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# Estimation of potential bacteriological hazards and their genetic virulence determinants in beef meals provided to intensive care patients

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**Abstract**

Meat is a good source of protein which makes it a good substrate for microbial growth. In this study, the prevalence, antibiogram and virulence genes of some foodborne pathogens from hospital beef meals provided to intensive care patients were evaluated. Therefore, a total of 60 beef meat samples (30 raws and 30 cooked samples) were randomly collected from different hospital kitchens in Ismailia province, Egypt. Samples were subjected to bacteriological evaluation for *Staphylococcus aureus*, *Escherichia coli* and *Salmonella*. The obtained results revealed that *S. aureus*, *E. coli* and *Salmonella* were isolated by a prevalence of 38.3% (23/60), 16.6% (10/60), and 8.3% (5/60), respectively. *S. aureus* was highly resistant to penicillin and streptomycin. *Escherichia coli* was highly resistant to amoxicillin, streptomycin, ciprofloxacin, gentamicin and ampicillin. *Salmonella* was highly resistant to amoxicillin, penicillin and cefpodoxime, ampicillin, gentamicin, ciprofloxacin and streptomycin. *Staphylococcus aureus* harboured *sea*, *seb*, *sec* and *sed* enterotoxin genes with a prevalence of 13.04%, 4.34%, 13.04 % and 4.34%, respectively. *Escherichia coli* isolates harboured $stx_1$ and $stx_2$ with a prevalence of 83.33% and 66.66%, respectively. *Escherichia coli* was serotyped as O128, O142, O26 and O157. *Salmonella* harboured *inv*A and *hil*A genes by 100% and 80%, respectively. In conclusion, the application of good manufacturing practices with well-trained food handlers is necessary to control and prevent contamination of hospitable meals by foodborne bacteria in order to protect and save intensive care patients.

## 1. Introduction

Meat is an important source of protein, essential amino acids, B complex vitamins and minerals. Therefore, it offers a highly favourable environment for the growth of pathogenic bacteria. The importance of food safety for hospitalized patients and the detrimental effect that contaminated food could have on their recovery have been emphasized (Hassan *et al.,* 2015).

In hospitals, outbreaks of foodborne infections are preventable but encouraged by many factors, including personnel carriers, poor kitchen hygiene conditions, carelessness, and lack of food handler training. In hospitals, the risk of tainted food is that such food is provided to customers in poor health (Teffo and Frederick, 2020; Young *et al.* 2020). This risk varies according to numerous parameters such as the microorganism's nature, the contamination level, the food nature and especially the consumer's physiological state. Therefore, the causes of foodborne illness and/or collective food poisoning may be microbiological food contamination by infected raw materials, insufficient cooking temperatures, improper preservation, contaminated facilities, cross-contamination and poor personal hygiene (Ndraha *et al.,* 2018).

Foodborne illness occurs when a pathogen is ingested with food and establishes itself in the human host, or when a toxigenic pathogen establishes itself in a food product and produces a toxin, which is then ingested by the human host. Thus, foodborne illness is generally classified into foodborne infection and foodborne intoxication. Foodborne pathogens are biological agents that can cause a foodborne illness event. A foodborne disease outbreak is defined as the

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occurrence of two or more cases of a similar illness resulting from the ingestion of a common food (CDC, 2012)

The most important bacterial pathogens in beef meat that are responsible for foodborne infections include *Escherichia coli*, *Salmonellae* and coagulase-positive *Staphylococcus aureus* (Abdaslam *et al.,* 2014). *E. coli* is mostly non-virulent, but pathogenic variants of *E. coli* (pathovars or pathotypes) cause much morbidity and mortality worldwide; many of these pathotypes are a major public health concern and are transmitted through ubiquitous mediums, including food and water (Croxen *et al.,* 2013).

Transmission of *E. coli* occurs when food or water that is contaminated with faeces of infected humans or animals is consumed. Contamination of animal products often occurs during the slaughter and processing of animals (Garcia *et al.,* 2010). *Salmonella* is one of the most widely implicated microorganisms in food-borne disease outbreaks. The most common causes of *Salmonella* food poisoning are meat products. Furthermore, *S. aureus* is one of the most causative agents of foodborne disease outbreaks causing gastroenteritis and is rarely acquired directly from raw meat but mostly occurs due to either excessive handling or contamination during or after cooking of meat and meat products (Addis and Sisay, 2015; Bou-Mitri *et al.* 2018; De Freitas *et al.* 2019).

The presence of *S. aureus* in food may be considered a public health hazard because of its ability to produce enterotoxin and the risk of subsequent food poisoning. Although there are nine identified staphylococcal enterotoxins, designated as A, B, C1, C2, C3, D, E, F, and G, types A and D are responsible for the majority of the outbreaks (Mossel *et al.,* 1995).

Therefore, the present study was performed to analyze sanitary-hygienic conditions and evaluate the microbiological status of hospital beef meals and investigate the prevalence of foodborne bacteria in hospital meat meals, perform antimicrobial susceptibility testing of bacterial isolates and detection of some virulence genes of the isolated foodborne pathogens.

## 2. Materials and methods

### *2.1 Sample collection*

A total of 60 random raw and cooked beef meat samples (30 each) were randomly collected from different hospital kitchens in Ismailia province from September 2020 to March 2021 for bacteriological evaluation. All samples were prepared according to techniques recommended by APHA (2001).

### *2.2. Isolation and identification of Staphylococcus aureus, Escherichia coli and Salmonella*

For isolation of *S. aureus*, a loopful from meat samples mixed with buffered peptone water was streaked on Baird-Parker agar media (Biolife, Italy) and incubated at 37°C for 24-48 hrs. The suspected *S. aureus* colonies were identified according to their colonial characters, hemolytic activity on blood agar, microscopical examination using Gram staining, biochemical reactions (catalase, coagulase, mannitol fermentation, oxidation fermentation and Voges–Proskauer test) as described by Cowan and Steel (1974).

For isolation of *E. coli*, a loopful from meat samples mixed with buffered peptone water was streaked on MacConkey's agar (Oxoid, Hampshire, UK) and eosin methylene blue (EMB) agar (Oxoid, Hampshire, UK) then incubated at 37°C for 24 hrs. The suspected colonies were identified according to colonial characters, microscopical examination by Gram's staining, motility test, hemolytic activity on blood agar, and biochemical reactions (oxidase, catalase, indole, lactose fermentation, methyl-red, citrate-utilization, $H_2S$, Voges-Proskauer, and urease tests) as described by Quinn *et al.* (2019).

For isolation of *Salmonella*, pre-enrichment of samples was done using Rapport–Vassiliadis broth (Oxoid, Hampshire, UK) then cultivation at 37°C for 18-24 hrs then selective cultivation from pre-enriched broth on Salmonella–Shigella (SS) agar (Oxoid, Hampshire, UK) and xylose lysine deoxycholate (XLD) agar (Oxoid, Hampshire, UK) then incubation at 37°C for 18-24 hrs. The suspected colonies were identified according to colonial characters, microscopical examination by Gram staining and biochemical reactions (oxidase test, catalase test, indole, methyl-red, citrate-utilization, $H_2S$, urease and Voges-Proskauer).

### *2.3 Serotyping of Escherichia coli and Salmonella*

*Escherichia coli* isolates were serotyped by slide agglutination test for detection of somatic (O) antigen with the aid of standard polyvalent and monovalent commercial *E. coli* antisera (Denka Seiken-Co., Ltd., Tokyo, Japan) as described by Starr (1986). *Salmonella* isolates were serotyped according to Kauffman – White scheme Kauffman (1974) for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum.

### *2.4 Antimicrobial susceptibility of Staphylococcus aureus, Escherichia coli and Salmonella*

Isolates were tested against 12 antibacterial agents (Oxoid Hampshire, UK) including amoxicillin/clavulanic acid, amoxicillin, cefpodoxime, ampicillin,

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ciprofloxacin, erythromycin, gentamicin, flumequine, cefotaxime, cefradine, enrofloxacin and streptomycin. Antibiogram was performed using Kirby Bauer disk diffusion method on Muller Hinton agar (Oxoid Hampshire, UK) as described before by Bauer *et al.* (1996). Briefly, 4-5 colonies from re-enriched preserved identified isolates, were inoculated in 5 mL Muller Hinton broth (Oxoid Hampshire, UK) and incubated for 4-5 hrs until turbidity was observed. The inoculated broth was then adjusted to a density equivalent to 0.5 MacFarlane standards. Sterile swabs were used to streak the bacterial suspension in different directions to wet the entire surface of labelled Muller Hinton agar plates and left for 30 mins. Antibiotic discs were then applied on the surface of the streaked plates using sterile forceps and the antibiotic dispenser. After overnight incubation, the inhibition zone was measured for each antibiotic using a calibre. The size of the inhibition zone was interpreted according to the prescribed guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015).

### *2.5 Molecular detection of some virulence genes in Staphylococcus aureus, Escherichia coli and Salmonella isolates*

PCR was used for detection of *sea*, *seb*, *sec* and sed enterotoxin genes of *S. aureus* additionally detection of *stx*$_1$ and *stx*$_2$ of *E. coli* and detection of *inv*A and *hil*A genes of *Salmonella* according to the previously published protocols (Table 1). QIAamp DNA Mini kit (Qiagen, GmbH, Germany/Catalogue No.51304) was used for DNA extraction according to the manufacturer's recommendations. DNA amplification of *S. aureus* was performed in a thermal cycler using the following conditions: initial denaturation for 5 mins at 94°C followed by 30 cycles of denaturation (94°C for 2 mins), annealing (50°C for 1 min), and extension (72°C for 1 min). A final extension step (72°C for 5 mins) was performed after the completion of the cycles. DNA amplification of *E. coli* virulence genes, *stx*$_1$ and *stx*$_2$ were detected by the following program, initial denaturation (3 mins at 94°C), followed by 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 55°C) and extension (1 min at 72°C), as well as a final extension at 72°C for 7 mins. Concerning DNA amplification of *Salmonella* as the following conditions, initial denaturation at 94°C for 60 s, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 64°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 7 mins. The reaction volume (25 µL) consisted of 6 µL of genomic DNA, 12.5 µL of 2×Master Mix and 20 pmol of each primer (Biobasic, Canada), and the final volume was completed to 25 µL by DNAse RNAse free water. Positive controls and negative controls (DNAse free water) were included in all reactions.

## 3. Results and discussion

### *3.1 Prevalence of foodborne pathogens in hospital beef meals*

Isolates were identified as *S. aureus* based on their morphology and biochemical characteristics. Microscopically, the bacteria appeared as Gram-positive small size, non-motile and non-sporulated cocci arranged in grape-like clusters. On Baired-Parker agar suspected *S. aureus* colonies appeared as dark grey to black colonies surrounded by a clear zone. On blood agar, the colonies were hemolytic. *S. aureus* was identified on the basis of coagulase test, catalase test, indole production, methyl red test, Voges-proskauer reaction, urease production, citrate utilization and sugar fermentation.

Table 1. Target genes, primer sequences, cycling conditions, specific amplicon size of *S. aureus*, *E. coli* and *Salmonella*

| Microorganism | Gene | Primer sequence (5'-3') | Length of amplified product | Reference |
|---|---|---|---|---|
| *S. aureus* | *sea* | TTGGAAACGGTTAAAACGAA<br>GAACCTTCCCATCAAAAACA | 120 | Rall *et al.* (2008) |
| | *seb* | TCGCATCAAACTGACAAACG<br>GCGGTACTCTATAAGTGCC | 478 | |
| | *sec* | GACATAAAAGCTAGGAATTT<br>AAATCGGATTAACATTATCC | 257 | |
| | *sed* | CTAGTTTGGTAATATCTCCT<br>TAATGCTATATCTTATAGGG | 317 | |
| *E. coli* | *stx*$_1$ | ACACTGGATGATCTCAGTGG<br>CTGAATCCCCCTCCATTATG | 614 | Dipineto *et al.* (2006) |
| | *stx*$_2$ | CCATGACAACGGACAGCAGTT<br>CCTGTCAACTGAGCAGCACTTTG | 779 | |
| *Salmonella* | *inv*A | GTGAAATTATCGCCACGTTCGGGCA<br>TCATCGCACCGTCAAAGGAACC | 284 | Shanmugasamy *et al.* (2011) |
| | *hil*A | CTGCCGCAGTGTTAAGGATA<br>CTGTCGCCTTAATCGCATGT | 497 | Guo *et al.* (2000) |

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Isolates were identified as *E. coli* based on their morphology and biochemical characteristics. Microscopically, the bacteria appeared as Gram-negative moderate size, motile, and non-sporulated rods. On MacConkeys's agar, *E. coli* gave characteristic pink colonies due to lactose fermentation. On blood agar, the colonies were hemolytic, while on EMB the bacteria gave characteristic metallic sheen colonies. Biochemically, all isolates were positive for catalase, lactose fermentation, indole, and methyl-red tests. Simultaneously, they were negative for cytochrome oxidase, Voges-Proskauer, citrate-utilization, $H_2S$ production, and urease tests.

*Salmonella* appeared as Gram-negative moderate size, motile, and non-sporulated rods microscopically. On XLD, *Salmonella* appeared as red colonies with a black centre while on SS agar appeared as colourless colonies with a black centre. Biochemically, all isolates were positive for catalase, methyl-red, citrate-utilization and $H_2S$ production tests. Simultaneously, they were negative for oxidase, indole, Voges-Proskauer and urease tests.

The prevalence of *S. aureus*, *E. coli* and *Salmonella* species in examined beef samples were 38.3% (23/60), 16.6% (10/60), and 8.3% (5/60), respectively, as shown in Table 2. Foodborne diseases persist to be an inclusive public health crisis with an estimated 600 million people falling ill annually (Faour-Klingbeil and Todd, 2020). Egypt as one of the Middle East countries is a particular one of concern in regard to food poisoning diseases according to Havelaar *et al.* (2015). In 2013, 160 Egyptian students have been recorded ill after consumption of food contaminated with *Salmonella* (Herriman, 2019).

*Staphylococcus aureus* was the most prevalent isolate from both raw and cooked beef meals, where it was isolated from raw beef by a percentage of 60% (18/30), while it was isolated from cooked beef by a percentage of 16.6% (5/30). *Escherichia coli* was isolated from raw beef meals by a percentage of 26.6% (8/30), while it was isolated from cooked beef meals by a percentage of 6.6% (2/30). *Salmonella* was isolated from raw meat and cooked meat meals by a percentage of 13.3% (4/30) and 3.3% (1/30), respectively as described in Table 2.

According to the obtained results, it could be confirmed that raw meat was the most contaminated with *S. aureus* than other samples. *S. aureus* inhabits the human skin and mucous membranes, where they exist mostly as commensal flora. Humans are the natural carriers of *S. aureus*, and the organism can be found in a healthy human population (Collins *et al.,* 2012).

The hospital feedings, sterile ready-to-use ones are exempted from the potential microbial contamination (Patchell *et al.,* 1998). Clinical consequences of contaminated feedings are abdominal pain, vomiting, diarrhoea, infection, fever, bacteremia, septicemia, and pneumonia (Oliviera *et al.,* 2000; Desport *et al.,* 2004). Since the microbial population receives the limit which causes food poising that is mainly affected by suppressed immune function and is often at high risk of developing and/or progressing malnutrition (Mokhalalati *et al.,* 2004).

The current results agreed with Wu *et al.* (2018) who isolated *S. aureus* from meat hospital meals by 35.0%. In addition, Shaltout *et al.* (2020) isolated *E. coli* from hospital meat meals by 13.33% and 11.7%, respectively. On the other hand, Jakabi *et al.* (2002) isolated *Salmonella* by 9%. The presence of these pathogens in hospital meals may reflect the bad hygienic practices during different stages of food chains from slaughtering, handling, transportation and excessive handling during preparation and serving of the meals.

*3.2 Serotyping of Escherichia coli and Salmonella isolates*

The results in Table 3 revealed that 10 (16.7%) *E. coli* isolates (8 from raw beef, 2 from cooked beef) were serologically typed. Where, 4 isolates (3 from raw beef, one from cooked beef) were typed as O128 (40%), 3 isolates were O142 (30%) in raw beef only, and one isolate was O26 (10%) in raw beef only. Finally, 2 isolates (one from raw beef, one from cooked beef) were typed as O157 (20%).

Serotyping is a common way to characterize Shiga toxin-producing *Escherichia coli* (STEC) strains and is based on the O antigen (somatic antigen) and H antigen (flagellar antigen) (Gyles, 2007). In concern to *E. coli* serovars, O128, O142, O26 and O157 were the detected serotypes. Similar results were observed by Saad *et al.* (2018). Detection of different *E. coli* O-serogroups in

Table 2. Prevalence of different foodborne pathogens from raw (n = 30) and cooked (n = 30) beef meals

| Bacterial isolates | No. of positive samples | Percentage (%) | Beef meals (n = 60) | | | |
|---|---|---|---|---|---|---|
| | | | Raw (n = 30) | | Cooked (n = 30) | |
| | | | No. of positive samples | % | No. of positive samples | % |
| *S. aureus* | 23 | 38.30% | 18 | 60% | 5 | 16.60% |
| *E. coli* | 10 | 16.60% | 8 | 26.60% | 2 | 6.60% |
| *Salmonella* | 5 | 8.30% | 4 | 13.30% | 1 | 3.30% |

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food are an indication of great public health hazards. Beef serves as a potential vehicle for transmitting *E. coli* O157:H7. The importance of proper handling and cooking of beef is probably as important in preventing *E. coli* O157:H7 infections. These include the application of Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP), followed by the implantation of the Hazard Analysis of Critical Control Points (HACCP) system at all food chains serving hospitable meals (Hiko *et al*., 2008).

Table 3. Prevalence of *E. coli* serotypes (n = 10) in hospital beef meals

| *E. coli* strains | Beef meals | | Strain Characteristic |
|---|---|---|---|
| | Raw no. (%) | Cooked no. (%) | |
| O128 | 3 (30) | 1 (10) | ETEC |
| O142 | 3 (30) | 0 (0.0) | EPEC |
| O26 | 1(10) | 0 (0.0) | EHEC |
| O157 | 1 (10) | 1 (10) | EHEC |
| Total | 8 (80) | 2 (20) | 10 (16.7) |

ETEC: Enterotoxigenic *E. coli,* EPEC: Enteropathogenic *E. coli,* EHEC: Enterohemorrhagic *E. coli*

The results in Table 4 revealed that five isolates of *Salmonella* were serologically identified as follows, *Salmonella enterica* serovar Typhimurium by 40% (2/5) in raw beef meals. While *Salmonella enterica* serovar Enteritidis by 20% (1/5) in raw beef meals and 20% (1/5) in cooked beef meals. *Salmonella enterica* serovar Infantis by 20% (1/5) in raw beef meals. The obtained results came in to agree with those obtained by Saad *et al.* (2018) who detected *S. enterica* ser. *Typhimurium* and *S. enterica* ser. *Enteritidis* in beef and hamburger.

*Salmonella enterica* serovar Infantis was confirmed as the cause of human salmonellosis in several countries and is the third most frequently isolated serovar of *Salmonella* after *S. enterica* ser. *Enteritidis* and *S. enterica* ser. *Typhimurium* (Ishihara *et al., 2009). Salmonella enterica* ser. *Typhimurium* was not detected in cooked beef samples, this is attributed to many stress factors which injured these bacteria, such as heat, which inhibits the repair mechanisms (Varnum and Evans, 1991).

Table 4. Prevalence of *Salmonella* serotypes (n = 5) in hospital beef meals

| Identified | Group | Antigenic structure | | Beef meals | |
|---|---|---|---|---|---|
| | | O | H | Raw | Cooked |
| *S. enterica* ser. Typhimurium | B | 1,4,5,12 | i : 1,2 | 2 (40%) | 0(00.0) |
| *S. enterica* ser. Enteritidis | D1 | 1,9,12 | g,m | 1 (20%) | 1(20%) |
| *S. enterica* ser. Infantis | C1 | 6,7 | r : 1,5 | 1 (20%) | 0 (00.0) |
| Total | | | | 4 (80%) | 1 (20%) |

O: Somatic antigen, H: Flagellar antigen

*3.3 Antimicrobial resistance of the isolated pathogens*

The results of antimicrobial susceptibility testing of isolated pathogens are shown in Table 5. The antimicrobial susceptibility testing of *S. aureus* (n = 23) from raw and cooked beef meals were as follows: antibiotics of choice against *S. aureus* were gentamicin and ciprofloxacin at 60.9%, followed by amoxycillin at 56.5%, enrofloxacin at 52.2%, cefotaxime and cefpodoxime at 43.5%, erythromycin and ampicillin at 21.7%, flumequine at 13.1% and cephradine at 8.7%. While *S. aureus* was highly resistant to penicillin, chloramphenicol (100% for each) was followed by streptomycin (78.3%). *S. aureus* was resistant to penicillin and streptomycin this nearly agreed with those detected by Akanbi *et al.* (2017).

The results of antimicrobial susceptibility testing of *E. coli* (n = 10) from raw and cooked beef meals were as follows; antibiotics of choice against *E. coli* were cefpodoxime 80% and cefotaxime 70%, while, *E. coli* was highly resistant to the following antibiotics, amoxycillin (100%), streptomycin and ciprofloxacin (80% for each) and ampicillin (60%). *Escherichia coli* was highly resistant to amoxycillin, streptomycin, ciprofloxacin, gentamicin and ampicillin, this was in agreement with Kibret and Abera (2011).

The findings of antimicrobial susceptibility testing of *Salmonella* isolates (n = 4) from raw and cooked beef meals were as follows; antibiotics of choice against *Salmonella* spp. were flumequine and cefotaxime by 75% for each. While *Salmonella* spp. were highly resistant to the following antibiotics, amoxycillin, penicillin and cefpodoxime (100% for each), ampicillin, gentamicin, ciprofloxacin and streptomycin (75% for each). *Salmonella* spp. was highly resistant to amoxycillin, penicillin, cefpodoxime, ampicillin, gentamicin, ciprofloxacin and streptomycin this was agreed with previous studies by Miko *et al.* (2005).

The careless use of antibiotics in veterinary practice is the main cause of the development of multidrug-resistant bacteria. Moreover, it is important to highlight that the use of any antimicrobial agent should not substitute good hygiene practices and, wherever possible, alternative management methods should be sought and used. In addition, veterinarians should respect and follow the sanitary legislation concerning the use of antimicrobial agents in their country.

*3.4 Prevalence of virulence genes of Staphylococcus aureus, Escherichia coli and Salmonella*

PCR proved that the tested *S. aureus* isolates (n = 23) were harboured the virulence genes (Table 6) *sea*, *seb*, *sec* and *sed* enterotoxin genes with a prevalence of

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Table 5. Antimicrobial resistance of *S. aureus* (n = 23), *E. coli* (n = 6) and *Salmonella* (n = 5) isolates

| Antimicrobial agent | *S. aureus* | | | | | | *E. coli* | | | | | | *Salmonella* spp. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sensitive | | Intermediate | | Resistant | | Sensitive | | Intermediate | | Resistant | | Sensitive | | Intermediate | | Resistant | |
| | no | % | no | % | no | % | no | % | no | % | no | % | no | % | no | % | no | % |
| Penicillin | 0 | 0 | 0 | 0 | 23 | 100 | 2 | 20 | 3 | 30 | 5 | 50 | 0 | 0 | 1 | 25 | 3 | 100 |
| Streptomycin | 0 | 0 | 5 | 21.7 | 18 | 78.3 | 0 | 0 | 2 | 20 | 8 | 80 | 0 | 0 | 1 | 25 | 3 | 75 |
| Flumequine | 3 | 13.1 | 7 | 30.4 | 13 | 56.5 | 6 | 60 | 4 | 40 | 0 | 0 | 3 | 75 | 0 | 0 | 1 | 25 |
| Cephardine | 2 | 8.7 | 8 | 34.8 | 13 | 56.5 | 2 | 20 | 5 | 50 | 3 | 30 | 1 | 25 | 1 | 25 | 2 | 50 |
| Erythromycin | 5 | 21.7 | 6 | 26.1 | 12 | 52.2 | 3 | 30 | 3 | 30 | 4 | 40 | 1 | 25 | 1 | 25 | 2 | 50 |
| Ampicillin | 5 | 21.7 | 8 | 34.8 | 10 | 43.5 | 0 | 0 | 4 | 40 | 6 | 60 | 0 | 0 | 1 | 25 | 3 | 75 |
| Enrofloxacine | 12 | 52.2 | 5 | 21.7 | 6 | 26.1 | 2 | 20 | 6 | 60 | 2 | 20 | 1 | 25 | 2 | 50 | 1 | 25 |
| Amoxycillin | 13 | 56.5 | 4 | 17.4 | 6 | 26.1 | 0 | 0 | 0 | 0 | 10 | 100 | 0 | 0 | 0 | 0 | 4 | 100 |
| Cefotaxime | 10 | 43.5 | 9 | 39.1 | 4 | 17.4 | 7 | 70 | 3 | 30 | 0 | 0 | 3 | 75 | 0 | 0 | 1 | 25 |
| Cefpodoxime | 10 | 43.5 | 10 | 43.5 | 3 | 13 | 8 | 80 | 2 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 100 |
| Gentamicin | 14 | 60.9 | 7 | 30.4 | 2 | 8.7 | 1 | 10 | 1 | 10 | 8 | 80 | 0 | 0 | 1 | 25 | 3 | 75 |
| Ciprofloxacin | 14 | 60.9 | 9 | 39.1 | 0 | 0 | 1 | 10 | 1 | 10 | 8 | 80 | 1 | 25 | 0 | 0 | 3 | 75 |

13.04% (3/23), 4.34% (1/23), 13.04 % (3/23) and 4.34% (1/23), respectively. These findings agreed with Elsherif *et al.* (2020).

On the other hand, the tested *E. coli* isolates (n = 6) were harboured the virulence genes $stx_1$ and $stx_2$ with a prevalence of 83.33% (5/6) and 66.66% (4/6), respectively. Concerning *inv*A and *hil*A genes of *Salmonella*, the tested isolates (n = 5) were harboured *inv*A gene by 100% (5/5) while *hil*A by 80% (4/5).

The current results proved that *E. coli* isolates were harboured $stx_1$ and $stx_2$. This agreed with Osek (2003) and Dipineto *et al.* (2006). Shiga toxin-producing *Escherichia coli* (STEC) are one of the most important pathogens transmitted by food. These strains, in addition to causing food poisoning, can cause severe diseases such as diarrhoea, bleeding colitis, hemolytic uremic syndrome, thrombocytopenic purpura, and death (Dipineto *et al.,* 2006).

The present data in Table 6 show that *Salmonella* (n = 5) was harboured *inv*A and *hil*A genes. These findings were in accordance with previous data detected by Thung *et al.* (2018) and Sallam *et al.* (2014). *Salmonella* harbouring *inv*A and *hil*A virulence genes is capable of producing gastroenteric illness in humans (Chaudhary *et al.,* 2015). On the other hand, *Salmonella* serotyped as *S. enterica* ser. Typhimurium, *S. enterica* ser. Enteritidis *and S. enterica* ser. Infantis and this was in accordance with Saad *et al.* (2018) who *detected S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis in beef kofta and burger. *Salmonella enterica* ser. Typhimurium was not detected in cooked beef this is attributed to many stress factors which injured these bacteria, such as heat, which inhibits the repair mechanisms (Varnum and Evans, 1991).

In conclusion, the presence of *S. aureus*, *E. coli* and *Salmonella* in hospital meat meals presents a great danger to public health as a result of the ability of these bacteria to produce foodborne poisoning, especially in immunosuppressed persons as intensive care patients. The application of good manufacturing practices with well-trained food handlers is necessary to control and prevent contamination of the hospitable meals by foodborne bacteria in order to protect and save intensive care patients.

Table 6. Prevalence of virulence genes in the isolated foodborne pathogens

| Prevalence genes of the isolated pathogens | | | | | |
|---|---|---|---|---|---|
| *S. aureus* (n = 23) | | *E. coli* (n = 6) | | *Salmonella* (n = 5) | |
| Genes | No. (%) | Genes | No. (%) | Genes | No. (%) |
| *sea* | 3 (13.04) | $stx_1$ | 5 (83.33) | *inv*A | 5 (100) |
| *seb* | 1 (4.34) | $stx_2$ | 4 (66.67) | *hil*A | 4 (80) |
| *sec* | 3 (13.04) | | | | |
| *Sed* | 1 (4.34) | | | | |

## Conflict of interest

The authors declared that the present study was performed in absence of any conflict of interest.

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