Biotyping Analysis of Methicillin-Resistant *Staphylococcus aureus* in Poultry from Wasit Markets, Iraq

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are widely acknowledged as significant contributors to foodborne infections. In this study, a total of thirteen MRSA isolates were obtained from raw and frozen chicken meat sold in Wasit markets. The aim was to investigate the origin of these isolates. To achieve this, the isolates were subjected to the Biotyping system developed by Devriese, which involved several biochemical assays, including staphylokinase (K) synthesis, beta-hemolysis (β), bovine plasma coagulation (CPB), and growth in the presence of crystal violet (CV). Among the thirteen MRSA isolates, five distinct biotypes were identified. Two of these biotypes were host-specific, classified as avian (07) and human (03). Additionally, three non-host-specific biotypes, each consisting of three isolates, were identified with the following biochemical profiles: K-; β+; CPB-; CV: A (01), K-; β+; CPB-; CV: E (01), and K-; β-; CPB-; CV: C (01). These biotypes can be found in birds, cattle, and humans. The results suggest that inadequate management of animals may contribute to public health risks, as evidenced by the presence of these biotypes. Further research is necessary to explore additional epidemiological factors related to the MRSA isolates found in various food samples.

Keywords: *Biotyping, Biochemical behavior,* Methicillin-resistant *Staphylococcus aureus, Poultry products.*

1. INTRODUCTION

Recent studies in Iraq have raised concerns about food safety, highlighting the contamination of meat by various 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 Abdullah, 2019; Kanaan and Abdullah, 2021; Kanaan et al., 2023b), and *Campylobacter* spp. (Ghaffoori, 2017; Kanaan and Khashan, 2018; 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 temperatures, producing toxins that cause illness. The distribution of *S. aureus* is widespread globally; nevertheless, the primary cause of *S. aureus* infections is food (Kanaan et al., 2023a). It is responsible for approximately 241,000 cases of foodborne illness annually 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* has a significant ability to adapt to diverse environmental conditions and rapidly develop resistance to most 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). 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). MRSA is well known as a

superbug that causes life threatening infections of humanity which is difficult to treat (Lakshmi et a., 2021). 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. Therefore, this study aims to analyze the distribution of MRSA isolates based on their biochemical behavior and biotype classification. 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. These isolates were identified and isolated using conventional microbiological and biochemical assays, as detailed by 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 °C, 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 thawed in a refrigerator for 24 h, subcultured onto Tryptone Soya Agar (Oxoid, CM0131B), and incubated for 24 h at a temperature range of 35 to 37°C.

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. MRSA isolates were incubated on Tryptone Soya Agar (Oxoid, CM0131B) plates with 1% bovine fibrin, with or without 5% dog serum as a plasminogen source, to assess the effect of staphylokinase production on bovine fibrinogen or canine plasminogen. The formation of staphylokinase is identified by 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%, while human-based biotypes accounted for 15.38%. 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, there was a significant difference in the prevalence of host-specific and non-host-specific biotypes in poultry meat samples ($P \leq 0.05$, $X^2 = 7.247$).

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; Ndip et al., 2021). 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), 17 of them pertained to the human ecovar, 11 to the chicken ecovar, nine to the ecovar of the bovine, 47 to the NHS ecovars, and four seemed not assigned to any particular ecovar. Previous results 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. Although some studies found no differences between human strains and NHS isolates

in their ability to cause illness (Isigidi et al., 1992), 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 76.93% and with NHS species it was 23.07% in this investigation. Additionally, Table 2 and Figure 1 show that 23.08% 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 higher 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. 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 slaughtering process, contamination is often transferred through workers' hands. 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 (Soltan Dallal et al., 2010). 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.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

All authors contributed to the study's conception and design. M.H.G.K., and S.S.A. both worked on the study's design. The document was written by M.H.G.K. S.S.A. collected data and conducted statistical analyses. M.H.G.K. and S.S.A. revised the manuscript. The final text was reviewed and approved by all authors.

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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 frozen chicken meat	7	_	_	_	A	1 (7.69)	Poultry
	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			

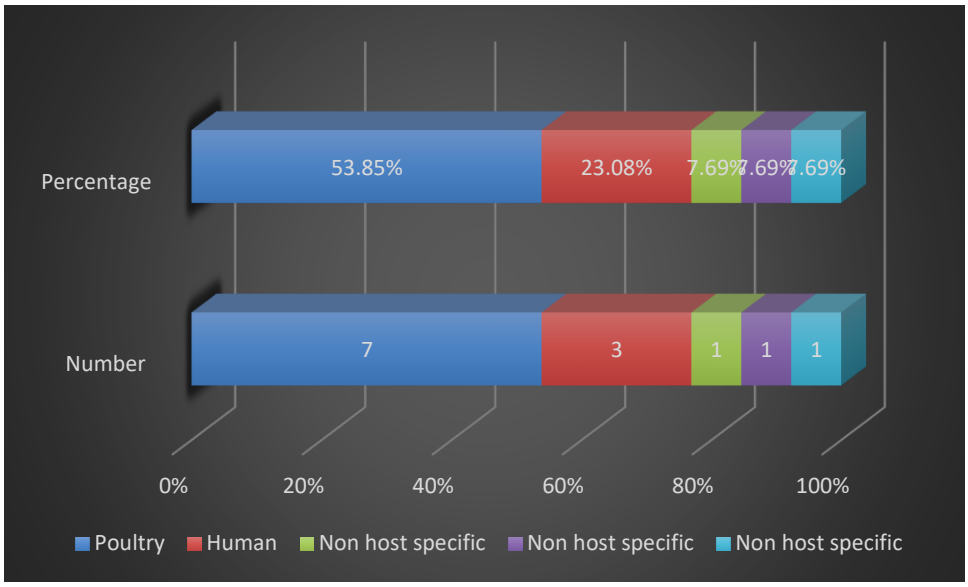

Figure 1: Distribution of Methicillin-resistant *Staphylococcus aureus* biotypes based on sample sources