

Assessment of Environmental Contamination with Methicillin-Resistant *Staphylococcus aureus* at some Hospitals in Kerbala City, Iraq

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the bacteria that causes hospital-acquired illnesses that is most resistant to antibiotics. The study aimed to examine the prevalence of MRSA in the environment of two hospitals in Kerbala city: Maternity Hospital (MH), and Kerbala Instructional Pediatric Hospital (KIPH) from (beds , Incubators of premature infants, floor, tables, walls, and Kitchenware) from October 2022 to December 2022 to identify the source of spread to develop appropriate control measures. The isolates were identified according to cultural, microscopic characteristics, biochemical tests, and Api-Staph system. The results showed that the floors and beds were the most contaminated sites in the wards of MH. The highest percentage of antibiotic resistance in MH and KIPH was to Kanamycin 88.8%, and Lomefloxacin 54.5%. The contamination in MH was due to the high numbers of pregnant women and it` companions thus the lack of time available to perform infection control program as well as lack of interest in the cleaning the tables in the KIPH making them as a source of survival and proliferation of MRSA. This study reveals widespread contamination of the hospitals environment, highlights the complexities of the problem of contamination, and confirms the need for more-effective cleaning of the hospital environment to eliminate MRSA.

Keywords: Hospital contamination; MRSA; Resistance; *Staphylococcus aureus*; Vancomycin.

1. Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is one common pathogen that is frequently acquired in hospitals, which may cause a variety of diseases. Particularly in hospitals, MRSA has become a serious public health problem in recent years. Burn units and intensive care units (ICUs) are high-risk locations for MRSA infections acquired in hospitals, which can raise healthcare expenses and increase morbidity and death. Multiple antibiotic resistance is exhibited by MRSA, which can lead to severe infections such as pneumonia, endocarditis, and cutaneous infections, especially in individuals with burn injuries [1]. Although the bacterium primarily spreads through direct touch, certain infections can spread through other means [2]. Regular cleaning is required since MRSA may infect the majority of equipment, including catheters, surgical tools, breathing equipment, and even wound dressings in intensive care units. To stop *S. aureus* from colonizing burn sites, it's critical to remove both endogenous and external sources [3]. Numerous investigations linking the environment to MRSA transmission were carried out during outbreaks that required extensive cleaning before they could be contained [4].

Worldwide (MRSA) strains have been discovered to be resistant to cephalosporins, penicillin, cephamycins, and other beta-lactam (β -lactam) antibiotics, with the exception of ceftaroline (fifth-generation cephalosporins), which is used to treat MRSA infections. Additionally, *Staphylococcus* species frequently become resistant to many antibiotic classes, including aminoglycosides, macrolides, and quinolones [5]. However, the role of the environment as a source of MRSA acquisition in a hospital in which endemic cross-infection occurs over an extended period is not clear. Due to the lack of information about the sources and rates of spread of MRSA bacteria in hospitals in Kerbala city, and due to the clinical importance that this bacteria poses in terms of its ability to cause disease and its multiple resistance to most known drugs, hence the idea of this research came, which includes the following aims: A study of the prevalence of MRSA bacteria in the wards and kitchens of two hospitals in Kerbala city: Maternity Hospital (MH), and Kerbala Instructional Pediatric Hospital (KIPH)), determine the role of the environment in the transmission of MRSA, determine the source of MRSA bacteria isolates spread in the two hospitals' environments for the purpose of developing appropriate procedures to control them and prevent their spread, Investigating the sensitivity and resistance pattern of MRSA isolates to some antibiotics.

2. Materials and Methods

2.1. Collection of Samples

20 isolates out of 163 samples of MRSA were isolated from the wards and kitchens of two hospitals in Kerbala city: Maternity Hospital, and Kerbala Instructional Pediatric Hospital for the period between October 2022 to December 2022. Sterile cotton swab (Sterlin, UK) moistened with normal saline solution was used to take swabs from the environment of the hallways and kitchens of the two hospitals and from various locations that included (beds, incubators, floors, tables, walls, and kitchen utensils). The swabs were then transferred within two hours to the laboratory and cultured on Mannitol Salt Agar (Himedia, India). The cultures were incubated under aerobic conditions at a temperature of 37°C for 24-48 hours [6]. After the appearance of growth, individual and fermented colonies of mannitol, which appeared golden yellow, were taken for the purpose of diagnosis.

2.2. Laboratory diagnosis

The samples were initially diagnosed by observing the cultural characteristics of the growing colonies in terms of colony size, height, edges, and color. They were purified by replanting them on Nutrient Agar medium, then incubated at a temperature of 37 °C, and for 24 hours after that, thin smears were made from them and stained. With Gram stain, the shapes of the cells, their arrangement, and their ability to stain with this dye were observed [7, 8]. Biochemical tests were conducted, such as the catalase test, the oxidase test, the free and bound types of the plasma coagulase enzyme test, the blood hemolysis test, and DNase test. The diagnosis was confirmed using Api-Staph system [9, 10].

2.3. Antibiotic susceptibility test

The sensitivity of bacteria to five types of antibiotics (Bioanlys, Turkey) was tested using Mueller Hinton Agar medium, represented by Gentamycin (10 mcg), Imipenem (10 mcg), Kanamycin (30 mcg), Lomefloxacin (10 mcg), Vancomycin. (30 mcg).

As for testing the sensitivity of the isolates to the antibiotic methicillin (5 mcg). The results were recorded by measuring the diameters of the inhibition zones in millimeters around each disc and were compared to the standard averages for the diameters of the inhibition zones for antibiotics as stated in [11,12].

3. Results and discussion

3.1. Sources and rates of spread of MRSA bacteria in the two hospitals

The wards of the Maternity Hospital were the most contaminated with MRSA bacteria compared to the wards of the Kerbala Instructional Pediatric Hospital, as the prevalence of bacteria in the two hospitals, respectively, was 22.5% and 10.8%, while the kitchens of both hospitals were not contaminated with MRSA bacteria, as shown in the **Tables 1, 2, and 3.**

Table 1. Numbers and percentages of MRSA isolates isolated from the wards of the Maternity Hospital.

Ward name	Percentage of contaminated sources %					Contamination rate %
	Incubators	Walls	Bedside Tables	Floor s	Beds	
Surgical ward	-----	0	33.3	40	42.8	30
Pregnant women ward	-----	0	0	66.6	0	28.5
Emergency room	-----	0	0	0	33.3	16.6
Neonatal intensive care unit	,	0	0	0	-----	0
Contamination rate of sources %	0	0	14.2	40	33.3	22.5

Table 2. Percentages of MRSA isolates isolated from the wards of Kerbala Instructional Pediatric Hospital

Ward's name	Percentage of contaminated sources %					Contamination rate %
	Incubators	Walls	Bedside Tables	Floors	Beds	
Neonatal intensive care unit	0	0	0	0	0	0
Wards of patients with thalassemia	-----	0	0	0	0	0
The first wards	-----	50	0	0	0	6.6
Emergency room	-----	0	33.3	0	0	10
The third wards	-----	0	0	0	20	11.1
The second wards	-----	0	41.6	16.6	18.1	23.5
Contamination rate of sources %	0	7.6	24	6.6	8.3	10.8

Table 3. Percentages of MRSA isolates isolated from the kitchens of the two hospitals.

Hospital`s Name	Percentage of contaminated sources %				The level of contamination in the kitchen %
	Instruments and tools	Walls	Tables	Floors	
Maternity Hospital	0	0	0	0	0
Kerbala Instructional Pediatric Hospital	0	0	0	0	0
Contamination rate of sources %	0	0	0	0	0

The wards most contaminated with MRSA bacteria in the Maternity Hospital were the surgical ward was 30% and the pregnant lounge was 28.5%, while the wars of the second ward in the Kerbala Instructional Pediatric Hospital were the most polluted, and the prevalence of bacteria in them was 23.5%.

The sites most contaminated with MRSA bacteria in the wards of the Maternity Hospital were the floors, 40%, followed by the beds, 33.3%. This is consistent with the findings of [13] in Hong Kong hospital, which showed that the Staphylococcal contamination was detected on 83% and 77% of the bedside surfaces of MRSA-positive and MRSA-negative patients, respectively, at 8:00 a.m., and the staphylococcal concentrations rose by 80% at 1200 h over a 4-hour period with standard ward and clinical activity. While [14] indicated that beds and floor were the most polluted sites in surgical ICU and Non-surgical ICU ward of Rohani hospital in Babol, Iran, at a rate of 89.4%, and 68.4% for floor, also 73.6%, 57.5% for beds which is more than the current study. On the other hand, tables were the most polluted sites in the wards of Kerbala Instructional Pediatric Hospital 24%.

The floors and beds in the Maternity Hospital were more contaminated with MRSA bacteria compared to the beds and floors of the Kerbala Instructional Pediatric Hospital, as the percentage of contamination (floors, beds) in the two hospitals, respectively, was (40, 33.3)% and (6.6, 8.3)% The highest percentage of floor contamination in the lobbies of the Maternity Hospital was in the floor of the pregnant lounge at 66.6%, followed by the floor of the surgical lobby at 40%. The floor of the hallways of the second ward in the Kerbala Instructional Pediatric Hospital was polluted at a rate of 16.6%, while the highest percentage of bed contamination was in the wards of the hospital.

Gynecology and obstetrics had 42.8% of surgical ward beds, followed by 33.3% in the emergency room. While the percentage of contamination of the wards beds of the Kerbala Instructional Pediatric Hospital in the wards beds of the third and second wards was 20% and 18.1%, respectively.

The high rate of contamination of the floors of the Maternity Hospital may be due to the large number of pregnant women and postpartum women and their companions in patient wards, which contributes to the transfer of bacteria and their persistence on the floors of these wards, while bed contamination may be due to high bed occupancy and thus lack of time. Available for sterilization operations. Since MRSA bacteria have the ability to survive from several weeks to several months on various dry surfaces [15]. This makes the beds a major reservoir of MRSA bacteria, and thus it is transmitted to the fingers that touch these surfaces [16], which constitutes a danger that threatens the lives of patients, as the bacteria may be transmitted to the patient himself. In a study [17], it was found that 31% of volunteers who touched iron bars and bedside tables had their hands contaminated with *Staphylococcus aureus* bacteria, of which MRSA bacteria constituted 35%. Therefore, sterilization of bed parts (iron bars) is necessary to prevent the transmission of bacteria to the patient who is in contact with the bed.

The wards tables of the Kerbala Instructional Pediatric Hospital were more polluted than the wards tables of the Maternity Hospital, as the percentage of contamination of the tables in the two hospitals, respectively, was 24% and 14.2%, and the highest percentage of contamination of the wards tables of the Maternity Hospital was in the surgical lobby tables, 33.3% in While the hallway tables of the second ward were the most polluted, with a rate of 41.6% in the Kerbala Instructional Pediatric Hospital, followed by Emergency wards desks 33.3%. The percentage of contamination of hospital bedside tables in the current study differs from what was found by [18] in Ukrainian acute care hospitals, 20.5%.

The study showed that the walls of the Maternity Hospital wards were uncontaminated, while the contamination rate of the walls of Kerbala Instructional Pediatric Hospital was 7.6%. The highest percentage was 50% in the walls of the first wards. As for the incubators for premature babies in both hospitals, they were not contaminated. This may be due to the sterilization of the incubators for premature babies with chlorine before and after the child left the hospital.

The beds, floors, and tables in the current study are considered a source of storage for MRSA bacteria and therefore pose a threat to the lives of patients lying in the hallways of these hospitals. Therefore,

it is preferable that these sources be sterilized with disinfectants, in addition to conducting a survey of these sites on an ongoing basis to investigate their contamination with this dangerous bacteria.

3.2. Antibiotic susceptibility test

The results showed that the highest percentage of resistance of bacterial isolates isolated from the Maternity Hospital was to the antibiotics lomefloxacin and kanamycin at 88.8%, followed by the antibiotic Gentamicin at 66.6%, while the bacterial isolates isolated from the Kerbala Instructional Pediatric Hospital had the highest resistance to the antibiotics lomefloxacin and Kanamycin at 54.5% as shown in **Table 4**.

The high rate of resistance of isolates from the two hospitals to antibiotics belonging to the group of fluoroquinolones, to which the antibiotic lomefloxacin belongs, is consistent with what was found by [19] in Japan, where it was found that the previous bacteria were highly resistant to fluoroquinolones antibiotics at a rate of 85%. This may be due to the occurrence of chromosomal point mutations in the *grlA*, *gyrA* and *norA* genes, as the first and second genes encode the enzymes DNA topoisomerase and DNA gyrase, which represent the primary target of antibiotics from the group of fluoroquinolones. These two enzymes are necessary for the replication of the DNA of bacterial cells, while The gene encodes *norA* The protein NorA is responsible for the Efflux pump mechanism for this group of antibodies outside the bacterial cells [20].

The resistance of bacteria to the antibiotic Kanamycin may be due to the presence of the transposon factor (Tn4001) carried on the pSK1 plasmid, which induces resistance to this antibiotic [21].

On the other hand, the study showed that the isolates from the Maternity Hospital were highly sensitive to the antibiotics vancomycin and imipenem, as the rate of resistance to these two antibiotics, respectively, was 11.1% and 22.2%, while the isolates from the Kerbala Instructional Pediatric Hospital were sensitive to the previous two antibiotics in addition to the antibiotic Gentamicin, as The resistance percentage for each of them was 0%, 9%, and 18%, respectively. The high sensitivity of bacterial isolates to the antibiotics vancomycin and imipenem may be due to the fact that the two antibiotics have high activity against Gram-positive bacteria such as MRSA [22]. Therefore, it is preferable to use these two antibiotics to treat infections resulting from MRSA bacteria acquired in hospitals. It is noted in **Tables 5**, and **6** that the resistance type (ME, CN, K, LOM) was the most frequent in MRSA isolates isolated from the Maternity Hospital, as it was 55%, while the resistance type was (ME, K, LOM) is the most common in bacterial isolates isolated from Kerbala Instructional

Pediatric Hospital, at a rate of 27%, which may indicate the seriousness of the isolates found in The environment of these halls had multiple resistance to more than one antibiotic.

Table 4. Percentages of bacterial antibiotics resistance isolated from hospital`s wards.

Antibiotics codes	Antibiotics Resistance Rates %	
	Kerbala Instructional Pediatric Hospital	Maternity Hospital
VA	0	11
IMP	9	22.2
CN	18	66.6
LOM	54.5	88.8
K	54.5	88.8

Note: LOM:Lomefloxacin (10 µcg), K: Kanamycin (30 µcg), CN: Gentamycin (10 µcg), IMP: Imipenem (10 µcg), VA:Vancomycin (30 µcg).

Table 5. Antibiotic resistance pattern of MRSA isolates isolated from hospitals.

Hospital name	Ward Name	Sources of bacterial isolates	Antibiotic resistance pattern
Maternity Hospital	Surgical ward	Bed	ME , CN , K , LOM
		Bed	ME , CN , K , LOM
		Floor	ME , CN , K , LOM
		Floor	ME , CN , K ,
		Bed	ME , CN , K , LOM
		Table	ME , CN , K , LOM , IPM
	Pregnant women ward	Floor	ME , IMP , K , LOM , VA
		Floor	ME , K , LOM
	Emergency room	Bed	ME
Kerbala Instructional Pediatric Hospital	The first ward	Wall	ME , CN , K , LOM , VA
	The second ward	Table	ME , K , LOM
		Bed	ME
		Table	ME
		Table	ME , K , LOM
		Floor	ME
		Table	ME , K
		Bed	ME , CN , K , LOM
		Table	ME , K , LOM
	The third ward	Bed	ME
	Emergency room	Table	ME , LOM

Note: LOM: Lomefloxacin (10 µcg), K: Kanamycin (30 µcg), CN: Gentamycin (10 µcg), IMP: Imipenem (10 µcg),VA: Vancomycin (30 µcg).

Table 6. Percentages of MRSA isolates of the two hospitals relative to the number of antibiotics they are resistant to.

The number of antibiotics that bacteria have become resistant to	1	2	3	4
Maternity Hospital	11%	11%	55%	22%
Kerbala Instructional Pediatric Hospital	18%	27%	9%	9%

4. Conclusions

According to this study the results showed that the floors and beds were the most contaminated sites in the wards of Maternity Hospital. The highest percentage of antibiotic resistance in both hospitals was to the antibiotics kanamycin , and lomefloxacin. The contamination in Maternity Hospital was due to the high numbers of pregnant women and it` companions thus the lack of time available to perform infection control program as well as lack of interest in the cleaning the tables in the Kerbala Instructional Pediatric Hospital making them as a source of survival and proliferation of MRSA.

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