RESEARCH ARTICLE

Antibiotic Resistance Profile of *Staphylococcus aureus* isolated from Imported and Domestic Meat Samples

Esraa M. Kazem, Eman M. Jarallah

Department of Biology, College of Science, University of Babylon, Hillah, Iraq

Received: 02nd November, 2022; Revised: 30th November, 2022; Accepted: 07th December, 2022; Available Online: 25th December, 2022

ABSTRACT

Food-borne microbes are considered one of the most important health risks at the present time, as well as an economic problem where the contaminated material must be destroyed and large sums of money lost to the producing companies. The result of examining the samples of cans using the pour method was that imported cans are more polluted than local cans. The sellers, as these bacteria cause food poisoning and most of them are resistant to antibiotics, which increases their danger. The highest percentages of antibiotic resistance were detected for trimethoprim followed by gentamicin and penicillin G, while most isolates were sensitive to ciprofloxacin and nitrofurantoin. The 60 samples were collected from canned food. They were randomly collected from different supermarkets in Hilla, Iraq. It also showed the results of microscopy and biochemical tests. In addition to the recognition of isolates by the Vitek 2 system, it was found that 30% of them contain *Staphylococcus aureus*, 20% *Enterobacteriaceae*, and 10% different bacteria, including *Streptococcus* and other species. Sensitivity tests were conducted for all the samples.

Keywords: Antibiotic Resistance, Canned meat, Foodborne diseases, *Staphylococcus aureus*

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.4.51

How to cite this article: Kazem EM, Jarallah EM. Antibiotic Resistance Profile of *Staphylococcus aureus* isolated from Imported and Domestic Meat Samples. International Journal of Drug Delivery Technology. 2022;12(4):1797-1801.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Food-borne diseases (FBDs) are the presence of physical, chemical and biological factors in food that lead to disease in humans. Biological factors include viruses, fungi, bacteria, and parasites or toxins that render them unfit for human consumption. Bacteria are among the most important pathogens in humans, resulting in a high morbidity and mortality rate. Diseases may be mild or fatal.[1,2]

Bacteria are classified according to their need for oxygen into aerobic and anaerobic, and aerobic bacteria are classified into spore-forming bacteria, which are *Bacillus*, and they are very dangerous because their spores remain for a long time and resist high temperatures and bacteria that do not form spores. These bacteria are contaminated with food and infect humans with diseases.[3] Prevent possible food poisoning, examining food before distributing it to the consumer reduces the risk of poisoning and death.

Food can be stored for long periods of time if proper procedures are followed, such as cleaning food contact surfaces and preventing microbial enzymes from spoiling the food.[2]

Meat is of high nutritional value as it provides a protein for the consumer, which has a major role in building the human body as it provides amino acids. But during the slaughter and canning operations, meat is contaminated with various pollutants harmful to humans, it is a suitable environment for the growth of microbes.[4]

Meat includes two types of white and red meat, and the quality of these meat depends on several factors including: temperature, humidity, pH, the cooking process and preservatives added to those meat and the presence of inhibitory and activating substances for microbes.[5]

Food-borne diseases constitute a public health, economic and social burden worldwide, The world health organization (WHO) has estimated that there are 600,000 food-borne diseases and 420,000 deaths, attributed to 31 microbes.[2,6-8] Meat may contain bacteria, containing antibiotic resistance genes, which are transmitted to humans and cause diseases that are resistant to antibiotics, including the beta-lactam group. And sometimes the meat may be uncontaminated, but it becomes contaminated during barbecue operations, including *Staphylococcus aureus* bacteria carrying methicillin-resistant genes, which reach the consumer due to lack of attention to hygiene operations.[9]

In 2017, the WHO recorded 12 types of bacteria that threaten human life, because they are resistant to antibiotics, Bacteria were categorized by priority: critical, high, and

**Author for Correspondence: emanm.jarallah@yahoo.com*

medium priority. *Pseudomonas aeruginosa* was among the critical priority group, *Enterococcus faecium*, and *Staphylococcus aureus, Campylobacter* and *Salmonella* are on the list high priority group. These bacteria cause symptoms including fever, vomiting, diarrhea, and stomach cramps.[10,11]

Staphylococcus aureus is considered one of the most pathogenic bacteria transmitted by food because it grows at a temperature of 15–45°C, as well as bears a high concentration of NaCl. It can multiply quickly at room temperature.[12]

MATERIAL AND METHODOLOGY

This research aims to study pathogenic bacteria isolated from canned meat.

Collection of Samples

The 60 meat canned samples were randomly collected from different markets from Hilla city in Iraq, including luncheon meat 15(25%), sausage 10(16.6), tuna 9(15%), Chicken nuggets pieces 9(15%), Minced meat 7(11.6), roasted meat 5(8.3), and hamburger 5(8.3%) (Table 1). The samples were collected during the period from november 2021 to january 2022 in a sterile, clean and dry, conditions (Figure 1).

Preparation of Samples

Under sterile conditions, one gram of each sample was add to 9 mL of distilled water, then series of tenfold dilution were done (Figure 2).[13,14]

Enumeration and Isolation of Bacteria

From 100 µL was taken from and the previously prepared serial dilution was cultured in nutrient agar by pour-plate technique and incubated at 37°C for 48 hours. were counted and isolated for more identification. After incubation period the bacteria colonies were counted according the following equation:

CFU per mL: No. of colonies *Inverted dilution factor/ inoculum volume.[15]

Identification of Bacterial Isolates

The isolated bacteria are purified by sub-culturing the colonies several times by plotting it on nutrient agar until we get pure colonies. Then it was grown on MacConky agar medium to isolate gram- negative bacteria. After that, it cultivated the isolates that grew on the medium of the MacConkey on another medium, the eosin methylene blue agar, where it distinguishes between *E. coli* and *Klebsiella*. Then the bacteria that did not grow on the MacConkey were re- cultivated on a medium mannitol salt agar medium to determine the types of *S. bacteria*, as they grow on this differential medium. Then gram stain was done for all samples, as well as biochemically characterized for catalase and oxidase testing before stock preparation.[16]

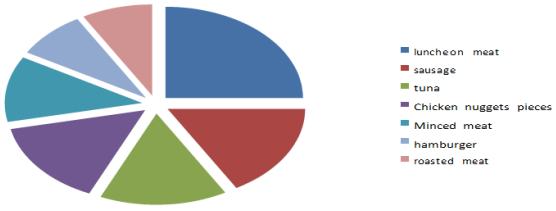

Figure 1: The distribution of isolated meat sample

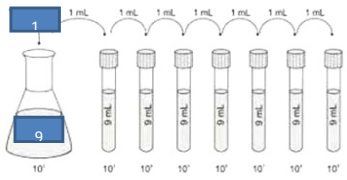

Figure 2: Series of tenfold dilutions

Figure 3: Isolation of bacteria from canned food

Table 1: Sample types and their proportions

Sample type	*Number of samples*	*Percentage (%)*
luncheon meat	15	25
sausage	10	16.6
tuna	9	15
Chicken nuggets pieces	9	15
Minced meat	7	11.6
Shawarma	5	8.3
hamburger	5	8.3

Antibiotic Susceptibility Testing

The Kirby-Bauer disc diffusion procedure was used to assess the antibiotic susceptibility of bacteria isolated from canned foods (Figure 3). Antibiotic discs were used in this study were 11 discs as shown in the Table 2 of antibiotics, their classification, abbreviation, potency and mechanism of action (Figure 4).

Cefalexin (FOX), erythromycin (E), chloramphenicol (C), ciprofloxacin (CIP), trimethoprim (TMP), nitrofurantoin (F), penicillin G (P), clindamycin (DA), vancomycin (VA), gentamicin (CN), rifampicin (RA), tetracycline (TE), levofloxacin (LEV).

Results were reported according to clinical and laboratory standards Institute guidelines (CLSI,2021). Colonies (2-4) of

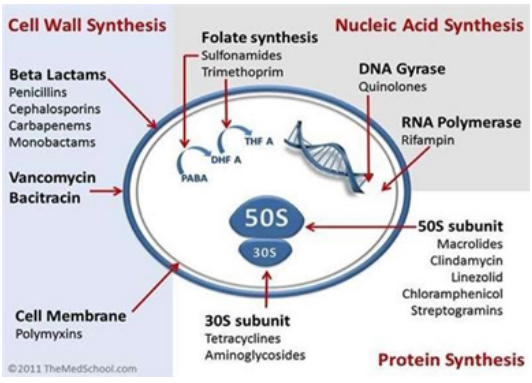

Figure 4: The mechanism of action of antibiotics

Table 2: Antibiotics, their classes, abbreviations, and their mechanism of action

Antibiotic	*Class*	*Abbreviation*	*Potency (Disc load (µg))*	*Mechanism of action*
Cefalexin	1st generation cephalosporin	FOX	30	Inhibition of cell wall synthesis
Erythromycin	Macrolides	E	15	Inhibition of proteins synthesis
Chloramphenicol	Miscellaneous	C	30	Inhibition of proteins synthesis
Ciprofloxacin	Fluoroquinolone	CIP	10	Inhibition of nucleic acid synthesis
Trimethoprim	Sulfonamides	TMP	5–10	
Nitrofurantoin	Miscellaneous	F-300 F-100	100–300	Inhibition of nucleic acid synthesis
Penicillin G	Penicillins	P	10	Inhibition of cell wall synthesis
Clindamycin	Lincosamides	DA	15	Inhibition of proteins synthesis
Vancomycin	Glycopeptides	VA	30	Inhibition of cell wall synthesis
Gentamicin	Aminoglycoside	CN	10	Inhibition of proteins synthesis
Rifampicin	Miscellaneous (others)	RA	5	Inhibition of nucleic acid synthesis
Tetracycline	Tetracyclines	TE	10	Inhibition of proteins synthesis
Levofloxacin	Fluoroquinolones	LEV	5	Inhibition of nucleic acid synthesis

the bacterial isolate were transferred to a test tube containing 5 mL of nutrition broth and incubated at 37°C for 2 hours then growth was reduced with physiological saline, and the growth in the tube was compared with the Macfarlaned tube (0.5) standards. The bacteria were spread on the Muller Hinton agar by cotton swab dipped in the broth. Then the discs were placed around the plate at the rate of four tablets and one in the center. The dishes were kept at 37°C for 18–24 hours for all types of antibiotics. Then the damping diameters were measured using a measuring ruler and compared with the standard values.

RESULTS AND DISCUSSION

A 60 samples of canned meat products were collected in a sterilized manner and randomly from different supermarkets of the city of Babylon in Iraq, part of which was fully cooked (luncheon, sardines, sauce, tuna and roasted meat (shawarma), another part was uncooked (minced meat, chicken nuggets pieces, hamburger). Most cases of disease treatment failure are due to bacterial resistance to many types of antibiotics due to the misuse of antibiotic.[17] A person acquires pathogenic bacteria that are resistant to antibiotics from eating foods contaminated with these bacteria, as they cause food poisoning from mild cases to severe cases and sometimes cause deaths. The results, after isolation and diagnosis, showed that 30% of the isolated bacteria were *Staphylococcus*, 20% *Enterobacteriaceae*, 10% of different bacteria types. According to the morphological and microscopic diagnosis and the final diagnosis by the Vitek device.

Gram stain was done for all samples to differentiate between bacteria based on the shape and composition of cells, into negative and positive (Figure 5). Gram stain reaction is based on the difference in the chemical essence of bacterial cell walls. Gram-positive cells have a thick peptidoglycan layer, whereas the peptidoglycan layer in gram negative cells is much thinner and surrounded by outer lipid-containing layers. As a result, gram positive bacteria appeared purple because iodine and crystal violet precipitate in the thickened cell wall and are not eluted by alcohol in contrast with gram negative bacteria where the crystal violet is freely eluted from the bacteria, which expressed the pink colour.

Table 3: The percentage of bacteria resistance to types of antibiotics and the percentage of bacteria sensitive to antibiotics

Antibiotic	*R (%)*	*S (%)*	*I (%)*
E15	98	2	0
DA15	98	2	0
TMP 5	100	0	0
TMP10	100	0	0
C30	96	4	0
P10	100	0	0
FOX10	98	2	0
F300	0	100	0
F100	14	84	0
CIP10	0	100	0
VA30	86	14	0
RA5	84	6	10
TE10	84	14	0
LEV5	0	100	0

Samples were cultured on nutrient agar by pour or diffusion method, many bacterial colonies were found on the medium, and some grew at the bottom of the dish, indicating that it is an anaerobic bacteria, meaning the presence of its spores in canned food. Then purified them by sub-culturing method on nutrient agar medium to obtain pure colonies for

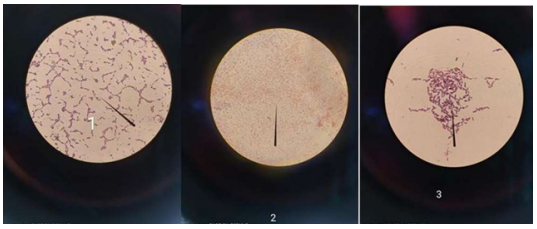

Figure 5: Gram stain of bacterial species

Figure 6: Bacterial growth on MacConkey agar

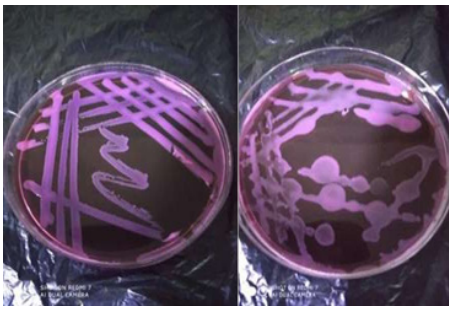

Figure 7: Bacterial growth on EMB agar

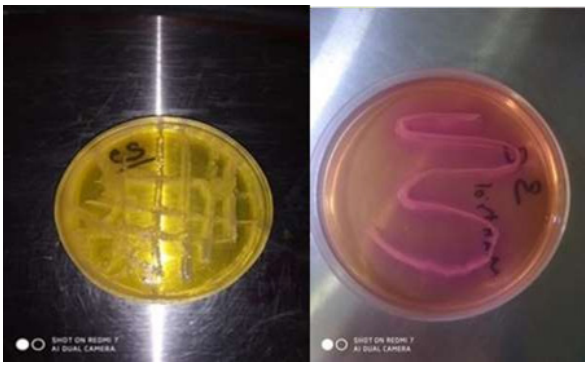

Figure 8: Bacterial growth on mannitol salt agar

diagnostic purpose. After purification was done first, has been cultivated the samples on MacConkey agar medium to isolate gram-negative bacteria and differentiate the lactose-fermenting bacteria present in it, as they appear in brighter color than the non-lactose- fermenting bacteria, which appear in pale color.

The media cultured on MacConkey agar was re-cultivated on eosin methylene blue to characterize *E. Coli* from the rest of the *Enterococcu*s, as it appeared in a bright green color. EMB is a differential microbiological medium, which slightly inhibits the growth of gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment

Figure 9: Antibitic susceptibility test bacterial isolates

lactose (e.g., *E. coli*) and those that do not (*e.g., Salmonella,* Shigella) (Figure 6, 7).

Then culturing the samples that did not grow on MacConkey agar and cultured them on mannitol salt agar medium for the purpose of diagnosing *Staphylococcus* bacteria of all kinds and we distinguished *Staphylococcus aureus* from the rest of the *S.* species. The medium will select organisms that can live in areas with a high concentration of salt (sodium chloride) and the fermentation of mannitol, demonstrated by the yellow turn of the pH indicator (phenol red), makes it possible to guide the diagnosis. It was found that 30% are staphylococci, and 36.6% are *S. aureus* (Figure 8).

After isolation and phenotypic determination of the bacteria initially, the determination of the type of bacteria was carried out with the Vitek device, and it showed the presence of the following types of bacteria:

S. aureus, Enterobacter cloacae ssp dissolvens, *Staphylococcus lentus, Streptcoccus agalactiae, Gemella sanguinis, Micrococcus luteus, Kocuria kristinae, Klebsiella pneumonia.*

Antibiotic Sensitivity Test

Sensitivity test was performed, which included the following antibiotics cefalexin (FOX), erythromycin (E), chloram phenicol (C), ciprofloxacin (CIP), trimethop rim (TMP), nitrofurantoin(F), penicillin G(P), clindamycin (DA), vancomycin (VA), gentamicin (CN), rifampicin(RA), tetracycline (TE) and levofloxacin (LEV), erythromycin, penicillin, vancomycin and trimethoprim are resistant to antibiotics. On the contrary, some antibiotics are highly sensitive to antibiotics, such as levofloxacin and ciprofloxacin. As shown in the Table 3 and Figure 9.

CONCLUSION

Currently, one of the biggest health hazards are those caused by food-borne microorganisms. Food-borne diseases constitute a public health, economic, and social burden worldwide. The pour method was used for examining the samples of cans that were imported and are more polluted than local cans. These bacteria can cause food poisoning and most of them are resistant to antibiotics. Hence, it can increase the risk of food poisoning and even can cause dealth. A 60 samples were collected from canned food and were randomly collected from different supermarkets in Hilla, Iraq. The microscopy and biochemical test showed the highest percentage of antibiotic resistance was detected from trimethoprim, followed by gentamicin and

penicillin G, while most isolates were sensitive to ciprofloxacin and nitrofurantoin. In addition to the recognition of isolates by the Vitek 2 system, it was found that 30% of them contain *Staphylococcus aureus*, 20% *Enterobacteriaceae*, and 10% different bacteria, including Streptococcus and other species. Sensitivity tests were also, conducted from all these samples.

REFERENCES

1. Rortana C, Nguyen-Viet H, Tum S, Unger F, Boqvist S, Dang-Xuan S, Koam S, Grace D, Osbjer K, Heng T, Sarim S. Prevalence of Salmonella spp. and Staphylococcus aureus in chicken meat and pork from Cambodian Markets. Pathogens. 2021 May;10(5):556.
2. HUSAIN DA, AZIZ ZS. Molecular study of bacteria isolated from meat and chicken frozen from Misan Governorate market in Iraq. Biodiversitas Journal of Biological Diversity. 2022;23(1).
3. Wang N, Jin Y, He G, Yuan L. Dynamic tracing of bacterial community distribution and biofilm control of dominant species in milk powder processing. LWT. 2022 Jan 15;154:112855.
4. Aduah M, Adzitey F, Amoako DG, Abia AL, Ekli R, Teye GA, Shariff AH, Huda N. Not All Street Food Is Bad: Low Prevalence of Antibiotic-Resistant Salmonella enterica in Ready-to-Eat (RTE) Meats in Ghana Is Associated with Good Vendors' Knowledge of Meat Safety. Foods. 2021 May 6;10(5):1011.
5. HAMEED MA, Muhammed HA, Al-Rrashedi AA. The bacterial load and contamination rate of raw meat in butcher shops in different areas of Karbala City. Pak J Med Health Sci. 2021 Mar;15:960.
6. Aguirre Garcia M, Hillion K, Cappelier JM, Neunlist M, Mahe MM, Haddad N. Intestinal Organoids: New Tools to Comprehend the Virulence of Bacterial Foodborne Pathogens. Foods. 2022 Jan 1;11(1):108.
7. Pires SM, Desta BN, Mughini-Gras L, Mmbaga BT, Fayemi OE, Salvador EM, Gobena T, Majowicz SE, Hald T, Hoejskov PS, Minato Y. Burden of foodborne diseases: Think global, act local. Current Opinion in Food Science. 2021 Jun 1;39:152-9.
8. Mi F, Guan M, Hu C, Peng F, Sun S, Wang X. Application of lectin-based biosensor technology in the detection of foodborne pathogenic bacteria: A review. Analyst. 2021;146(2):429-43.
9. Plaza-Rodríguez C, Mesa-Varona O, Alt K, Grobbel M, Tenhagen BA, Kaesbohrer A. Comparative Analysis of Consumer Exposure to Resistant Bacteria through Chicken Meat Consumption in Germany. Microorganisms. 2021 May 12;9(5):1045.
10. Qi W, Wang L, Rong N, Huo X, Li Y, Liao M, Lin J. A lab-on-a-tube biosensor for automatic detection of foodborne bacteria using rotated Halbach magnetic separation and Raspberry Pi imaging. Talanta. 2022 Mar 1;239:123095.
11. Beier R. Interactions and inhibition of pathogenic foodborne bacteria with individual dissociated organic acid species: a review. J. Food Chem. Nanotechnol. 2021 Feb 18;7:4-17.
12. 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.
13. Samaha IA, Ibrahim H, Hamada MO. Isolation of some enteropathogens from retailed poultry meat in Alexandria Province. Alex. J. Vet. Sci. 2012;37(1):17-22.
14. Khalafallah BM, El-Tawab A, Awad A, Nada S, Elkhayat ME. Phenotypic and genotypic characterization of pseudomonas species isolated from frozen meat. Benha Veterinary Medical Journal. 2020 Dec 1;39(2):47-51.
15. Hafez M, Ibrahim H, Amin R. Assessment of Bacterial Evaluation of imported Frozen Meat. Benha Veterinary Medical Journal. 2019 Dec 1;37(2):42-5.
16. Nair PS, Surendran PK. Biochemical characterization of lactic acid bacteria isolated from fish and prawn.
17. Gnanadhas DP, Ben Thomas M, Thomas R, Raichur AM, Chakravortty D. Interaction of silver nanoparticles with serum proteins affects their antimicrobial activity in vivo. Antimicrobial agents and chemotherapy. 2013 Oct;57(10):4945-55.