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Detection of Methicillin-Resistant *Staphylococcus Aureus* (Mrsa) Among Women with Breast Cancer In Iraq

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ABSTRACT

Background: Breast cancer (BC) is the most malignancies in worldwide among women. *Staphylococcus aureus* is one of the most common agents responsible for overt and subclinical BC infections. Methicillin-resistant *S. aureus* (MRSA) have a high ability to resist *β*-lactam antibiotics and multidrug resistance (MDR). These strains increase the risk and severity of infections associated with BC.
Objectives: According to our knowledge, this study is a first nationwide, and was aimed to detect MRSA strains by phenotypic methods and study correlation between infections caused by MRSA and BC among women.
Methods: The current study was conducted at oncology center in Babylon City during the period of November (2019) to January (2020). Ages of patients were ranged from (20-80) years. Two hundred and sixteen randomly patients with BC were enrolled. *S. aureus* was detected using microbiological and biochemical tests. Isolates of MRSA were detected by five different phenotypic methods using the cefoxitin disc diffusion (FOXDD) method as a reference standard for *mecA* gene detection. Other phenotypic methods including: chromogenic assay, oxacillin disc diffusion (OXDD) and oxacillin, methicillin HiComb E-test [OX, (Meth E-test)].
Results: Of the 130 *S. aureus* isolates, 40 (30.7%) and 90 (69.3%) were confirmed using FOXDD test as MRSA and methicillin-sensitive *S. aureus* (MSSA), respectively. However, chromogenic assay and OX (E-test) were highly effective for detecting MRSA because they were recorded isolation rates for MRSA 39 (30.0%) and 38 (29.3%), respectively. Meth (E-test) gave lower result compared with other phenotypic tests, detecting 35 (26.9%) MRSA isolates. All MRSA isolates were recognized as completely sensitive against levofloxacin, vancomycin, linezolid and nitrofurantoin. Tetracycline 28 (70%) and erythromycin 27 (67.5%) were determined to be agents with the highest resistance. 25 (62.5%) of MRSA isolates in the current study were MDR. The highest percentage of MDR-MRSA were observed in class three of antibiotics 9 (22.5%). The lowest percentage is observed in a class four and seven of antibiotics 3 (7.5%).
Conclusions: Isolates of *S. aureus* were more presented in BC infections and the elderly groups were most affected, while MRSA isolates were found in a low rate but worrisome because that all these isolates were resistant to all *β*-lactam antibiotics and most of these isolates were MDR. The FOXDD method is more efficient and accurate for MRSA detection than other phenotypic methods.

Keywords
Breast cancer, *Staphylococcus aureus*, MRSA, MDR

INTRODUCTION

Breast cancer is one of the most influential and common cause of carcinomas detected in women. It is the second greatest common reason for increasing the death rate from cancer among women affected by this disease in the world [1]. BC always evolves silently. Most of the women discover their disease during their routine screening. Others may present with an accidentally discovered breast lump, change of breast shape or size, or nipple discharge [2]. Bacterial communities within the host could be one additional environmental factor related to BC, which has been only recently considered in sporadic BCs of unknown etiology. Recently, there has been a strong attention in completely characterizing the microbiota linked with different parts of the body under various health situations [3]. The microbiota also has been involved in cancer expansion aggressiveness and progression at a different of body sites [4]. The human microflora in epidemiologic studies

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assume that share in 16 to18% and more of global malignancies [3]. *S. aureus* is one of the most important microbes associated with BC. In the majority of reports, *S. epidermidis* and *S. aureus* are the most prevalent germs responsible for overt and sub-clinical breast infections [5]. MRSA are strains of *S. aureus* but have a high ability to resist *β*-lactam antibiotics by mutation of a penicillin-binding protein, a chromosome- encoded protein [6], and these resistant strains increase the risk and severity of infections associated with BC. Previously MRSA was only acquired from hospitals, yet community acquired infection and colonization have become increasing prevalent. The risk in cancer patients for MRSA infection and colonization is even further raised [7, 8]. There are numerous factors that are interconnected with BC, such as microbiota presence, and it can be inferred through phenotypic or molecular laboratory investigations [3]. The goal of this study was to obtain baseline analyses in order to detect MRSA and their sensitivity among patients with BC by phenotypic methods.

MATERIALS AND METHODS

Patients

The current study was conducted at oncology center in Babylon City, Iraq during the period of November 2019 to January 2020. A total of 216 BC patients were enrolled in this study. Ages of patients were ranged from (20-80) years.

Sample Collection

All samples in this study were collected from women patients with BC after chemo and radiotherapy. These samples were collected under the supervision of the specialist doctor and the surgeon. *S. aureus* isolates were detected according to the morphological characteristics on culture medium and biochemical tests [9].

Detection of MRSA by Phenotypic Methods

A. Chromogenic Assay

All 130 *S. aureus* isolates were inoculated onto a BBL^{TM}CHROMagar^{TM}MRSA (Becton and Dickinson, France). The medium preparation protocol and bacterial culture conditions were followed depending on the manufacturer's instructions. Colonies of MRSA were seemed pink, brown to mauve on this medium.

B. Cefoxitin and Oxacillin Disc Diffusion Test

All confirmed *S. aureus* isolates were subsequently tested for MRSA detection by screening for susceptibility to cefoxitin and oxacillin using disc diffusion method according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) [10].

C. Methicillin and Oxacillin HiComb E-test

HiComb^{TM} MIC strips for methicillin Meth (E-test) and oxacillin OX (E-test) were applied on all isolates of *S. aureus* according to the manufacturer's instructions (Himedia, India).

Antibiotic Susceptibility Testing

Susceptibility of 40 MRSA isolates against 13 antimicrobial agents was determined by modified disc diffusion method. The antimicrobial agents were including: penicillin G (P, 10U), oxacillin (OX, 1µg), cefoxitin (FOX, 30µg), erythromycin (E, 15µg), tetracycline (TE, 30µg), levofloxacin (LE, 5µg), vancomycin (VA, 30µg), linezolid (LZ, 30µg), nitrofurantoin (NIT, 300µg), gentamicin (GEN, 10µg), rifampicin (R, 5µg), clindamycin (CD, 2µg), and trimethoprim-

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sulfamethoxazole (COT, 1.25/23.7µg) (HiMedia). Interpret the results according to the recommendations of CLSI [10].

RESULTS

A total of 216 samples from various BC clinical samples were randomly collected for *S. aureus* screening during the course of this study. *S. aureus* was the most prevalent agents that isolated from 60.2% of all BC cases. The isolation rate for *S. aureus* in BC samples was 65 (50.0%) for skin abscess, 42 (32.3%) for wound exudate and 23 (17.7%) for nipple discharge (Table 1). The elderly groups: (61-70) and (71-80) were the most affected by infections. These age groups were the most colonized by *S. aureus*, and recorded the highest rates, which were 35 (26.9%) and 30 (23.1%), respectively. While the age groups (20-30) and (31-40) were the least colonized by bacteria, it was found that the isolation rate of *S. aureus* 2 (1.5%) and 14 (10.8%), respectively. However, the rest age groups were showed a moderate effect by *S. aureus* (Table 2).

Table 1. Occurrence of *S. aureus* isolates according to source of breast cancer infections isolation

Breast cancer sample	No (%) of sample (n=216)	No (%) of *S. aureus* (n=130)
Skin abscess	37 (17.1)	65 (50.0)
Wound exudate	92 (42.6)	42 (32.3)
Nipple discharge	87 (40.3)	23 (17.7)

Table 2. Distribution of 130 *S. aureus* isolates in clinical breast cancer samples among various age groups

Patients profile	Age group	No (%) of sample (n = 216)	No (%) of *S. aureus* (n = 130)
Age group	20-30	12 (5.6)	2 (1.5)
	31-40	45 (20.8)	14 (10.8)
	41-50	52 (24.1)	20 (15.4)
	51-60	41 (19.0)	29 (22.3)
	61-70	50 (23.1)	35 (26.9)
	71-80	16 (7.4)	30 (23.1)

Phenotypic Detection of MRSA

One hundred and thirty *S. aureus* isolates were incorporated in the study. The FOXDD method was used as a reference standard for *mecA* gene detection [10]. The rate of MRSA in the *S. aureus* isolates was found to be 40 (30.7%) in the current study, and 90 (69.3%) of MSSA (Figure 1). However, chromogenic assay and OX (E-test) were highly effective for detecting MRSA because they were recorded isolation rates for MRSA 39 (30.0%) and 38 (29.3%), respectively. Meth (E-test) gave lower result compared with other phenotypic tests, detecting 35 (26.9%) MRSA isolates (Table 3) and (Figure 2, A, B, C and D).

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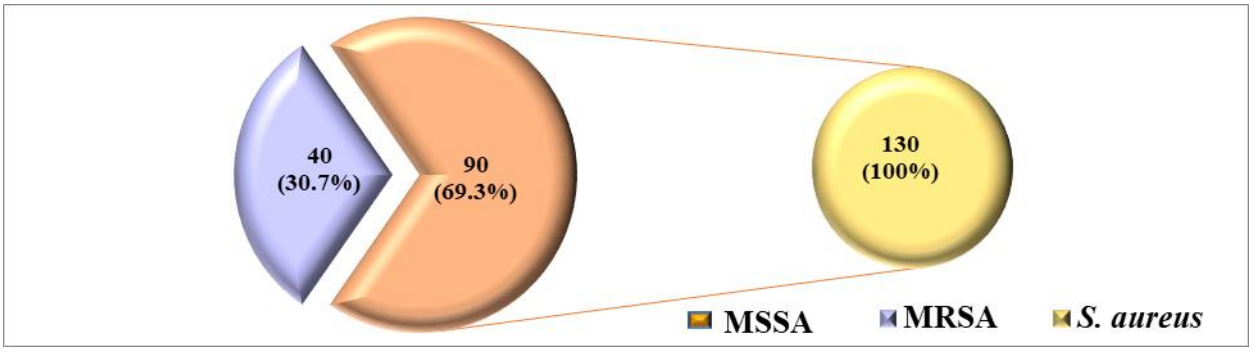

Figure 1. The percentage of MRSA and MSSA isolates among all 130 *S. aureus* isolates that were detected by using cefoxitin disc diffusion.

Table 3. Phenotypic detection of methicillin resistance among 130 *S. aureus* isolates were isolated from woman with breast cancer

Phenotypic test	MRSA n= 40 (30.7%)	MSSA n= 90 (69.3%)
FOXDD test	40 (30.7)	90 (69.3)
OXDD test	36 (27.7)	94 (72.3)
OX (E-test)	38 (29.3)	92 (70.7)
MET (E-test)	35 (26.9)	95 (73.1)
Chromogenic assay	39 (30.0)	91 (70.0)

CHROMagar, BBL ™CHROMagar ™ MRSA; FOXDD, cefoxitin disc diffusion; OXDD, oxacillin disc diffusion; OX (E-test), oxacillin (HiComb E-test); MET (E-test), methicillin (HiComb E-test).

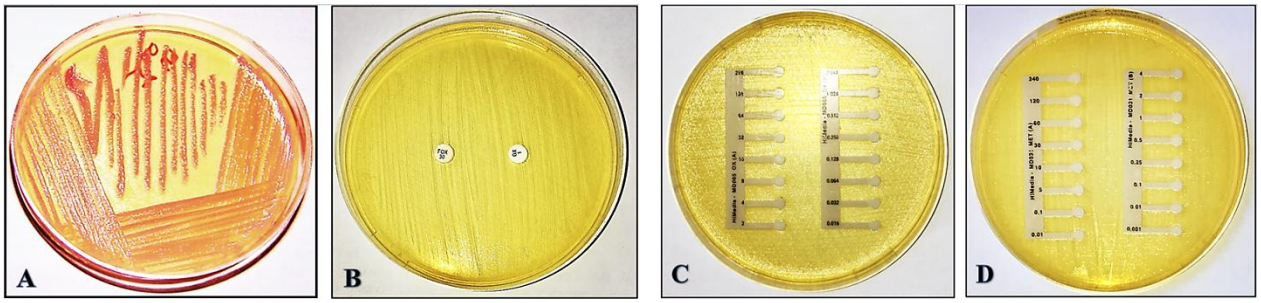

Figure 2. Phenotypic detection of methicillin resistance. (A) Positive result, colonies of isolate BC-7 were appeared pink to mauve on chromogenic agar; (B) Positive result, isolate BC-12, there was no inhibition zone around cefoxitin and oxacillin discs (FOX, OX)-30 µg; (C) Positive result, isolate BC-39 [OX (E-test) MIC ≥ 256 µg/ ml]; (D) Positive result, isolate BC-26 [Meth (E-test) MIC ≥ 240 µg/ ml].

Assessment of Antibiotic Susceptibility (AST)

According to AST results, all isolates of MRSA under study showed completely resistance to penicillin G, oxacillin, and cefoxitin. However, all these isolates were recognized as completely sensitive against levofloxacin, vancomycin, linezolid and nitrofurantoin. Tetracycline 28 (70%) and erythromycin 27 (67.5%) were determined to be agents with the highest resistance. Trimethoprim/sulfamethoxazole, rifampicin and gentamicin were highly effective against MRSA

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were determined as 29 (72.5%), 28 (70%) and 27 (67.5%), respectively. Moderate resistance 19 (47.5%) were recorded against clindamycin (Figure 2).

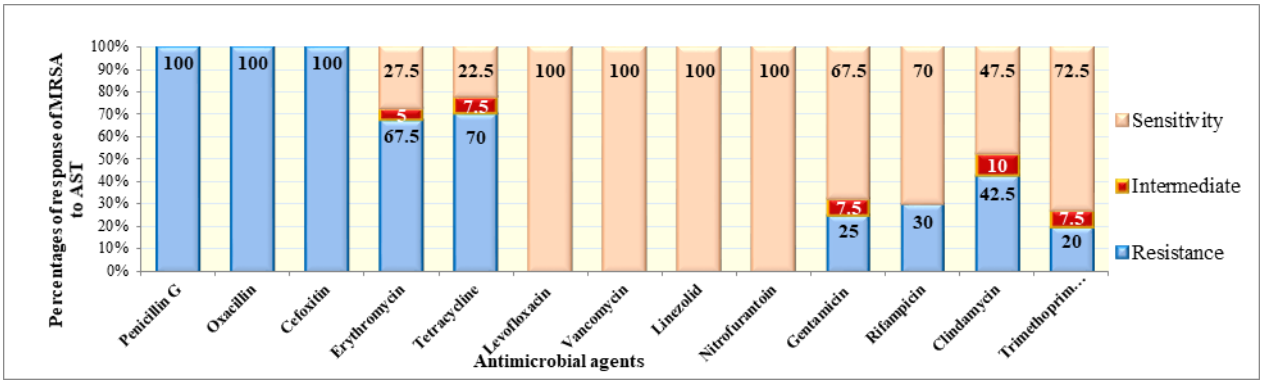

Figure 3. Antimicrobial susceptibility patterns of 40 MRSA isolates isolated from women with breast cancer.

Multidrug Resistance Patterns of MRSA

Twenty five (62.5%) of MRSA isolates in the current study were MDR. The highest percentage of MDR-MRSA were observed in class three of antibiotics 9 (22.5%) followed by class five and six of antibiotics 5 (12.5%). The lowest percentage is observed in a class four and seven of antibiotics 3 (7.5%). Resistance pattern to different classes of antibiotics are described in Table (4). MDR is well-defined as a strain that is resistant to at least one antibiotic in at least three antibiotic classes [11].

Table 4. Multidrug resistance among 40 isolates of MRSA were isolated from woman with breast cancer

Antibiotic susceptibility pattern	No. of MRSA isolates	No. (%) of MDR-MRSA isolates 25 (62.5%)	No. of antibiotic classes (n= 11)
FOX, E, TE	9	9 (22.5)	3
FOX, E, TE, R	1	3 (7.5)	4
FOX, E, TE, CD	2		
FOX, E, TE, R, CD	1	5 (12.5)	5
FOX, E, TE, GEN, CD	2		
FOX, E, TE, CD, COT	1		
FOX, GEN, R, CD, COT	1		
FOX, E, TE, GEN, R, CD	3	5 (12.5)	6
FOX, E, TE, R, CD, COT	2		
FOX, E, TE, GEN, R, CD, COT	3	3 (7.5)	7

MDR, multidrug resistance; FOX, cefoxitin; E, erythromycin; TE, tetracycline; LE, levofloxacin; VA, vancomycin; LZ; linezolid; NIT; GEN, gentamicin; R, rifampicin; CD, clindamycin and COT, trimethoprim-sulfamethoxazole.

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DISCUSSION

The incidence of virulent MRSA carriers and infections continues to rise. It was acquired from healthcare institutions as well as community. MRSA colonization has become increasing prevalent. In recent years, 1.5% to 8% of the general community have been found to be colonized by MRSA [8]. The risk for virulent MRSA infection and colonization in cancer patients is even further multiplied more than other patients. Importantly, many reports have revealed that preoperative colonization of MRSA is independently predictive of increased danger for MRSA wound infections after surgery. Previous reports have shown that MRSA is a common pathogen in chronic wounds and MRSA colonization increases the development of invasive MRSA infections [8, 12].

Data, whether in Iraq and other countries of the world, are scarce about the factors linked with surgical site infection among women who are discharged after BC surgery surgical, chemical and radiological procedures have been used as part of BC treatment for many years at wide levels. In this study, most of *S. aureus* isolation rate were diagnosed among women that subjected to surgery in rate 130 (60.2%). Other reports recorded that an estimated approximately 47%- 84% of surgical infections occur discharge; utmost of these are treated completely in the outpatient setting [13]. Microbiota can affect adiposity by changing energy efficiency and free fatty acids synthesizing, which, can lead to unbalance in hormones. Levels of steroid hormones, especially estrogen, are dependent on the metabolic activity of the microbiota, which are the dominant controllers of systemic immunity. Microbiota are produce many metabolites, including toxins, virulence factors, fatty acids, cofactors and vitamins, potent enough to trigger several signaling cascades. All of these factors can contribute to the overall risk of breast carcinogenesis [14]. Pek et al. [15] found that complicated wounds require readmission with the possibility of surgical interference while mild infections are commonly managed on outpatient centers. This is usually accompanied by serially delays of the start of postoperative adjuvant treatment and delay in wound healing. One study in Kuwait conducted by found that most of BC patients who developed surgical site infections were admitted again for treatment of their complicated wounds infections including new operation may worsen the patient outcome and adding more cost on the healthcare settings [13], also they were found that the percentage of *S. aureus* associated with postoperative BC infections (40%) and this percentage was high compared with current study. Another study that focused on finding the factors associated with infections in the surgery sites and found that the most infections in this vital site are caused by virulent *S. aureus*. So, the first factor that was most influential was bacterial colonization, skin flap necrosis and the last factor that was no less effective than other factors, which is age [16], and this corresponds to what was found in the current study, as the elderly groups: (61-70) and (71-80) were the most affected by surgical infections after drainage. These age groups are the most colonized by *S. aureus*, and recorded the highest rates.

The relationship between BC and ageing seems to be more than apparent in the current study. There are many studies that had linked between aging, the recurrence of bacterial infections and malignant tumors. Owusu and Berger [17] and Smetana et al. [18] emphasized in their studies that elderly persons commonly suffer from diseases dependent age. The treatment of malignant tumors can affected by polymorbidity, such an impending situation may be linked with multiple medical, economic and social issues. Ageing seems to be integral component in both the aforementioned concepts. Due to inherent genetic instability, ageing represents an implicit factor for cancer development. Cancer-affected elderly patients represent an immensely growing target

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population for cancer therapy in the near future. Smetana et al. [18] and Burkhalter et al. [19] explained the relationship between aging and changes at the cellular level, they were found that limited capacity of DNA repair allowing successful editing of stochastically occurring mutations is further restricted in the elderly by associated epigenetic mechanisms. By extension, a generalized decrease in gene-repair capacity in the process of ageing might be regarded as an integral regulatory check-point of natural human ontogeny. Greco [20] explained the association between aging and the increased possibility of infections and the opportunity to BC, he explained that the aging immune system becomes chronically inflammatory, a phenomenon often referred to as inflamm-aging. The increase in an aged person's systemic pro-inflammatory status with age is a main contributor to cancer development. These findings open further avenues for immuno-therapeutic development to target age-related BC.

Detection of MRSA

The use of checking, treatment programs have been shown to reduce the occurrence of virulent MRSA before and postoperative infections. Since MRSA are resistant to all β-lactam antibiotics and a wide range of other antibiotics as well as the treatment selections are limited significantly [21]. MRSA is a dangerous bacteria that shows antibiotic resistance, virulence and survival fitness. Selection pressure, exercised by cross-transmission through wide-spectrum antimicrobial treatment and healthcare workers' hands, facilitates its spreading. Hence, profiles to identify patients at great threat of MRSA carriage might improve prevention of MRSA infections because MRSA carriers, without symptomatic infection, are an important pathogen reservoir [22, 23]. Therefore, the current study was designed to diagnose MRSA in woman with BC by multiple phenotypic methods to be able to know the extent of these methods in early diagnosis and to predict the possibility of infection through easy, simple, fast and inexpensive methods compared to molecular methods. Detection of MRSA is important for patient care and use of appropriate antimicrobial therapy. Rapid, accurate identification of patients with MRSA is important in preventing its transmission and enabling early therapeutic decisions [6, 24].

Table (3) shows the results of the comparison of five phenotypic tests. Cefoxitin is an excellent potent inducer and stimulator of the *mecA* gene regulatory system than other the penicillins. The FOXDD results in this study were similar to *mecA* gene and sensitivity of this test were 100%, consistent with the reports published previously [25, 26]. Kumar et al. [27] emphasis on the fact that the FOXDD can be the superior method for classic MRSA detection where molecular methods are not available. FOXDD has the advantages of being easy, simple, giving results on the same day, rapid, low cost, high efficiency, more stable, capable for detecting heteroresistant MRSA, and its able to detection MRSA even if production of penicillin binding proteins (PBPs) in small quantities. In addition, this method isn't need skilled people to do it. Accordingly, the FOXDD was found to be the method of choice to identify MRSA. This result is in agreement with previous studies about the suitability of FOXDD for MRSA identification. It was reported that disc diffusion with cefoxitin is more reliable than that with oxacillin. It is a more potent inducer of the *mec*A gene with no special requirements of medium or temperature. Several studies indicate that FOXDD is superior with a specificity 96-100% and sensitivity 94–100%, and it can be used as an alternative to molecular methods like PCR. Cefoxitin disk was revealed to be suitable even in low-level heterogeneous strains [6, 10, 24, 26]. In Anand et al. [28] study, the FOXDD sensitivity and specificity reached to 100% and emphasized using this method as an alternative to molecular method. FOXDD has recommended at several recent reports compare with OXDD because of complicated determination of MRSA showed resistance at multiple levels [29].

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However, OXDD in this study showed less sensitivity to *mecA* detection. OXDD sensitivity reduced when hetero-resistant isolates of MRSA were experienced. Demir et al. [26] noted that sensitivity decreased when heterogeneous resistant strains were tested. Several studies indicate that oxacillin often failed to detect heterogeneous MRSA populations, and also should not be used in methicillin disc due to lower specificity test results. Asiimwe et al. [30] was mentioned that routine OXDD often fail to identify heterogeneous MRSA, which consequently are considered MSSA because of their common sensitive to most non-*β*-lactam antibiotics.
Chromogenic assay appeared more sensitive for detection *mecA* gene in the current study. The sensitivity could be increased to 100% by increasing the incubation period from 24 to 48 hours. Datta et al. [12] was confirmed this fact. Hraishawi [29] was also founded that the sensitivity of MRSA was raised with increasing the incubation period where increased from 71% to 96% after 24 hours and 48 hours of incubation, respectively in contrast low sensitivity even after 48 hours incubation 73.49 % [31].

Antibiotic Susceptibility Patterns of MRSA

Infections caused by MDR bacteria are linked with higher expenditures and worse health outcomes; however, few studies have studied the occurrence of MDR-MRSA in human populations [32]. Genetic versatility of MRSA which permits for antibiotic adaptation, such that many isolates can be MDR to many classes of antibiotics [6].
Interestingly, penicillin G, oxacillin and cefoxitin, which are widely used in Babylon City and other provinces of Iraq, this study found that 100% of MRSA strains were resistant to these antibiotics. This is a worrying sign, which highlights extensive use of wide spectrum antibiotics and other similar in clinical settings. MRSA produces a PBP2a enzyme encoded by *mecA* gene and *β*-lactamase enzyme encoded by *blaZ* gene and these enzymes are reduce the activity of *β*-lactams [6, 24].
In general, high percentages of MDR may appear from various isolates of *MRSA* under antibiotics pressure or as a result of extensive transmission of MDR isolates [24, 28]. Despite the widespread use of vancomycin, levofloxacin and nitrofurantoin in Iraq, but fortunately, the current study wasn't recorded any resistance to isolates of both MRSA and MSSA. There is a local study done in Al-Najaf by Al-Kudheiri [6], and it was found that HA-MRSA isolates were also 100% sensitive to previous antibiotics. Linezolid is included in the list of antibiotics that weren't recommended by the Iraqi Ministry of Health and it isn't available in pharmacies and perhaps for this reason, this study didn't recorded any resistance to this antibiotic. Boakes et al. [33] that recommended to use vancomycin as a second line in more severe cases of HA-MRSA for women with breast infections.
In the present study, tetracycline and erythromycin were determined to be agents with the highest resistance. Lim et al. [34] proved tetracycline-resistant isolates often showed co-resistance towards erythromycin and the *tetM* and *ermA* were the dominant genes identified in tetracycline and erythromycin-resistant strains, respectively. The presence of *tetM*, *tetK*, *ermA* and *ermC* genes was responsible for tetracycline and erythromycin resistance among MRSA isolates. Association of these resistance genes with mobile genetic elements possibly enhances the spread of resistant traits in MRSA.
This study found association between BC infections and MDR-MRSA colonization. Where it was found that the rate of MDR-MRSA 25 (62.5%), which is a disturbing proportion because the MDR strains limit the therapeutic choices, creating an economic and social burden to the healthcare system. Transfer of horizontal gene in the hospital setting is responsible for spreading

antibiotic resistant determinants. Gitau et al. [35] confirmed this fact and noted that chromosomal mutation is responsible for multiple antibiotics resistance.
The conclusions of the reality of Steinig et al. [36] study were very similar to those of the Iraqi health reality, where it was found that there is a relationship between poor antibiotic regulation, limited public health infrastructure, considering the widespread use of antibiotics, and high population density, the emergence and global dissemination of MDR clones (both Gram negative and Gram positive) are not surprising and alarming.
MRSA isolates were recorded percentage 30% among BC women. Ray et al. [37] found that the treatment with rifampicin has shown to induce MDR expression in healthy persons. Hence, MDR overexpression can be affected by gene rearrangement/ amplification, rifampicin induction. Another factor responsible in MDR-cancer is the mutation in the apoptotic gene p53. This gene induces apoptosis in cells which have undergone DNA damage. So, the drugs that increase DNA damage will come to no use in certain cancers. Thus MDR arising from genomic reactions affects a large diversity of anticancer medications and increases the rate of surviving mutant cells, which leads to greater tumor heterogeneity.

CONCLUSIONS

Isolates of *S. aureus* were more presented in BC infections and the elderly groups were most affected, while MRSA isolates were found in a low rate but worrisome because that all these isolates were resistant to all *β*-lactam antibiotics and most of these isolates were MDR. The FOXDD method is more efficient and accurate for MRSA detection than other phenotypic methods.

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