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Comparison of chromogenic agar medium and diffusion disk test for detection of hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) from patients and hospital environment in Nasiriyah city, Iraq

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To compare the culture screening protocols for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in the surgical wards, one hundred and forty eight samples (116 nasal swabs from 116 patients and 32 environment items swabs) were examined. 96 (64.8%) of the samples stowed S. aureus, 75 (64.6%) from patients, and 21(65.6%) from hospital environment. The detection of MRSA among the isolates of the *S. aureus* was carried out using direct culture and subculture from mannitol salt agar on HiCrome MeReSa agar medium compared with FOX, OX and ME DD test. Among the 96 *S. aureus* isolates, 63 (65.6%) were HA-MRSA of which 50 (66.6%) and 13 (61.9%) from the patients and environment, respectively. There is no significant difference in the detection rate of MRSA between HiCrome MeReSa Agar and DD test as each showed 63 isolates (50 from patients and 13 from environment). The prevalence of MRSA in both was 42.5% (63/148) and 22.3% (33/148) of MSSA. Lower detection of 47 and 11 MRSA isolates with prevalence 39.1% (58/148) was obtained after 24 h of incubation by direct culture method on HiCrome Agar, respectively. The report time was fastest (24 h) in direct culture on HiCrome agar and the slowest (48 h) in subculture on HiCrome Me ReSa Agar and OX, FOX, ME disc diffusion test. In the patients screening samples, the sensitivity, specificity, positive predictive value PPV and negative predictive value NPV between direct culture on HiCrome Agar medium and DD test were 94, 100, 100 and 95.6%, respectively, while in the environment samples were 84.6, 100, 100 and 90.4%, respectively. Patient's samples demonstrated higher sensitivity and NPV than environment samples but the same rate in specificity and PPV. In contrast, no observed differences between results from subculture on HiCrome Agar medium and (OX, FOX and ME) DD test, which agreed in their sensitivity, specificity, PPV and NPV. The study also revealed that all MRSA isolates were multidrug resistant (MDR) and they were highly resistant (100%) to Beta-lactam antibiotics: oxacillin, cefoxitin, methicillin, ampicillin and amoxicillin.

Key words: Methicillin resistant *Staphylococcus aureus* (MRSA), hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA), Iraq, chromogenic medium, surgical wards.

INTRODUCTION

Methicillin-Resistant Staphylococcus Aureus (MRSA) is a type of staph bacteria that is resistant to certain antibiotics called beta-lactams. These antibiotics include methicillin and other more common antibiotics such as oxacillin, cefoxitin, penicillin and amoxicillin (CDC, 2011). MRSA is a major health problem worldwide, found in symptomless carriage but also causing even life-threatening infections.

MRSA is able to cause a wide variety of different diseases, ranging from superficial skin inflammation to severe invasive infections in patients exposed to health care setting such as bacteremia, which leads to endocarditis and osteomyelitis (Vainio, 2012; Burdette et al., 2012; Bassetti et al., 2011; Changchien et al., 2011). Hospital-acquired MRSA is typically resistant to classes of antimicrobials other than β-lactams, which narrows treatment possibilities and changes the spectrum of antibiotics prescribed in hospitals.

Antimicrobial resistance (AMR) has been recognized by the World Health Organization (WHO) as one of the top three threats to human health, in particular, methicillin-resistant *Staphylococcus aureus* (Ferreira and Pinto, 2011; Drago et al., 2007; Peterson, 2012; Klevens et al., 2007).

Different policies have undergone test and are currently in use to control AMR/MRSA and one of them involves the screening of patients in hospitals (Ferreira and Pinto, 2011). AMR/MRSA is an important agent of hospital-acquired infection; seen mainly as a significant human pathogen (Lee, 2008; Tenover and Gorwitz, 2006).

According to National Nosocomial Infection Surveillance System (NNIS) report, 50% of hospital-acquired infections in ICUs in the USA are due to MRSA (Drago et al., 2007). The pre enrichment was an essential step to increase detection of MRSA in the nasal surveillance of ICU patients (Lee, 2007). MRSA-Select agar (the Hi-Media, India and Bio-Rad, France) a chromogenic selective agar for fast detection of MRSA been introduced, gave an overall results for detecting MRSA from nasal specimens (Denys et al., 2013).

This medium is selected based on the presence of an antibiotics/antifungal mixture and an optimized concentration of salt that inhibits the growth of yeast and the majority of gram positive and gram negative bacteria with the exception of MRSA, the identification is based on cleavage of achromogenic substrate by a specific enzymatic activity of MRSA (HiMedia Labs., India).

This study was undertaken to evaluate the MRSA detection rates obtained by the use of culture screening protocols including direct and subculture on HiCrome MeReSa Agar and oxacillin, cefoxistin and methicillin disc diffusion test for nasal swabs among patients and the environment in the surgical wards of Al-Hussein teaching hospital in Nasiriyah, Iraq.

MATERIALS AND METHODS

Collection of samples

This Study was performed from May to September 2012, in the surgical wards of the Al-Hussein teaching hospital in Nasiriyah city, Iraq. One hundred and forty eight samples (from 116 patients and 32 hospital environment items) were collected for detection of HA-MRSA. Informed consent was obtained from patients who were stayed in the hospital wards for at least 72 h. Nasal samples were taken from each patient. A Performa including age, gender, health status and relevant data were also collected from each patient. Sterile cotton swabs (dipped in normal saline 0.9) were used for nasal swabbing of the anterior nares of the patients. Swab was inserted simultaneously inside the anterior nares, first within one nostril and then within the other nostril and rubbed very well by rotating 5 times over the inner wall of the nasal septum and immediately processed for culture and isolation. For the environment, surfaces of frequently handled items (beds, sinks, door handles, surgical trays, and table surfaces) were swabbed.

Each swab was cultured directly on HiCrome MeReSa Agar plate (selective medium for MRSA, HiMedia, India) and subsequently on Mannitol Salt agar plate within one hour after collection by spreading as per the conventional technique. All effort was made to ensure equivalent specimen distributions between the two plates by each swab. The HiCrome culture plates were incubated at 35°C for 24 h, whereas the mannitol salt culture incubated at 37°C for 24 to 48 h. The predominant colonies per sample showing mannitol fermentation were selected and *S. aureus* was identified to species level using standard cultural and biochemical tests (MacFaddin, 2000). The biochemical properties were determined using API staph. System (bioMerieux, France). For Each *S. aureus* isolate, a bacterial suspension adjusted to 0.5 McFarland was used.

Subsequently, a swab was dipped in the suspension, streaked onto the HiCrome MeReSa agar plate and incubated at 35°C for 24 h. All cultures showing blue colored growth on this HiCrome MeReSa agar were considered as MRSA positive.

Detection of MRSA by chromogenic agar

The Hi-Media (India) made HiCrome MeReSa Agar Base (M1674) was used for detection of the MRSA among the clinical isolates of *S. aureus*. The medium was prepared by suspending 41.65 g of the medium into 500 ml ofthe distilled water and boiling. The medium was cooled to around 45 to 50°C and MeReSa selective supplement (FD229) reconstituted with 5 ml sterile distilled water into each methicillin vials having 2.0 mg of methicillin as per the direction of the supplier (HiMedia-India), was added and mixed very well. Soon after, the medium was poured into Petri plates and cooled then checked for sterility by keeping at 37°C overnight. In this study the detection of MRSA was determined by direct culture of each swab on HiCrome medium and by subculture of the identified *S. aureus* strains from mannitol salt agar onto the HiCrome MeReSa agar. Plates were incubated at 35°C for 24 h after which, all cultures showing blue colored growth were taken as MRSA positive strains, while all others are recorded as MSSA

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Table 1. Total number of *Staphylococcus aureus* isolated from patients and hospital environment.

Samples source	Total samples No.	No.	S. aureus%
Patients	116	75	64.6
Hospital environment	32	21	65.6
Total	148	96	64.8

Table 2. Frequency of MRSA and MSSA in patients and hospital environment.

Isolates	*S. aureus*		MRSA		MSSA	
	No.	%	No.	%	No.	%
Patients	75	64.6	50	66.6	25	33.3
Environment	21	65.6	13	61.9	8	38
Total	96	64.8	63	65.6	33	34.4

MRSA, Methicillin resistance *Staphylococcus aureus*; MSSA, Methicillin sensitive *Staphylococcus aureus.*

strains (HiMedia Labs. Products, India).

Antibiotic susceptibility testing

The susceptibilities of the isolates to 13 antibiotics (methicillin, cefoxitin, oxacillin, ampicillin, amoxicillin, meropenem, imipenem, ciprofloxacin, erythromycin, vancomycin, tetracycline, gentamycin and clindamycin) were determined on Mueller-Hinton agar, by the Kirby Bauer disk diffusion method. Results were interpreted basis on the guidelines by the Clinical and Laboratory Standards' institute (CLSI, 2011, 2012). MRSA was identified based on oxacillin, cefoxitin and methicillin resistance and confirmed by HiCrome Agar detection for comparison.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the MRSA HiCrome MeReSa agar test (direct and subculture) were calculated by comparing the results of these culture protocols with the results of the OX, FOX and ME DD Test. The Microsoft Excel data analysis tool was used to compare if there is a difference between HiCrome MeReSa agar and DD test result.

RESULTS

Out of 148 samples (116 nasal swabs and 32 environment swabs) 96 (64.8%) were identified as S. aureus [75 (64.6%)[from patients and 21 (65.6%) from environment (Table 1). The detection of MRSA among the isolates of the S. aureus was carried out using direct and subculture on HiCrome MeReSa agar medium (Figure 1) compared with cefoxitin, oxacillin and methicillin disc diffusion test. Out of 96 *S. aureus* isolates 63 (65.6%) were MRSA of which 50 (66.6%) and 13 (61.9%) from the patients and the environment, respectively; while the prevalence of MSSA was 33 (34.4%). The details of the results are shown in Table 2.

Table 3 demonstrates the relationship between MRSA and demographic variable among patients, the results showed the high prevalence of MRSA among the male than the female (70 and 30%, respectively), and the Maximum MRSA strains were found among the age group 21 to 41 years (36%) and the minimum MRSA among age group 1 to 20 years(12%).

From the results, Table 4 shows no difference in the detection rate of MRSA between subculture on HiCrome MeReSa Agar examined after *S. aureus* isolates grew on Mannitol salt agar and DD test at 48 h (50 from patients and 13 from hospital environment). The prevalence of MRSA in both was 42.5% (63/148), whereas the results showed a decrease in the detection and prevalence of MRSA identified by direct culture method on HiCrome Agar medium from patients and hospital environment were 47 and 11 [39.1% (58/148)], respectively. The report time was fastest (24 h) in direct culture on HiCrome agar and slowest (48 h) in subculture on HiCrome MeReSa Agar and OX, FOX, ME Disc Diffusion test.

In the patients screening samples, sensitivity, specificity, positive predictive value PPV and negative predictive value NPV between direct culture on HiCrome Agar medium and (OX, FOX and ME) DD test were 94, 100, 100 and 95.6%, respectively, whereas in the environment samples were 84.6, 100, 100 and 90.4%, respectively. Results demonstrated higher sensitivity and NPV in patient's samples than in environment samples but the same rate in specificity and positive predictive value PPV. In contrast, no observed differences between results from subculture on HiCrome Agar medium and (OX, FOX and ME) DD test, which agreed in sensitivity, specificity, PPV and NPV (Tables 4 and 5).

The antimicrobial susceptibility patterns of MRSA isolates against 13 different types of antibiotics as shown in Table 6 and Figure 3. The drug resistance patterns of MRSA isolated from clinical specimens was found to be

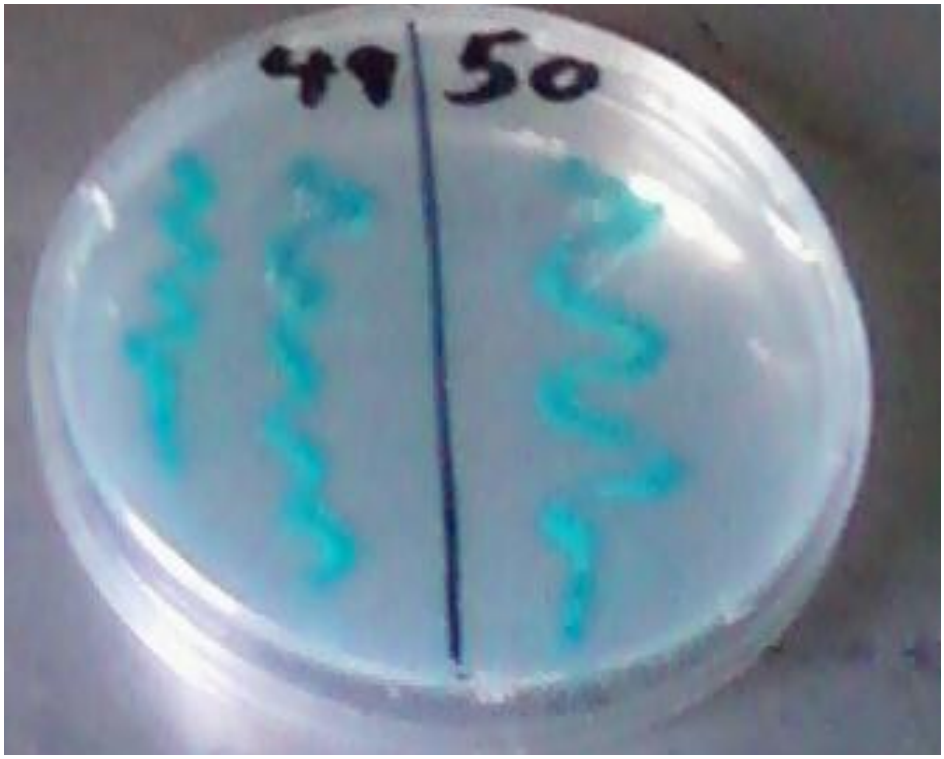

Figure 1. MRSA positive on Hi-Crome agar medium.

Table 3. MRSA and demographic variables among patients with MRSA.

Variable	Total n=116	S. aureus n=75		MRSA n=50		MSSA n=25	
		No.	%	No.	%	No.	%
Gender							
Female	36	26	34.6	15	30	11	44
Male	80	49	65.4	35	70	14	56
Age/year							
1-20	18	11	14.7	6	12	5	20
21-41	56	35	46.7	18	36	17	68
42-62	24	16	21.3	14	28	2	8
>62	18	13	17.3	12	24	1	4

Table 4. Comparison of direct culture, subculture on HiCrome MeReSa Agar and OX, FOX, ME DD test for MRSA prevalence (%) among all samples.

Isolate source n=148	*S. aureus*	Ox, FOX, ME DD test (48 h)		Direct culture on HiCrome (24 h)		Subculture on HiCrome (48 h)	
		MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
Patients n=116	75	50	25	47	28	50	25
Environment n=32	21	13	8	11	10	13	8
%	64.8	42.5	22.3	39.1	25.7	42.5	22.3
Prevalence	(96/148)	(63/148)	(33/148)	(58/148)	(38/148)	(63/148)	(33/148)

OX, oxacillin; FOX, cefoxitin; ME, methicillin.

Table 5. Sensitivity, specificity, PPV and NPV estimating with direct culture and subculture on HiCrome MeReSa Agar and OX, FOX and ME disc diffusion test.

Test	Subculture on HiCrome MeReSa agar and Oxacillin, Cefoxitin and Methicillin D D test					
	Patients isolates			Environment isolates		
Direct culture on HiCrome MeReSa agar	Positive	Negative	Total	Positive	Negative	Total
Positive	47	0	47	11	0	11
Negative	3	66	69	2	19	21
Total	50	66	116	13	19	32
sensitivity			94%			84.60%
specificity			100%			100%
PPV			100%			100%
NPV			95.60%			90.40%

PPV, Positive predictive value; NPV, negative predictive value.

Table 6. Antibiotics resistance patterns of MRSA.

Antibiotic	Disc potency (µg)	MRSA of patients= 50		MRSA of hospital environment=13	
		Resist. No.	Resist. %	Resist. No.	Resist. %
Methicillin	5	50	100	13	100
Cefoxitin	30	50	100	13	100
Oxacillin	1	50	100	13	100
Ampicillin	10	50	100	13	100
Amoxicillin	10	50	100	13	100
Imipenem	10	13	26	2	15.3
Meropenem	10	20	40	5	38.4
Erythromycin	15	33	66	9	69.2
Gentamycin	5	22	44	7	53.8
Tetracycline	30	15	30	5	38.4
Ciprofloxacin	5	21	42	6	46.1
Vancomycin	10	6	12	1	7.6
Clindamycin	2	13	26	3	23

highly variable. All the 63 MRSA strains screened from nasal samples and environment items were resistant to methicillin, oxacillin, cefoxitin, ampicillin, and amoxicillin (100%). In MRSA from patients, resistance to erythromycin, gentamycin, ciprofloxacin and meropenem was (66%, 33/50; 44%, 22/50; 42%, 21/50 and 40%, 20/50; respectively), whereas resistance to tetracycline, imipenem, clindamycin and vancomycin was relatively low (30%, 15/50; 26%, 13/50; 26%, 13/50 and 12%, 6/50; respectively). Results demonstrated the resistance of environment MRSA to antibiotics; erythromycin, gentamycin, ciprofloxacin, meropenem, tetracycline, imipenem, clindamycin and vancomycin were (69.2%, 9/13; 53.8%, 7/13; 46.1%, 6/13; 38.4%, 5/13; 38.4%, 5/13; 15.3%, 2/13; 23%, 3/13 and 7.6%, 1/13; respectively). However, high MRSA isolates tested in this study were recorded sensitive to vancomycin, and all MRSA provided were multidrug resistant MDR (Table 6).

DISCUSSION

Chromogenic agar containing substrates, which changes color in the presence of MRS was used in this study. Selectivity for MRSA is achieved by incorporation of antibiotics into the agar. Use of such agar allows identification of MRSA from primary isolation plates within 24 or 48 h after enrichment, obviating the need for additional biochemical tests (Malhotra-Kumar, 2008). This method may save time and money and provide overall results that are equivalent to the results of the PCR method (Wolk et al., 2009). This is to ascertain the prevalence of MRSA among patients and environment of surgical wards of Al-Hussein teaching hospital using HiCrome agar medium and (OX, FOX and ME) DD test. Ninety-six *S. aureus* isolates were identified. The prevalence of MRSA was 65.6% (63/96) among patients 66.6% (50/75) and hospital environment

61.9% (13/21) respectively. In this study MRSA prevalence was 42.5 (63/148) among all the samples for patients and environment, which is less than that reported in other studies. Kateete et al. (2011) showed that MRSA prevalence in the surgical wards of Kampala was 100 and 46% among *S. aureus*, (Perry et al., 2004) 78%, and (Naseer and Jayaraj, 2010) 77.9% from all *S. aureus* isolates in Indian hospitals. However, result from this study was more than that obtained by Al-Charrakh et al. (2012), who found that 32.3% of clinical *S. aureus* isolates in Al-Dewaniya /Iraq were MRSA, and Al-Khudheiri (2008), who found the prevalence of MRSA was 22.5% of *S. aureus* isolates in Najaf/Iraq.

The result from this study shows higher rates when compared to that from other countries, such as France at 14.5% (Lamy et al., 2012), Taiwan at 6.9% (Kang et al., 2012), Netherlands at 3.1% (Wassenberg et al., 2012), and in Western Europe, the percentage of MRSA among *S. aureus*. Clinical isolates ranged between 5 and 54%, but limited by the different methodologies used in several studies (Dulon et al., 2011). Khadri and Alzohairy (2010) also pointed out that MRSA isolates constituted 54.2% among all *S. aureus* isolates in India.

Regarding the prevalence of MRSA in relation to the age group, the highest rate (36%) was seen in the age group 21 to 41 years, which probably reflects the fact that people at this age group are more involved in different life activity (Table 3 and Figure 2). The results demonstrated higher MRSA prevalence in males (70%) than in the females (30%) which are different than reported by (Kang et al., 2012) who found that 68.4% in female and 52,6% in the age group 30 to 59 years. For comparison, the direct culture on the chromogenic agar is the basic tool to prevent the nosocomial spread of MRSA. MRSA-Select (HiCrome MeReSa Agar) which used in this study was the most rapid (24 h) and the most specific (100%) protocol for patients and environment screening samples.

Although, the Direct culture on MRSA-Select protocol showed slightly lower in sensitivity in both patients and environment screening (94 and 84.6, respectively), but the subculture of *S. aureus* isolates from manitol salt agar on HiCrome agar medium was increased the detection rate of MRSA (from 47/75 to 50/75 and 11/21 to 13/21, respectively). This is in turn, increased the rate of sensitivity in both patients and environment samples, though the report time could be delayed as much as 48 h (Tables 4 and 5). It is likely that the pre-enrichment step enhances the growth of MRSA with low-level resistance (Lee et al., 2008, 2007). The specificity was higher in all MRSA select protocols (100%) as there was no detection of any false positive sample. The specificity reported in the present study is higher than that reported by James et al. (2010).

Each isolate identified in this study as MRSA by DD test, was confirmed by subculture on HiCrome MeReSa agar medium. Results demonstrated similar detection rate of MRSA by subculture on HiCrome MeReSa agar compared with Ox, FOX and Me DD test in patients and environment isolates (50/75 and 13/21, respectively) while prevalence was 42.5 (63/148) for all samples.

In contrast, the direct culture on HiCrome medium showed lower detection rate of MRSA in patients and environment (47/75 and 11/21, respectively) and prevalence 39.1 (58/148) compared with the other two methods. Our result of direct culture was similar to that reported by Kateete et al. (2011).

Tables 4 and 5 shows the sources from which MRSA isolates were isolated and the relative sensitivities and Specificities of the different media for different specimens sources. There was a little difference in sensitivity, specificity, PPV and NPV among three methods for the isolation of MRSA from nasal swabs and environment items swabs (94, 84.6%; 100, 100%; 100, 100%; 95.6, 90.4%; respectively) which was agreed with results of Hoecke et al. (2011). The chromogenic agar gave the best overall results for detecting MRSA from nasal specimens (Denys et al., 2013).

Results in Table 6 and Figure 3 shows that all MRSA (from patients and environment) were multidrug resistant (MDR) and highly resistant (100%) to Beta-lactam antibiotics (oxacillin, cefoxitin, methicillin, ampicillin and amoxicillin). Also other antibiotics like erythromycin, gentamycin, ciprofloxacin, meropenem, tetracycline as imipenem and clindamycin, showed resistance rates 66, 44, 42, 40, 30, 26 and 26%, respectively, but showed much lower resistant to vancomycin. High resistance to Beta-lactam antibiotics can be attributed to the hyper production of Beta–lactamases and the low affinity penicillin binding protein 2a which encoded by mecA gene (Ausubel et al., 1996; Mariana et al., 2001).

In addition, bacteria that were at one time susceptible to an antibiotic can acquire resistance through mutation of their genetic material or by acquiring pieces of DNA that code for the resistance properties from other bacteria (CDC, 2010). MRSA resistance of meropenem and imipenem was lower than other Beta-lactams used in present study in both patients and environment isolates. This can be attributed low exposure because of the limited or little usage of these antibiotics as drug in the Iraqi hospitals.

On other hand, MRSA demonstrated very low resistance (12 and 7.6%) against vancomycin (Table 6). Vancomycin is a glycopeptides antimicrobial, it inhibits bacterial growth by binding to the C terminal end of late peptidoglycan precursors, preventing the effective formation of a bacterial cell wall and therefore, MRSA is sensitive to this type of antibiotics.

Conclusion

Based on the results of this study we conclude that chromogenic agar (HiCrome) medium for MRSA

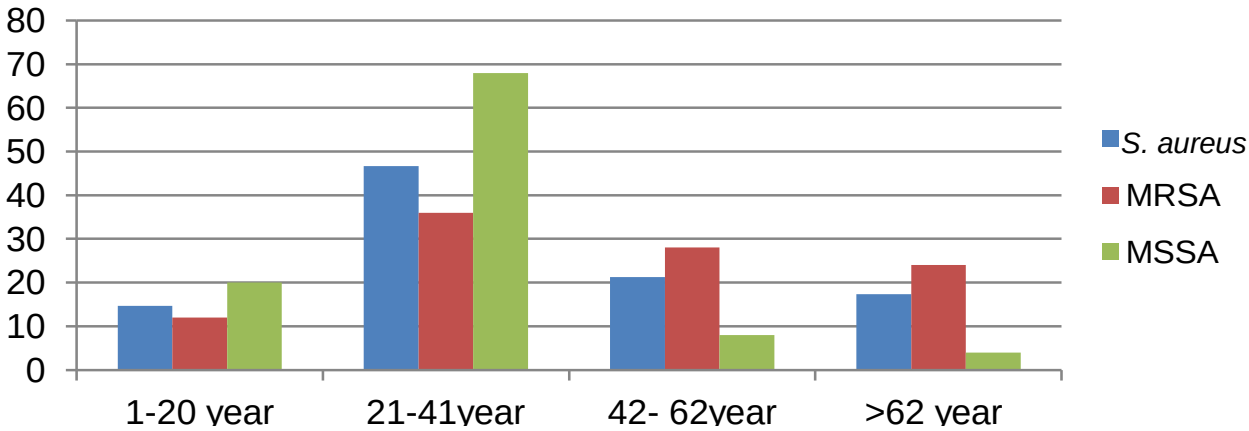

Figure 2. Prevalence of MRSA, MSSA and *Staphylococcus aureus* among different age groups.

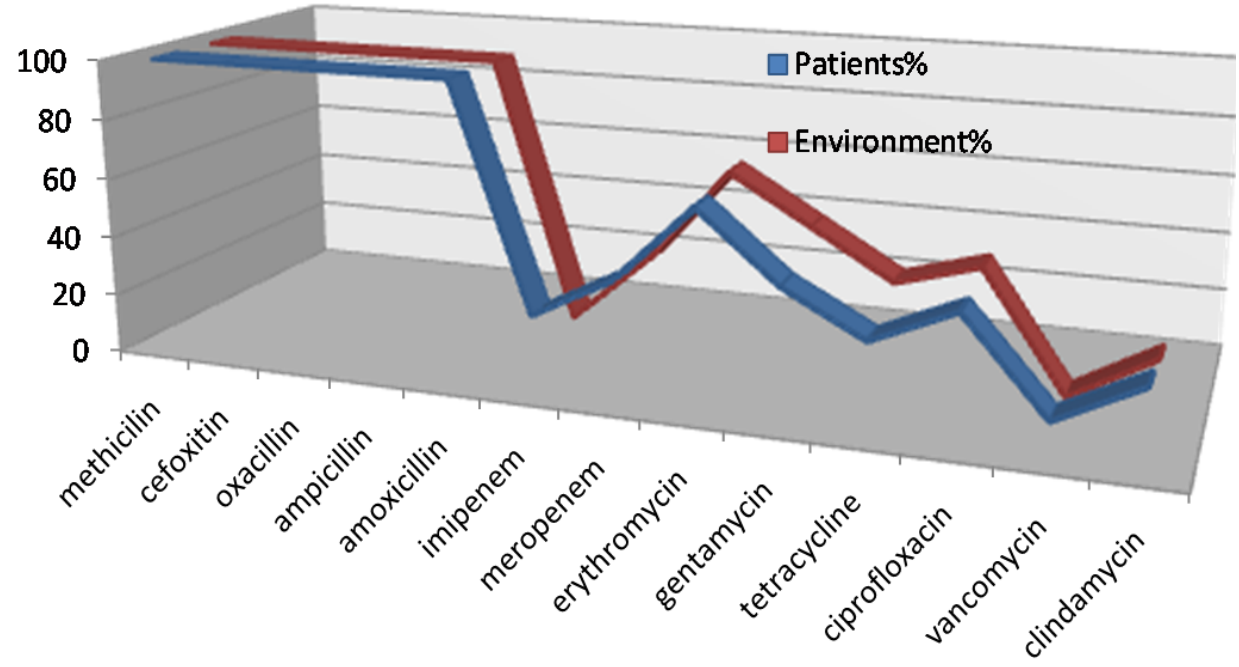

Figure 3. Antibiotics resistance patterns of MRSA.

screening demonstrated a higher sensitivity and specificity for detection of MRSA nasal colonization and hospital environment isolates, in particular by subculture on HiCrome medium, and provided lower time in generating positive and negative results.

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