Biotyping of Methicillin-Resistant *Staphylococcus Aureus* Isolated from Poultry Meat Sold in Wasit Markets of Iraq

ABSTRACT

Within the realm of food-borne infections, disease causing biotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) strains seem widely acknowledged as being among the most significant contributors. A total of thirteen MRSA isolates were obtained from raw and frozen chicken meat that was sold in Wasit markets. The purpose of this study was to establish the origin of these isolates. To accomplish this objective, the isolates were put through the Biotyping system that was presented by Devriese. This system was used to check the behavior of the isolates in several biochemical assays, including the synthesis of staphylokinase (K), beta-hemolysis (β), bovine plasma coagulation (CPB), along with growth in the presence of crystal violet (CV). Among the thirteen MRSA isolates, there were five distinct biotypes. Two of these biotypes were host-specific, and they were classified as avian (07) and human (03). Additionally, there were three non-host-specific biotypes, each consisting of three isolates. These biotypes exhibited the subsequent biochemical features: K-; β+; CPB-; CV: A (01), K-; β+; CPB-; CV: E (01), and K-; β-; CPB-; CV: C (01). These biotypes can be isolated from birds, cattle, and humans. It is possible that there is a lack of adequate management of animals that pose a threat to public health, as shown by the existence of this biotype which is present. We need to do more study in order to discover other epidemiological elements of the MRSA isolates that were detected in a variety of food samples.

Keywords: *Biotyping, Biochemical behavior, MRSA, Poultry products.*

1. INTRODUCTION

In recent years, numerous pathogens have raised concerns regarding food safety, with various studies conducted in Iraq highlighting the contamination of meat by several foodborne pathogens, including *Acinetobacter baumannii* (Kanaan et al., 2020; Kanaan and Khashan, 2022), *Arcobacter* spp. (Kanaan, 2021; Kanaan, 2024b), *Clostridium botulinum* (Kanaan and Tarek, 2020), *Salmonella enterica* (Kanaan et al., 2022; Kanaan, 2023), *Staphylococcus aureus* (Kanaan and Al-Isawi, 2019; Kanaan and Abdullah, 2019; Kanaan and Abdullah, 2021; Kanaan et al., 2023b), and *Campylobacter* spp. (Ghaffoori, 2017; Kanaan and Khashan, 218; Kanaan and Mohammed, 2020; Kanaan and Tarek, 2022; Kanaan et al., 2022; Kanaan et al., 2023; Kanaan, 2024a; Kanaan et al., 2024). *Staphylococcus aureus* is globally acknowledged as a significant foodborne pathogen in fresh and ready-to-eat foods, responsible for several illnesses (Kanaan, 2018; Kanaan et al., 2023a). This bacterium proliferates rapidly at ambient temperature, generating toxins that induce sickness. The distribution of *S. aureus* is widespread globally; nevertheless, the primary cause of *S. aureus* infections is food (Kanaan et al., 2023a). Annually, it is implicated in approximately 241,000 cases of foodborne illness in the USA (Wu et al., 2018). In 2013, *S. aureus* accounted for 12.5% of foodborne bacterial outbreaks in China, ranking as the third most prevalent pathogen behind *Vibrio parahaemolyticus* (27.8%) and *Salmonella* (23.1%) (Wei-Wei et al., 2018). In recent decades, the extensive application of antibiotics in bacteria has led to the rise of multidrug-resistant strains (MDR), presenting significant concerns to public health (Kanaan, 2024 b). *S. aureus* exhibits a significant adaptation capacity, enabling it to adjust to diverse

environmental circumstances and swiftly develop resistance to nearly all antibiotics (Mccallum et al., 2010). Currently, an increasing incidence of multidrug-resistant *S. aureus* has been documented in food poisoning outbreaks and isolated from food items in prior studies (Gharsa et al., 2012; Papadopoulos et al., 2018). Also, MRSA has been a hot topic as of late. In 2017, the World Health Organization (WHO) identified this bacterium family as one of the twelve most dangerous to human health due to its tendency to exhibit MDR (Govindaraj and Vanitha, 2018). Knowing whether MRSA strains originate from animals or humans can be crucial when foods containing animal products or components are involved (Blaiotta et al., 2006; Wendlandt et al., 2013). It is possible for *S. aureus* to spread across the host's surroundings. The matter has taken on more significance due to the fact that *S. aureus* strains may be classified into several biotypes, each with its own unique set of microbiological traits (Blaiotta et al., 2006; Wendlandt et al., 2013). A practical way to identify the specific pathways of food contamination and to compare and contrast their microbiological and epidemiological characteristics is to identify the *S. aureus* biotypes (Dehkordi et al., 2017). According to Dehkordi et al. (2017), it is vital to perform Biotyping on MRSA isolates obtained from a variety of food samples in order to trace their origin and identify the relevance of these strains to public health, as well as to examine the link between the strains and to determine the diversity of these strains both within and between samples. As a result of the fact that chicken is frequently regarded as the most popular sort of meat in several territories around our country. Thus, the objective of this study was to analyze the distribution of MRSA isolates according to their behavior in biochemical tests and their categorization according to their biotypes. This would be done in order to get a better knowledge of the epidemiology of this bacteria in poultry meat that is sold at Wasit markets.

2.METHODOLOGY

2.1. Bacterial Isolates Preparation

According to the findings of a recent study (Kanaan, 2018), a total of thirteen MRSA isolates were recovered from poultry flesh. The isolation and identification of these isolates were carried out by employing the conventional microbiological and biochemical assays that were detailed earlier (Kanaan, 2018). Biochemical tests using the Electronic RapID™ Staph PlusCode Compendium Panel System (ERIC®) with Installation ERIC® CD, Standard Color Differential Chart, and Online ATCC Codes (Remel, R8311009) were used to validate *S. aureus* species level. MRSA isolates were identified using the dry SPOT Staphytect Plus kit (Oxoid, DR0100M) to identify MRSA-specific virulence factors. The latex agglutination PBP2 test kit (Oxoid, DR0900A) detected PBP2a in MRSA isolates, identifying them further (Kanaan, 2018). At a temperature of minus 80 degrees Celsius, frozen single-use stocks were stored in a double-strength Tryptic Soy broth (Oxoid, CM0129) - Yeast Extract (Oxoid, LP0021B) with 20% medical glycerin. These stocks were then thawed in a refrigerator for a full 24 hours, then subculture onto Tryptone Soya Agar (Oxoid, CM0131B), and then incubated for a full 24 hours at a temperature range of 35 to 37 degrees Celsius.

2.2. Biotyping of MRSA Isolates

Although there were some minor adjustments made, the Biotyping of the MRSA strains was carried out in accordance with the technique outlined by Devriese (1984). MRSA isolates were subjected to a series of tests, including the generation of staphylokinase and β-hemolysin, the ability to coagulate bovine plasma, and the proliferation capacity on crystal violet agar. By incubating MRSA isolates on Tryptone Soya Agar (Oxoid, CM0131B) plates with 1% bovine fibrin plates with or

without 5% dog serum as a plasminogen source, the staphylokinase production of the MRSA isolates was investigated in order to validate the effect of staphylokinase on bovine fibrinogen or canine plasminogen. The formation of staphylokinase can be identified by observing the presence of Lear zones on bovine fibrin plates that have been treated along with dog serum.

In addition, MRSA isolates have been grown on blood agar base (Oxoid, CM0055) with 5% defibrinated sheep blood in order to determine the amount of beta-haemolysin that was produced. A marker for the generation of beta-haemolysin is the appearance of large discolored zones with sharp edges that clear up at a temperature of four degrees Celsius. The process of studying the coagulation of bovine plasma involves introducing 0.1 mL of an overnight culture of MRSA isolates in Brain Heart Infusion broth (Oxoid, CM1135) to 0.5 mL volumes of plasma that have been diluted 1:3 and then incubating the mixture at 37 degrees Celsius. The formation of large clots during a period of six hours was determined to be a beneficial reaction. Meyer (1967) provided the interpretation that was used for the verification of growth in the presence of crystal violet as well as the features of MRSA isolates. Colonies that were blue or violet in hue were referred to as type C colonies, colonies that were light or brilliant yellow and had a blue edge were referred to as type A colonies, and colonies that were white or bluish-white were referred to as type E colonies. The growth test was conducted on plates containing nutritional agar (Oxoid, BO0336) and crystal violet at a concentration of 1μg/mL, as described by Devriese in 1984. After performing the biochemical tests, the reactions were interpreted and the strains were classified according to criteria proposed by Devriese (1984) and Shimizu et al. (1986).

2.3. Data Analyses

Through the use of MedCalc Software bvba Version 23.1.3 (Belgium, United States of America, https://www.medcalc.org/), the accessible data were evaluated. An investigation into the significance between proportions was conducted by employing a Chi-square (χ^2), which is a statistical method that compares two samples with a significance threshold of 5% (version 23.1.3).

3. RESULTS

The data that has been publicized in Table 1 are a representation of the prevalence of numerous biotypes of MRSA isolates that were obtained from samples of chicken. In the MRSA isolates that were isolated from raw chicken flesh samples, the prevalence of poultry-based biotypes was 38.46%, whereas the frequency of human-based biotypes seemed 15.38% more prevalent. 7.69% of the MRSA isolates were found to have biotypes that were either non-host-specific or unknown throughout the analysis. Furthermore, the prevalence of poultry, human, and non-host-specific biotypes in the MRSA isolates was 7.69%, 7.69%, and 7.69%, respectively, in the local frozen chicken meat. On the other hand, imported frozen chicken meat only exhibited one host-specific biotype (the poultry biotype) and one non-host-specific biotype, with the percentages being 7.69% and 7.69%, respectively.

Based on the biochemical behavior of MRSA isolates, our findings (Table 2) proved that ten out of thirteen isolates displayed two host-specific biotypes (poultry and human) at a rate of 53.85% and 23.08%, respectively. This represents the prevalence of MRSA isolates. On the other hand, three different isolates displayed three different non-host-specific biotypes, with the percentages being 7.69%, 7.69%, and 7.69%, respectively (Table 2 and Figure 1). Furthermore, the prevalence of host-

specific and non-host-specific biotypes in poultry meat samples was found to be statistically significant ($P \leq 0.05$) ($X^2 = 7.247$, $P = 0.0071$).

4. DISCUSSION

Methicillin-resistant *Staphylococcus aureus* is a leading cause of food poisoning in many populations (Sergelidis and Angelidis, 2017). Contamination of meat can occur as a result of diseased animals being harvested or as a result of poor manufacturing practices, such as through the butcher's tools or in the meat boards for buyers, which are suitable vehicles for the transmission of the pathogen (Kanaan and Al-Isawi, 2019). Biotyping is a straightforward approach for determining the derivation of *S. aureus* extracted from food samples (Cunha et al., 2011). The current study is the first to reveal the Biotyping of MRSA isolates from raw and frozen chicken meat samples sold at Wasit markets. Our findings revealed that MRSA isolates from raw and locally frozen chicken meat contained two different host specific biotypes and one non-host specific biotype, whereas imported chicken meat samples contained only two biotypes, one host specific and one non-host specific (Table 1). Using the simplified system developed by Devriese (1984), researchers from different parts of the world who conducted epidemiological studies of *S. aureus* isolated from different food samples obtained different percentages compared to those found in this study. These percentages were related to the classification of these isolates into specific and non-host biotypes. Our findings are comparable to those of other research conducted from across the world. According to the findings of Normanno et al. (2007), the predominance of the human biotype was found to be 50.4% among the 125 strains that were examined among the Italian isolates found in dairy and meat. This was followed by the ovine biotype comprising 23.2%, the bovine

biotype including 7.2%, the poultry-like ecovar comprising 1.6%, and the NHS strains comprising 17.6%. Another study that was carried out by Soltan Dallal et al. (2010) revealed that out of a total of one hundred isolates that were recovered from food (dairy and meat products), seventeen of them pertained to the human ecovar, eleven to the chicken ecovar, nine to the ecovar of the bovine, forty-seven to the NHS ecovars, and four seemed not assigned to any particular ecovar. It is clear from all of the evidence shown above that the presence of human ecovar in food products seems rather high level. In another Iranian study (Maktabi et al., 2021), it was revealed that the prevalence of HS and NHS species among samples was sixty percent and twenty-eight percent, respectively. In this study, sixteen percent of the isolates seemed of the human biotype, and three isolates were not characterized in the Biotyping technique. Despite the fact that certain studies discovered that there were no differences between the human strains and the NHS isolates in terms of their capacity to produce sickness (Maktabi et al., 2021), it has been shown that the human ecovar has a higher frequency of some severity factors compared to the NHS ecovars (Soltan Dallal et al., 2010).

Sample contamination with HS species was 76.33% and with NHS species it was 23.17% in this investigation. Additionally, Table 2 and Figure 1 show that 23.07% of the isolates were human biotype. While 48.64% of MRSA isolates coming from the hospital food samples in a prior study were human origin (Dehkordi et al., 2017), which is lower than those of our outcomes. The study's restriction to MRSA isolates from raw and frozen poultry meat likely contributed to the disparity, since it restricted the particular biotypes among isolates compared to other studies that used a wider variety of samples. In addition, our examination confirmed the significant frequency of poultry-based biotypes (53.8%), which has been reported in earlier

studies (Kitai et al., 2005; Dehkordi et al., 2017). According to their findings, a significant majority of *S. aureus* food-borne isolates (71.42%) were from poultry, while a smaller percentage (22.10%) were from humans and 48.64% were from other animals. In general, the findings showed that infected people and those who work with food have a role in the spread and transfer of MRSA strains to food products. Cruha et al. (2011), Ferreira et al. (2014), Costa et al. (2015), and Castro et al. (2016) are among the research that have reported a discussion on the role that food handlers have in the transmission of MRSA strains among food samples. It is possible that slaughtering involves handling the carcass in various ways is the cause of the presence of human biotypes in the meats, particularly in the case of raw chicken meat. During the visceral evacuation process, there is a stage that is responsible for the transfer of contamination through the hands of workers. Due to the increased manipulation of these goods, the human contamination original materials are believed to be original materials for additional contribution to human food poisoning. This is because of the high rate of contamination that occurs in slaughterhouses that affect human ecovar. In particular, a number of studies have shed light on the prevalence of a number of parameters that are implicated in staphylococcal poisoning among the human ecovar (Maktabi et al., 2021). It is possible to reduce the amount of contamination caused by this sort of adulteration by preventing infection from the hands.

5. CONCLUSION

There was a significant frequency of poultry- and human-based biotypes in poultry meat samples, that was observed in the current analysis, which is the first report of the Biotyping of MRSA isolates from raw and frozen chicken meat that was sold in Wasit markets. The sources of human contamination are regarded to be the

key sources that further contribute to the occurrence of food poisoning in humans. It is imperative that the emergence of many biotypes of MDR *S. aureus* be regarded as a significant threat to public health. The complete cooking of food is essential; nonetheless, it is advised that human agents avoid the contamination of meat during slaughter operations and throughout the supply chain of meat in order to alleviate the risk of food poisoning. In order to have a better understanding of the higher epidemiological characteristics of MRSA isolates found in a variety of food samples, more study is necessary.

REFERENCES

Asokan, G. V., & Vanitha, A. (2018). WHO global priority pathogens list on antibiotic resistance: an urgent need for action to integrate One Health data. *Perspectives in public health*, *138*(2), 87-88.

Blaiotta, G., Fusco, V., von Eiff, C., Villani, F., & Becker, K. (2006). Biotyping of enterotoxigenic Staphylococcus aureus by enterotoxin gene cluster (egc) polymorphism and spa typing analyses. *Applied and environmental microbiology*, *72*(9), 6117-6123.

Castro, A., Santos, C., Meireles, H., Silva, J., & Teixeira, P. (2016). Food handlers as potential sources of dissemination of virulent strains of Staphylococcus aureus in the community. Journal of infection and public health, 9(2), 153-160.

Costa, W. L. R., Ferreira, J. D. S., Carvalho, J. S., Cerqueira, E. S., Oliveira, L. C., & Almeida, R. C. D. C. (2015). Methicillin-resistant Staphylococcus aureus in raw meats and prepared foods in public hospitals in Salvador, Bahia, Brazil. Journal of Food Science, 80(1), M147-M150.

Cunha Cunha Neto, A., Lopes Hardoim, E., & da Cruz Monte, S. (2011). Staphylococcus aureus biotypes isolated from raw milk of cows with subclinical mastitis. *Acta Vet. bras.*, 54-60.

Devriese, L. A. (1984). A simplified system for biotyping Staphylococcus aureus strains isolated from different animal species. Journal of Applied Microbiology, 56(2), 215-220.

Ferreira, J. S., Costa, W. L. R., Cerqueira, E. S., Carvalho, J. S., Oliveira, L. C., & Almeida, R. C. C. (2014). Food handler-associated methicillin-resistant Staphylococcus aureus in public hospitals in Salvador, Brazil. Food Control, 37, 395-400.

Ghaffoori, M. H. (2017). Prevalence of Campylobacter jejuni in chicken meat marketed in Baghdad province. *Int. J. Adv. Res. Biol. Sci*, *4*(6), 1-11.

Gharsa, H., Slama, K. B., Lozano, C., Gómez-Sanz, E., Klibi, N., Sallem, R. B., Gómez, P., Zarazaga, M., Boudabous, A. & Torres, C. (2012). Prevalence, antibiotic resistance, virulence traits and genetic lineages of Staphylococcus aureus in healthy sheep in Tunisia. *Veterinary microbiology*, *156*(3-4), 367-373.

Hadi Ghaffoori Kanaan M, Jebur Obayes Al-Isawi A. & Ahmad Mohamme F. (2022). Antimicrobial Resistance and Antibiogram of Thermotolerant Campylobacter Recovered from Poultry Meat in Baghdad Markets, Iraq. Arch Razi Inst., 77(1):249-255. doi: 10.22092/ARI.2021.356362.1828. PMID: 35891742; PMCID: PMC9288606.

Kanaan, M. H. (2018). Antibacterial effect of ozonated water against methicillin-resistant Staphylococcus aureus contaminating chicken meat in Wasit Province, Iraq. *Veterinary world*, *11*(10), 1445.

Kanaan, M. H. (2021). Prevalence, resistance to antimicrobials, and antibiotypes of Arcobacter species recovered from retail meat in Wasit marketplaces in Iraq.

Kanaan, M. H. (2023). Prevalence and antimicrobial resistance of Salmonella enterica serovars Enteritidis and Typhimurium isolated from retail chicken meat in Wasit markets, Iraq. *Veterinary World*, *16*(3), 455.

Kanaan, M. H. (2024) a. Effect of biofilm formation in a hostile oxidative stress environment on the survival of Campylobacter jejuni recovered from poultry in Iraqi markets. *Veterinary World*, *17*(1), 136.

Kanaan, M. H. G. (2024) b. Effectiveness of gaseous ozone on Arcobacter butzleri and bacterial loads on retailed meat sold at Iraqi Wasit markets. *Open Veterinary Journal*, *14*(11), 2794.

Kanaan, M. H. G., & Al-Isawi, A. J. O. (2019). Prevalence of methicillin or multiple drug-resistant Staphylococcus aureus in cattle meat marketed in Wasit province. *Biochemical & Cellular Archives*, *19*(1).

Kanaan, M. H. G., & Khashan, H. T. (2022). Molecular typing, virulence traits and risk factors of pandrug-resistant Acinetobacter baumannii spread in intensive care unit centers of Baghdad city, Iraq. *Reviews and Research in Medical Microbiology*, *33*(1), 51-55.

Kanaan, M. H. G., & Mohammed, F. A. (2020). Antimicrobial resistance of Campylobacter jejuni from poultry meat in local markets of Iraq. *Plant Arch*, *20*(1), 410-415.

Kanaan, M. H. G., & Tarek, A. M. (2020). Clostridium botulinum, a foodborne pathogen and its impact on public health. *Ann. Trop. Med. Publ. Health*, *23*(5), 49-62.

Kanaan, M. H. G., & Tarek, A. M. (2022). Innovative modern bio-preservation module of meat by lytic bacteriophages against emergent contaminants. *Open Veterinary Journal*, *12*(6), 1018-1026.

Kanaan, M. H. G., Al-Shadeedi, S. M., Al-Massody, A. J., & Ghasemian, A. (2020). Drug resistance and virulence traits of Acinetobacter baumannii from Turkey and chicken raw meat. *Comparative immunology, microbiology and infectious diseases*, *70*, 101451.

Kanaan, M. H. G., Khalil, Z. K., Khashan, H. T., & Ghasemian, A. (2022). Occurrence of virulence factors and carbapenemase genes in Salmonella enterica serovar Enteritidis isolated from chicken meat and egg samples in Iraq. *BMC microbiology*, *22*(1), 279.

Kanaan, M. H. G., Mohammed, F. A., & Abdullah, S. S. (2024) b. Prevalence patterns, virulence indices, and antibiotics resistance in Campylobacter. *Journal of Applied Pharmaceutical Science*.

Kanaan, M. H. G., Sheriff, N. A., & Abdullah, S. S. (2023) a. Analysis of the Responses to Pasteurization Temperatures of Methicillin Resistant Staphylococcus aureus Derived from Milk Items. *Microbiology Research Journal International*, *33*(7), 9-16.

Kanaan, M. H. G., Tarek, A. M., & Abdullah, S. S. (2023). Prevalence and antimicrobial resistance of Staphylococcus aureus in some types of dairy products in Baghdad Markets. *J Dis Global Health*, *16*(1), 1-7.

Kanaan, M. H., Salim, I. D., Tarek, A. M., & Abdullah, S. S. (2023) b. Knowledge, attitude, and hygiene practices of food handlers related to food safety in Al-Suwaira City, Wasit Province in Iraq. *International Journal of One Health*, *9*(2), 150-158.

Kanaan, M., & Abdullah, S. (2021). Evaluation of aqueous Ozone as a method to combat multidrug-resistant Staphylococcus aureus tainting cattle meat sold in Wasit marketplaces. *Mansoura Veterinary Medical Journal*, *22*(3), 117-123.

Kanaan, M., & Abdullah, S. S. (2019). *Methicillin-Resistant Staphylococcus Aureus: As A Superbug Foodborne Pathogen*. LAP LAMBERT Academic Publishing.

Kanaan, M., & Khashan, H. T. (2018). Prevalence of multidrug resistant thermotolerant species of Campylobacter in Retail Frozen Chicken meat in Baghdad Province. *Curr Res Microbiol Biotechnol*, *6*(1), 1431-1440.

Kanaan, M.H.G. (2024) b. Knowledge, attitudes, and practices regarding antibiotic use and resistance among veterinarians and animal health professionals in Wasit Governorate, Iraq, International Journal of One Health, 10(2): 230-241.

Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Kitagawa, H., Fujio, K., Matsumura, K., Yasuda, R. & Inamoto, T. (2005). Prevalence and characterization of Staphylococcus aureus and enterotoxigenic Staphylococcus aureus in retail raw chicken meat throughout Japan. Journal of veterinary medical science, 67(3), 269-274.

Li, W. W., Guo, Y. C., Liu, Z. T., Chu, Z. H., Liang, J. J., Meng, C., & Fu, P. (2022). Analysis of foodborne disease outbreaks in China Mainland in 2016.

Maktabi, S., Zarei, M., Ghafuri, Z., & Ataee, R. A. (2021). Biotyping and enterotoxigenicity of coagulase-positive and coagulase-negative staphylococci isolated from different raw meat. *Iranian Journal of Veterinary Research*, *22*(2), 113.

McCallum, N., Berger-Bächi, B., & Senn, M. M. (2010). Regulation of antibiotic resistance in Staphylococcus aureus. *International Journal of Medical Microbiology*, *300*(2-3), 118-129.

Meyer, W. (1967). A proposal for subdividing the species Staphylococcus aureus. International Journal of Systematic and Evolutionary Microbiology, 17(4), 387-389.

Normanno, G., La Salandra, G., Dambrosio, A., Quaglia, N. C., Corrente, M., Parisi, A., Santagada, G., Firinu, A., Crisetti, E. & Celano, G.V. (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic Staphylococcus aureus isolated from meat and dairy products. International journal of food microbiology, 115(3), 290-296.

Papadopoulos, P., Papadopoulos, T., Angelidis, A. S., Boukouvala, E., Zdragas, A., Papa, A., Hadjichristodoulou, C. & Sergelidis, D. (2018). Prevalence of Staphylococcus aureus and of methicillin-resistant S. aureus (MRSA) along the production chain of dairy products in north-western Greece. *Food microbiology*, *69*, 43-50.

Safarpoor Dehkordi, F., Gandomi, H., Basti, A. A., Misaghi, A., & Rahimi, E. (2017). Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial resistance & infection control, 6, 1-11.

Sergelidis, D., & Angelidis, A. S. (2017). Methicillin-resistant Staphylococcus aureus: a controversial food-borne pathogen. Letters in applied microbiology, 64(6), 409-418.

Shimizu, A., Kawano, J. & Kimura, S. (1986). Biotyping of coagulase-positive Staphylococcus aureus and Staphylococcus intermedius strains isolated from various animals in Japan. Japan Journal Veterinary Science. 48(6): 1227 – 1235.

Soltan Dallal, M. M., Salehipour, Z., Eshraghi, S., Fallah Mehrabadi, J., & Bakhtiari, R. (2010). Occurrence and molecular characterization of Staphylococcus aureus strains isolated from meat and dairy products by PCR-RFLP. Annals of microbiology, 60, 189-196.

Wendlandt, S., Schwarz, S., & Silley, P. (2013). Methicillin-resistant Staphylococcus aureus: a food-borne pathogen?. *Annual review of food science and technology*, *4*(1), 117-139.

Wu, S., Huang, J., Wu, Q., Zhang, F., Zhang, J., Lei, T., Chen, M., Ding, Y. & Xue, L. (2018). Prevalence and characterization of Staphylococcus aureus isolated from retail vegetables in China. *Frontiers in microbiology*, *9*, 1263.

Table 1: Prevalence of different biotypes among the MRSA isolates isolated from poultry samples

Source	Biotype	Staphylokinase	β-hemolysis	Bovine plasma coagulation	Crystal violet reaction	+ve No. (%)	Classification
Raw chicken meat	1	_	_	_	A	5 (38.46)	Poultry
	2	+	_	_	C	2 (15.38)	Human
	3	_	+	_	A	1 (7.69)	NHS
Local frozen chicken meat	4	_	_	_	A	1 (7.69)	Poultry
	5	+	_	_	C	1 (7.69)	Human
	6	_	+	+	E	1 (7.69)	NHS
Imported	7	_	_	_	A	1 (7.69)	Poultry

frozen chicken meat	8	_	_	_	C	1 (7.69)	NHS
MRSA= methicillin-resistant *Staphylococcus aureus*; NHS= non host specific							

Table 2: Prevalence of MRSA isolates according to their behavior in biochemical tests and their classification according to their biotypes

Biochemical behavior	MRSA isolates and their prevalence		
	Biotype	Number (%)	P value
K-; β-; CPB-; CV: A	Poultry (HS)	7 (53.85)	P = 0.0071
K+; β -; CPB-; CV: C	Human (HS)	3 (23.08)	X^2= 7.247
K-; β+; CPB-; CV: A	NHS	1 (7.69)	
K-; β+; CPB+; CV: E	NHS	1 (7.69)	
K-; β-; CPB-; CV: C	NHS	1 (7.69)	
MRSA= methicillin-resistant *Staphylococcus aureus*; NHS= non host specific; HS= host specific			

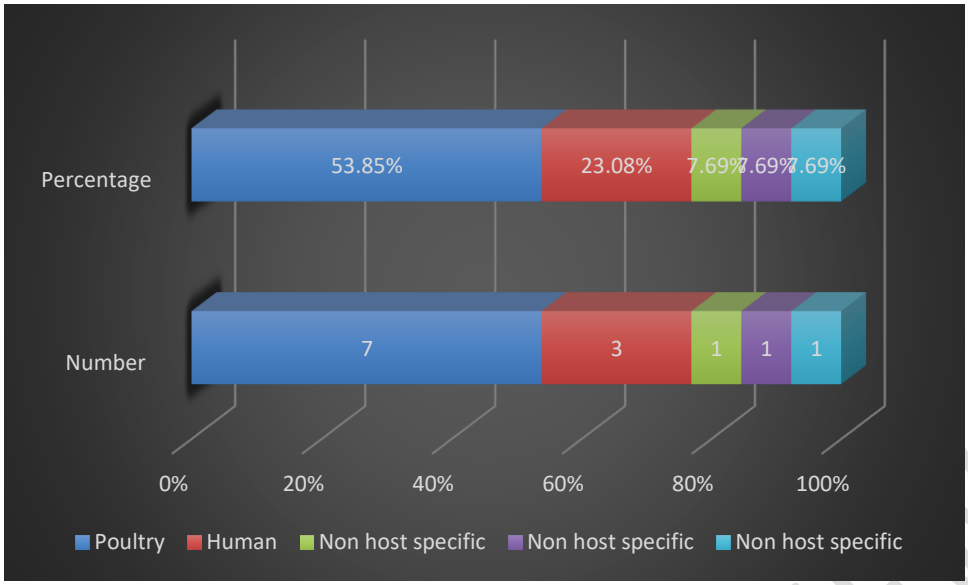

Figure 1: Distribution of Methicillin-resistant *Staphylococcus aureus* biotypes based on sample sources