https://doi.org/10.15407/microbiolj84.02.040

H.K. YAKOB [1]*, L.R. HAMAD [2], M.B. FARHAN [3]

[1] Department of Biology, College of Education for Pure Sciences, University of Anbar, Ramadi Iraq,.

[2] Department of Pathological Analysis, College of Applied Science, University of Fallujah, Fallujah, Iraq.

[3] Department of Biology, Education College for Women, University of Anbar, Ramadi, Iraq.

* Author for correspondence; e-mail: halsalamany@uoanbar.edu.iq

ISOLATION AND DIAGNOSIS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN MEAT AND BUTCHER TOOL SURFACES IN BAGHDAD

Due to the medical and epidemiological importance of the spread of Methicillin-Resistant Staphylococcus aureus, this study was conducted for ***the purpose*** *of isolating and diagnosing these bacteria from local sheep meat and butcher's tools in Baghdad.* ***Methods.*** *200 samples were collected. Mannitol salt agar and Staph.110 medium were used to isolate the bacteria. The isolates were identified using standard cultivation methods, biochemical tests, the GP24 diagnostic system, and an integrated Vitek 2 device. The isolates were tested for sensitivity to methicillin by the disk diffusion method. DNA was extracted and the mecA gene was detected in the isolates that showed methicillin resistance by polymerase chain reaction.* ***Results.*** *S. aureus was diagnosed in 83 (41.5%) of the samples. Of them, 35 (42.2%) were methicillin-resistant. Out of these, 24 (68.6%) were found to have the mecA gene.* ***Conclusions.*** *Methicillin-Resistant S. aureus strains were detected with a high prevalence due to the underdeveloped reality of slaughter places .*

Keywords: *MRSA, mecA gene, sheep meat, Iraq.*

Food safety is one of the important issues in the United States and Europe, and many regulations on food safety in the agri-food division have been applied [1]. In Iraq, there is a lack of inclusive evaluation of agri-food throughout the last fifteen years. There are many sources of food contamination from bacteria to fungi that lead to spoilage of feed and food, through contamination with chemical pollutants such as heavy metals and pesticides or misuse of antibiotics , which leads to a lot of health risks and economic losses [1, 2].

Food is considered contaminated if it contains something that makes it unfit for human consumption. This may be pathogenic germs mixed with some toxic chemicals or exposure to lethal

Citation: Yakob H.K., Hamad L.R., Farhan M.B. Isolation and Diagnosis of Methicillin-Resistant *Staphylococcus aureus* in Meat and Butcher Tool Surfaces in Baghdad. *Microbiological journal.* 2022 (2). P. 40—46. https://doi.org/10.15407/microbiolj84.02.040

radioactive materials, which results in food poisoning, represented by human infection with severe diseases of the stomach and intestine [3].

Meat is an essential source for supply of proteins, fats, and some vitamins. Its nutritional value is related to the content of proteins, fats, carbohydrates, vitamins, and minerals. Also, meat is an ideal medium for the growth of many microorganisms due to the availability of moisture, nitrogenous compounds, other essential elements, and some vitamins. Since the sources of pollution are different, such as water, air and soil, so there are numerous microorganisms on fresh meat [4].

There are many foodborne bacteria such as *Salmonella*, *Listeria monocytogenes*, *Enterobacter sakazakii*, *Escherichia coli*, and *Staphylococcus* spp. [1]. Staphylococcus includes many types of bacteria that cause pathogenicity to humans and animals. *Staphylococcus aureus* is one of the most common pathogens for humans. Although it is part of the natural microorganisms present in different parts of the body such as the skin and respiratory tract, it is considered one of the most clinically important types of the staphylococcus genus It is responsible for a wide range of diseases such as pneumonia, septicemia, endocarditis, osteomyelitis, postoperative wound infection, and food poisoning [3, 5, 6].

The pathogenicity of this bacterium and its ability to invade the host's tissue and spread due to the possession of many virulence factors such as a capsular that helps in resisting to the process of phagocytosis, in addition to having a cell wall that works to resist the host's immune system [7, 8]. Some strains of *Staphylococcus aureus* produce biofilms that facilitate the process of attachment and settlement of the bacterial cell with the host cells. *Staphylococcus aureus* has the ability to produce many toxins and extra-cellular enzymes that help the bacteria in causing infection, such as the coagulant enzyme of blood plasma. *Staphylococcus aureus* is capable of producing other enzymes that represent proliferation factors such as staphylokinase, proteinase, and lipase, which contribute to the invasion of bacteria to tissues and the spread of infection. They produce blood-dissolving toxins type alpha, beta, gamma, and delta, in addition to producing enterotoxins, causing food poisoning [9,10]. Methicillin belongs to the group of β-lactam antibiotics and has a complex structure. It was used in treatment in the 1960s. Its effect is fatal as it inhibits cell wall formation by stopping the transpeptidation [10].

Methicillin-Resistant *Staphylococcus aureus* (MRSA) was first isolated in 1972 from bovine udder infections [11]. Since then, this bacterium has been isolated from various animals, including sheep, dairy cows, calves, horses, pigs, dogs, cats, and birds [12].

The emergence of MRSA strains became one of the major health problems because the spread of these strains reduces the treatment options available to doctors, in addition to the fact that their resistance can be transmitted to various bacterial species and genera [13]. Because of the medical and epidemiological importance of the spread of such strains and the spread of the phenomenon of indiscriminate slaughter of sheep in different areas of Baghdad along with the lack of health monitoring, so this study aimed to isolate and diagnose MRSA in local meat in the Al-Karkh side of Baghdad.

Materials and Methods. Meat Samples. 100 samples of fresh sheep meat were collected randomly from butcher shops in five different areas on the Al-Karkh side of Baghdad (Al-Mansour, Al-Daoudi, Al-Amiriyah, Al-Khadra, and Abu Ghraib). All samples were kept in a refrigerated container before transferring to the laboratory for the required tests.

Samples of the Surface of Butcher's Tools. At the same time, 100 swabs (50 swabs from the surfaces of meat cutting knives and 50 swabs from the surfaces of meat chopping boards) were collected from the same butcher shops using sterile cotton swabs.

Isolation and Diagnosis of *Staphylococcus aureus*. Each 25 g meat sample taken from examined places of the animal's body was weighed by three times, then transferred to 225 mL of buffered peptone water and mixed with it for 3 min. 1 mL of such samples was transferred and added to test tubes each containing 9 mL of Tryptone Soya Broth medium with 10% NaCl added and incubated for 48 hr at 35 °C. As for knife swabs and chopping boards, they were grown in Tryptone Soya Broth with 10% NaCl added and incubated for 48 hr at 35 °C.

After the incubation period, 0.1 mL of each tube was spread onto Staph.110 medium and mannitol salt agar by a sterile glass spreader, and the dishes were incubated for 24 hr at 35 °C. Then the dishes in which the colonies appeared were examined, and confirmatory tests were performed to diagnose *S. aureus* through studying the morphological characteristics of colonies and bacterial cells and conducting biochemical tests based on the techniques described in [11].

The diagnosis of *S. aureus* isolates was confirmed using the Diagnostic GP24 System and a Vitek 2 compact device.

Test for Sensitivity to Methicillin. The sensitivity of *S. aureus* isolates to methicillin (5 micrograms/ disk) was tested using the Kirby-Bauer disk diffusion technique on Muller-Hinton agar, and the resistance and sensitivity were determined depending on the standard diameters as per Clinical and Laboratory Standards Institute [14].

Screening for the *mec*A gene in MRSA Isolates. The isolated bacteria that showed resistance to methicillin were grown on a mannitol salt agar medium. Single colonies were transferred to a test tube containing 3 mL of Tryptone Soya Broth. DNA was extracted using a Promega DNA extraction and purification kit. DNA concentration and purity were measured with a Nanodrop device. The *mec*A initiator supplied by Macrogen Korea was used in the PCR.

The *mec*A gene, which gives the resistance characteristic of *Staphylococcus aureus* to methicillin, was detected using *mec*A forward primer (5′-AAAATCGATGGTAAAGGTTGGC-3′) and *mec*A reverse primer (5′-AGTTCTGCAG TACCGGATTTTGC-3′) with the amplification program as follows: denaturation at 95 °C for 30 sec, annealing at 55 °C for 45 sec, and extension at 72 °C for 60 sec with a final extension at 72 °C for 5 min [15].

The PCR products were detected by migrating the samples on agarose gel electrophoresis at

Prevalence of *Staphylococcus aureus* and MRSA in sheep meat and the surfaces of butcher's tools

Study area	Source	No. of tested samples	No. (%) of *S. aureus* positive isolates	No. (%) of MRSA-positive isolates	No. (%) of *mec*A gene-positive MRSA
Al-Mansour	Sheep meat	20	4 (20)	1(25)	1(100)
	Butcher's tools	20	5 (25)	2(40)	1(50)
Al-Daoudi	Sheep meat	20	7 (35)	2(28.6)	1(50)
	Butcher's tools	20	5 (25)	2(40)	2(100)
Al-Amiriyah	Sheep meat	20	8 (40)	4(50)	2(50)
	Butcher's tools	20	9 (45)	3(33.3)	2(66.7)
Al-Khadra	Sheep meat	20	9 (45)	5(55.5)	3(60)
	Butcher's tools	20	11 (55)	5(45.5)	4(80)
Abu Ghraib	Sheep meat	20	10 (50)	5(50)	3(60)
	Butcher's tools	20	15 (75)	6(40)	5(83.3)
Total	—	200	83 (41.5)	35(42.2)	24(68.6)

a concentration of 1.5%. The products and the marker were electrically migrated and the power supply was set at 100 V for 1 hr. After the migration, the gel was checked with ultraviolet light and photographed with a digital camera.

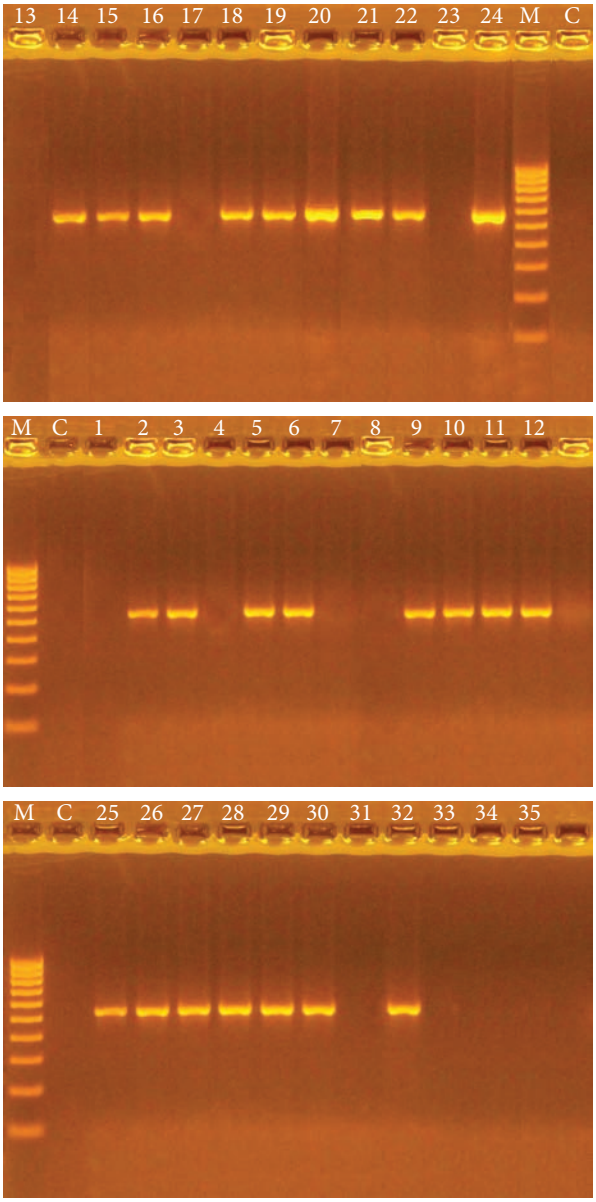
Results. Prevalence of *Staphylococcus aureus* and MRSA. 100 samples of sheep meat and 100 swabs of butcher tools were collected from study areas in Baghdad to determine the presence of *S. aureus* bacteria resistant to methicillin. Mannitol salt agar and Staph.110 medium were used to isolate the bacteria in the form of yellow colonies on the medium of mannitol salt agar and pale ones on the medium Staph-110. The colony was transferred onto a glass slide and stained with a gram stain, then there appeared microscopically spherical and gram-positive cells. After that, the isolates were identified on the basis of the catalase activity, oxidase test, mannitol fermentation test, and hemolytic activity. The result was positive for the catalase test and negative for the oxidase test, while the result of the mannitol fermentation test was positive and the hemolysis was of the beta type. The diagnosis was confirmed using the Diagnostic System GP24 and the Vitek 2 compact technology.

83 isolates (41.5%) were diagnosed as such containing *S. aureus*. According to the antibiotic disk diffusion test, 35 (42.2%) of them were resistant to methicillin .

The results of the study showed that the highest rate of isolation of *S. aureus* (30.1%) was in Abu Ghraib, while the lowest rate (10.8%) was in Al-Mansour (Table).

Molecular detection of the *mec*A gene in MRSA isolates. DNA was extracted from the *S. aureus* isolates that were identified as methicillin-resistant. Then, the presence of the *mec*A gene (533 bp) responsible for the resistance of this bacterium to methicillin was detected using PCR technology. The results showed its presence in 24 (68.6%) isolates (Table, Figure).

Discussion. Food poisoning caused by microbial toxins is an important cause of morbidity and mortality worldwide. Meat is an ideal medium for the growth of various microorganisms, including bacteria because it is high in moisture, rich in nitrogenous compounds, and supplied with minerals and accessory growth factors. The



1.5% agarose gel electrophoresis with ethidium bromide staining showing the result of PCR amplification product of *mec*A gene (533 bp) of methicillin-resistant *Staphylococcus aureus* isolates (1—35). *Note:* M — DNA marker; C — negative control

present study was carried out to isolate and identify Methicillin-Resistant *S. aureus* in local sheep meat, as well as on different butchers' tools in Baghdad. To isolate bacteria from the samples, the mannitol salt agar medium and Staph.110 medium were used due to the high content of sodium chloride, which inhibits the growth of most of the gram-negative bacteria and activates the growth of *S. aureus*, which appeared in the form of yellow colonies on the mannitol salt agar and pale ones on the medium Staph-110.

S. aureus was isolated by 41.5% and compared with local studies. Our result is higher than the rates obtained for other different areas [7, 16, 17]. This difference may be related to the different extents of the application of sanitary conditions in each place and the nature and intensity of production. Through our study, the underdeveloped reality of places of slaughter was observed, and it was far from the simplest sanitary elements.

The carcass is exposed to bacteria contamination during slaughter and after slaughtering from a variety of sources, including tools and hands, and during the removal of the skin from the carcass. Food poisoning occurs as a result of unhealthy handling of the carcass, so the tender meat is contaminated with bacteria that may be harmful to humans because the bacteria cause biochemical changes and thus the occurrence of diseases [18, 19].

The source of meat contamination with bacteria is the natural bacteria present in animal tissues, the air, and the environment, or pollution occurs as a result of unhealthy slaughtering and contaminated hands, as well as during meat preparation Meat is a good medium for the growth of pathological germs or those that cause meat spoilage because each step in preparing the carcass after slaughtering leads to the entry of bacteria and thus contaminates the meat [3]. Contamination of carcasses with bacteria is a result of contamination of either the animal itself or the hands of butchers, so the intensification of health awareness and hygiene care during the handling of meat are important issues to reduce bacterial contamination [20].

The molecular detection of the *mecA* gene showed its presence in 24 (68.6%) isolates. *S. aureus* resistance to oxacillin and methicillin is linked to the presence of the *mec*A gene as this gene encodes for penicillin-binding proteins, which lead to resistance to these antibodies [19, 21]. Like other types of bacteria, these bacteria contain genetic elements called plasmids formed as circular annular structures of a double strand of DNA, which can duplicate independently of the bacterial chromosome and be steadily inherited and widely spread in the cells of different bacteria [9].

In summary, Methicillin-Resistant *S. aureus* was diagnosed in 35 isolates. 24 of them were found to have the *mec*A gene. The high prevalence of the bacteria may be due to the underdeveloped reality of places of slaughter. Having compared these results with the International and European quality standards for meat and meat products, we can conclude that Methicillin-Resistant *S. aureus* strains have been detected in the areas studied in Baghdad with a highp-revalence due to the underdeveloped reality of places of slaughter.

Conclusions. Methicillin-Resistant *S. aureus* strains were detected with a high prevalence (42.2%) based on an antibiotic disk diffusion test. Moreover, the result of molecular detection of the *mec*A gene showed the presence of the gene with a percentage of 68.6%.

Acknowledgments. The authors would like to thank Dr. Laith K. Tawfeeq from the Department of Biology, College of Science, the University of Baghdad for his assistance in the molecular part. The authors also appreciate the efforts of the staff of the Central Public Health Laboratory, Iraqi Ministry of Health and Environment.

REFERENCES

1. Nørrung B, Buncic S. Microbial safety of meat in the European Union. Meat science. 2008; 78 (1–2):14–24. DOI: 10.1016/j.meatsci.2007.07.032
2. Jaja I, Iwu C, Chigor V, Anyanwu M, Ezealisiji K, Oguttu JW, Green E. Antimicrobial resistance phenotype of *Staphylococcus aureus* and *Escherichia coli* isolates obtained from meat in the formal and informal sectors in south Africa. BioMed Res Int. 2020; 1–11. PMID: 3979482. DOI: 10.1155/2020/3979482
3. Kluytmans JA. Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency? A review. Clin Microbiol Infect. 2009; 16(1):11–15. DOI: 10.1111/j.1469-0691.2009.03110.x.
4. Pesavento G, Ducci B, Comodo N, Lo Nostro A. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). Food Control. 2007; 18(3):196–200. doi.org/10.1016/j. foodcont.2005.09.013.
5. Bhandare SG, Sherikar AT, Paturkar AM, Waskar VS, Zende RJ. A comparison of microbial contamination on sheep/goat carcasses in modern Indian abattoir and traditional meat shops. Food Control. 2007; 18:854–858. DOI:10.1016/j.foodcont.2006.04.012.
6. Jaradat ZW, Tarazi YH, Ababneh QO. Molecular characterization of *Staphylococcus aureus* isolated from meat and their antibiotic resistance profiles. Pak Vet J. 2014; 34(1):58–62.
7. Jarallah EM, Sahib SI, Yassen K. Isolation and identification of some pathogenic bacterial species contaminated from meats in butcher shops and kebab restaurants in AL-Kut city. Euph J Agr Sci. 2014; 6 (4):30–37.
8. Kanaan MHG. Antibacterial effect of ozonated water against methicillin-resistant *Staphylococcus aureus* contaminating chicken meat in Wasit Province, Iraq. Vet World. 2018; 11(10):1445–1453. DOI: 10.14202/vetworld.2018.1445-1453.
9. Ranjan KD. Textbook of diagnostic microbiology. Medical college and hospital, medical publishers (p) Ltd Newdelhi. PP: 2007; 124.
10. Yalcin S, Nizamlio M, Gürbüz Ü. Fecal coliform contamination of beef carcasses during the slaughtering process. J Food Safety. 2007; 21:225–231. DOI: 10.1111/j.1745-4565.2001.tb00321.x.
11. Phillips D, Sumner J, Alexander JF, Dutton KM. Microbiological quality of Australian sheep meat. J Food prot. 2001; 64(5):697–700.
12. Wu S, Huang J, Wu Q, Zhang J, Zhang F, Yang X, Wu H, Zeng H, Chen M, Ding Y, Wang J, Lei T, Zhang S, Xue L. *Staphylococcus aureus* isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. Front Microbiol. 2018; 9:2767. PMID: 30498486. DOI: 10.3389/fmicb.2018.02767.
13. Holt JK, Krieg NR, Staleg JT. Bwrgys manual of determinative bacteriology, Baltimor, USA. 1994; 175–191.
14. CLSI (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing. Twenty-second information supplement M100-S25.CLSI, Wayne, PA, USA. 2015.
15. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol. 1991; 29(10):2240–2244. DOI: 10.1128/JCM.29.10.2240.
16. Mukhopadhyay H, KPillai RM, Pal U, Kumar AJ. Microbial quality of fresh chevon and beef in retail outlets of Pondicherry. TNJVAS. 2009; 5(1):33–36.
17. Hamzah AM, Abbas MS, Ahmed WA. The isolation and identification of the important pathogenic bacteria from fresh meat. Iraqi J Vet Med. 2010; 34(1):44–50.
18. Arjyal C, Kc J, Neupane S. Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Shrines. Int J Microbiol. 2020. PMID: 7981648. DOI: 10.1155/2020/ 7981648.
19. Torki Baghbaderani Z, Shakerian A, Rahimi E: Phenotypic and genotypic assessment of antibiotic resistance of *Staphylococcus aureus* bacteria isolated from retail meat. Infect Drug Resist. 2020; 7(13):1339–1349. DOI: 10.2147/IDR.S241189.
20. Assafi MS, Hado HA, Abdulrahman IS. Detection of methicillin-resistant *Staphylococcus aureus* in broiler and broilers farm workers in Duhok, Iraq by using conventional and PCR techniques. Iraqi J Vet Sci. 2020; 34(1):15–22.
21. Jackson CR, Davis JA, Barrett JB. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. J Clin Microbiol. 2013; 51(4):1199–207. DOI: 10.1128/JCM.03166-12.

Received 13.05.2021

Х.К. Джейкоб [1], Л.Р. Хамад [2], М.Б. Фархан [3]

[1] Кафедра біології, Педагогічний коледж теоретичних наук Університету Анбара, Рамаді, Ірак
[2] Кафедра патологічного аналізу, Коледж прикладних наук, Університет Фаллуджи, Фаллуджа, Ірак
[3] Кафедра біології, Освітній коледж для жінок, Університет Анбара, Рамаді, Ірак

ІЗОЛЯЦІЯ ТА ДІАГНОСТИКА МЕТИЦИЛІН-РЕЗИСТЕНТНОГО *STAPHYLOCOCCUS AUREUS* НА ПОВЕРХНІ М`ЯСА І М'ЯСНИХ ІНСТРУМЕНТІВ У БАГДАДІ

З огляду на медичну та епідеміологічну важливість поширення метицилін-резистентного золотистого стафілокока, це дослідження було проведено з метою виділення та діагностики цих бактерій з м'яса овець та інструментів м'ясника в Багдаді. **Методи.** Було зібрано 200 зразків. Для виділення бактерій використовували манітний сольовий агар та середовище Staph.110. Ізоляти ідентифікували за допомогою стандартних методів культивування, біохімічних тестів, діагностичної системи GP24 та інтегрованого пристрою Vitek 2. Ізоляти перевіряли на чутливість до метициліну методом дискової дифузії. ДНК екстрагували, і ген *mec*A виявили в ізолятах, стійких до метициліну, за допомогою полімеразної ланцюгової реакції. **Результати.** *S. aureus* діагностовано у 83 (41,5%) проб. З цих ізолятів 35 (42,2%) були метицилін-резистентними, а з них 24 (68,6%) мали ген *mec*A. **Висновки.** Метицилін-резистентні штами *S. aureus* були виявлені з високою поширеністю через нерозвиненість місць забою.

Ключові слова: MRSA, ген *mec*A, м'ясо овець, Ірак