactose fermentation & Lactose non fermentation Figure (1) and (2) this may be *Citrobacter spp, Enterobacter spp, Klebsiella spp,* and *E.coli, the* second group non-fermenter *pseudomonas spp, Shigella spp, salmonella spp, Proteus spp* and *Serratia spp*.

Figure (1): Lactose fermenter and pink colonies on MacConkey agar

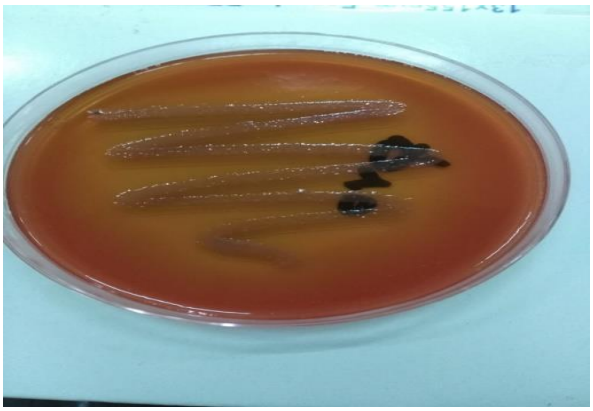

Figure (2): Lactose non-fermenter and pale colonies on MacConkey agar.

Fermenter and non-fermenter colony cultured on selective media (Salmonella Shigella agar, eosin methylene blue agar, salmonella chrome agar and orientation chrome agar the results were shown in the following Figures (3, 4, 5, 6, 7).

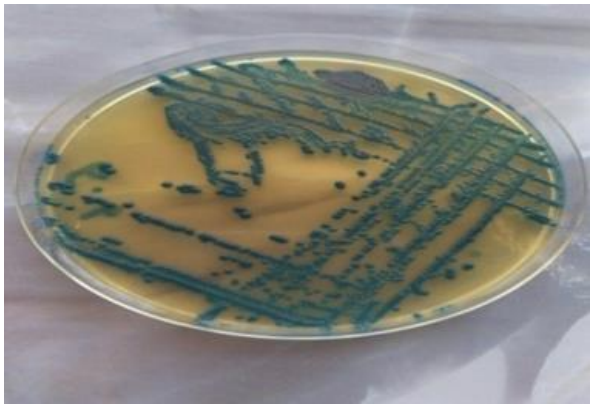

Figure (3): metallic blue (suspect Klebsiella spp, Enterobacter or Citrobacter spp) Depending on the general characteristic (color) on Chromagar orientation

Figure (4): *E coli* on Eosin methylene blue agar (metallic shine)

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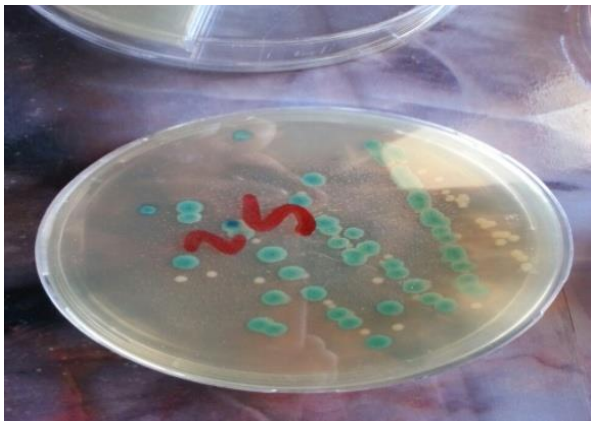

Figure (5): Turquoise blue colonies (suspect *Enterococcus spp*) on Chromagar Orientation.

Figure (6): Rounded pink colonies on salmonella chrome agar (*salmonella* spp).

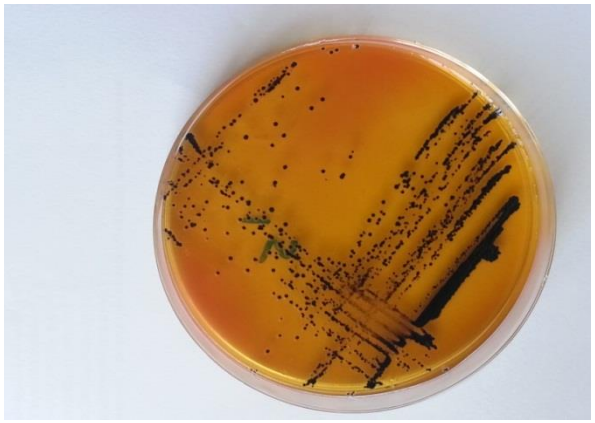

Figure (7): positive H2S production colonies on Salmonella Shigella agar suspect *Salmonella spp or Proteus spp*).

The samples when cultured on blood agar media appear colonies surrounded by zones of clear beta-hemolysis (suspect *Staphylococcus* spp or Streptococcus spp),this colonies cultured on selective media for *staphylococcus spp* (mannitol salt agar) yellow colonies appear (*staphylococcus aureas).* All isolates stained by Gram stain for detection type of it (G+ Or G- Bacteria)

Diagnostic colonies by Vitek 2:

The characteristic of growth on selective agar is not a final detection, all isolates should be examine biochemically, and the result of vitek2 technique (64 biochemical test), the results showed in Figure (8) and Table (1) as below.

Table (1): Included the bacteria and its percentage by use Vitek 2.

Type of Bacteria	Percentage %
E coli	*55%*
Klebsiella pneumonia	38%
Klebsiella oxytoca	2%
staphylococcus aureus	44%
Proteus mirabilis	13%
salmonella typhimurium	18%
Enterococcus faecalis	8%
Serratia marcescens	3%

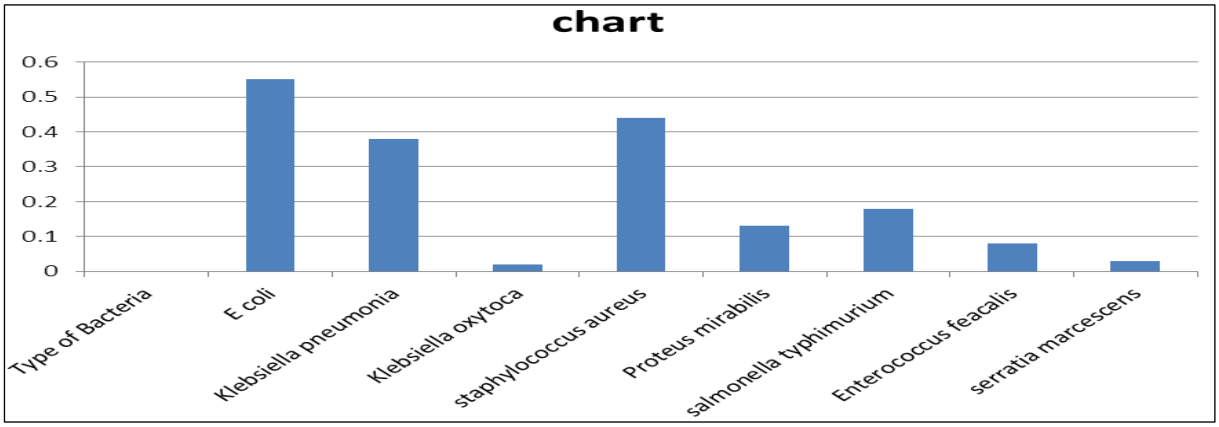

Figure (8): Chart show the per-cent of organism that isolated from beef hamburger

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Discussion

According to our study, the etiological agent more predominant that isolated from contaminated hamburger is *E.coli*, that agreement with (20,21) where they found *E.coli* is more prevalence in the meat also they found many of bacteria ,that bacteria isolated too in our study such as *Klebsiella pnemoniae, Proteus mirabilis, Staphylococcus aureus and Enterococcus faecium.* In addition (22) and (23) found Two food borne bacterial pathogens, *E. coli* and *Salmonella*, it's were associated with contaminated meat products. According to our results, the percentage of *E.coli* isolates in hamburger is (55%), that close with results of (24) where recorded percentage is (58.26%) in hamburger, while (25, 26) and (27) found the percentage of *E.coli* was (92%), (95%) and (88.4%) respectively, that represent more than our prevalence. The (28), (29) and (30) found low percentage of *E.coli* isolates in hamburger are (38.88%), (2.2%) and (2.3%) respectively, that less than our study. Meat products contaminated by *E. coli* isolates transmitted from infected animals, there no satisfactory hygienic measures applied during packing and handling (31). Depend on our percentage, *Klebsiella pneumonia* is (38%) in hamburger, where (32) and (33) recorded (33.33%) and (35.5%) respectively, that near to our results. while (34,35) and (21) record low prevalence (15%), (3%) and (*23.53%)* respectively, as percentage of *K. pneumoniae* in different type of hamburger meat, all that less than our results. while (36) recorded percentage of *K. pneumoniae* is very high (80%) that considered more than our results. (37) In Kolkata and (38) in Egypt recorded prevalence more than our percentage of *Klebsiella oxytoca* are (35%) and (17.3%) respectively, where our study found prevalence of *Klebsiella oxytoca* is (2%) in hamburger. *Staphylococcus aureus* represent *(*44%) according to our prevalence, (27) that near to our results Where was founded percentage (46.3%) in Cooked Hamburger, while (25, 36) and (39) recorded high percentage if compare with our study (85%), (96%) and (62%) respectively, (16.5%) represent percentage of *Staphylococcus aureus* in Meat Products, that less than our results (40). Distribution of *Staphylococcus aureus* in the meat products occur due to excessive human handling, where bacteria resides normally on the skin of the human and animal, degree of the contamination depend on type and number the transmitted bacteria (41). Percentage of *Proteus mirabilis* is (13%) depend on our study, where (40), (21) and (41) found (3.2%), (2.94%) and (4%) respectively, that is less than our results, while (36) and (37) recorded prevalence more than our study, it were (61.3%) and (50%) in fresh raw beef meat. *Salmonella typhimurium (*18%) in hamburger meat, according to our study, while (36) found (42.5%), and (42) found (11.1%), the first is higher while the second in lower than our results. This study showed meats are could contaminated by *Salmonella spp*, it was discover in the meat, The presence salmonella in meat should take it great attention because contaminated meat causing problems in public health hazard and causing food borne poisoning (43). Differences in production system of meat and contamination of meat at slaughterhouse process(44), variability in sampling of isolates from different sources(45), geographical features, socioeconomic and cultural differences between countries, national or international control and surveillance program differences, all that result variations in the prevalence (46) and (47). (21) Found percentage of *Enterococcus feacalis* in pork meat (8.82%). That is very close for our results in hamburger meat, where our results found (8%) represent prevalence of *Enterococcus feacalis* in hamburger meat, while (42) isolated *Enterobacter spp* as (13.9%) that is considered more than our results. the biochemical tests showed a high rate of contamination by *Enterobacter spp*. as

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(49.01%) in meat products (32). *prevelance of Srratia marcescens* is (3%) in hamburger meats according to our results, it was (11.1%) was founded by (42). Bacteria can be transmitted by incomplete cooking meat products, it has relatively short life; therefore, also should be rapid detection as soon as in these foods (48).Occurrence of infection usually associated with poor sanitary environment and dirty places during slaughtered, transported and processed (49). the many of factors which affecting on variety prevalence rates of contamination of meat and meat products among the studies; these differences in are mainly related to samples (type, source/location of the isolate), the environment and season plat great role in the prevalence (50), also food poisoning has seasonal occurrence, in the hot summer more than cold seasons (51). Finally, the hamburger is very dangerous food if do not applied hygiene measurements during slaughtering, cleaning, transportation, packing and marketing, it could be contaminated with many of bacteria that resulting matter on public health level.

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