Cefoxitin screening agar for rapid identification of methicillin-resistant *S. aureus* in healthcare settings

Shaima'a R. Al-Salihy,[1] Nedhal S. Ayoub[2] and Abdul-Razak Sh. Hasan[1]

[1]Department of Microbiology, College of Medicine, Diyala University, Baqubah, Iraq.
[2]Department of Microbiology, College of Medicine, Baghdad University, Baghdad, Iraq.
Correspondence to Shiama'a R. Al-Salihy (email: sh.r802011@gmail.com).
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Objective This study was aimed to explore the validity of Cefoxitin-Mueller Hinton broth for detection of MRSA from different pathological specimens and to explore the susceptibility to β-lactam antibiotics and other selected locally used antimicrobials.

Patients, Materials and Methods The present study was carried out during the period from December 2009 to October 2010. A total of 200 specimens were collected from Baghdad Teaching Hospital and Baquba Teaching Hospital including; 50 specimens from each of hospital inpatients, medical and paramedical staff, and hospitals environments. several types of clinical specimens comprising (burn, wound, conjunctival, ear, nasal, and throat swabs, pleural and ascetic fluids, sputum, urine, and urethral discharge, and nasal swabs). Collected swabs were inoculated in 5 milliliters of Cefoxitin-Mueller-Hinton broth tubes supplemented with 7.5% NaCl (wt. /vol.) and 6μg/ml of cefoxitin and incubated for 24 hours at 37°C. Positive tubes were subcultured on blood agar and Mannitol-salt agar. Suspected colonies were identified by biochemical reactions and Staphytect-Plus test (Oxoid, UK). Susceptibility testing was performed by disk diffusion on Mueller-Hinton agar. Statistical analyses were done using SPSS version 18 computer software (Statistical Package for Social Sciences), and P values < 0.05 were considered significant.

Results The results revealed that 62(31%) isolates were MRSA. The resistant rate to methicillin was significantly higher ($P = 0.004$) among those isolates recovered from inpatients compared to other study groups. All MRSA isolates were found to be multi-drug resistant since, and that (17.7%), (30.6%) of them were resistant to vancomycin and ciprofloxacin, respectively.

Conclusions About one third of *S. aureus* isolates were MRSA with multiple antimicrobial resistances. Thus, mandatory MRSA surveillance system should be implemented in healthcare settings, represented by admission screening of patients using MRSA rapid tests, such as rapid culturing method.

Keywords *s. aureus*, MRSA, CA-MRSA, HA-MRSA

Introduction

Since its emergence in 1961, MRSA represents a major agent of hospital infections worldwide.[1] The homogeneous resistance to all β-lactams, characteristic of MRSA strains, together with the continuous accumulation and organization of many resistance genes, has made the treatment and prevention of this species is particularly difficult.[2] The cause of resistance to methicillin and all other β-lactam antibiotics is the penicillin-binding protein 2α, which is encoded by *mecA* gene, which is situated on a mobile genetic element of staphylococcal cassette chromosome *mec* (SCC*mec*).[3]

For decades, MRSA has been considered the prototype of multi-resistant nosocomial pathogens, causing healthcare-associated (HA) infections in high-risk patients. New lineages of MRSA, defined as community-associated (CA-MRSA), have emerged that have a propensity to cause infections in young individuals without risk factors.[4,5] The prevalence of HA- and CA-MRSA infections continues to increase with excessive morbidity and mortality compared with infection caused by methicillin-sensitive *S. aureus* (MSSA).[6] Hence, rapid and accurate laboratory diagnosis of MRSA is a vital constituent in both implementation of infection control measures and prevention of the nosocomial spread of this microorganism.[7]

Several phenotypic and genotypic methods are used to detect MRSA strains. These techniques vary from an effective culture screening method, through rapid latex agglutination with antibodies directed against PBP2a, to the detection of the *mecA* gene by automated PCR methods.[8–10] Different culture media containing methicillin, oxacillin, and more recently cefoxitin to identify MRSA have been evaluated.[11,12] Multiple studies have reported that cefoxitin-containing media can be alternative to PCR for the detection of MRSA from different clinical specimens.[13–15]

Patients, Materials and Methods

Patients and Samples

A total of 200 specimens were included in this study; 50 specimens from each of hospital inpatients during their residing in Baghdad Teaching Hospital and Baquba Teaching Hospital, outpatients attending the Emergency Wards and Outpatient Clinics, medical and paramedical staff working in the hospitals (HCWS), and hospitals environments for the period from 1st December, 2009 to 30th October, 2010. Trying to be a comprehensive, this study adopts several types of pathological specimens comprising (burn, wound, conjunctival, ear, nasal, and throat swabs, pleural and ascetic fluids, sputum, urine, and urethral discharge, and nasal swabs from HCWs as well as swabs from the environment of hospital setting.

Cefoxitin-Mueller Hinton Broth

Collected swabs were inoculated in 5 ml of cefoxitin-Mueller-Hinton broth tubes supplemented with 7.5% NaCl

(wt. /vol.) and 6 µg/ml of cefoxitin as soon as they arrived to the laboratory, incubated for 24 h at 37°C.[11]

Confirmatory tests for MRSA

All positive test tubes were subcultured on blood agar (Oxoid. UK) and Mannitol-salt agar (Oxoid, UK) to obtain the organisms responsible for growth. Colonies suspected to be *S. aureus* were first identified be means of Gram's stain, catalase reaction, and Staphytect-Plus test (Oxoid, UK).

Susceptibility Testing

Susceptibility testing was performed by disk diffusion on Mueller-Hinton agar, a procedure which was accepted by Clinical and Laboratory Standard Institutes (CLSI), formerly National Committee for Clinical Laboratory Standard (NCCLS), employed it as described by previous research.[16] The antibiotic disks from Bioanalyse Company, Ankara, Turkey were used with the following potencies; ceftriaxone (CRO 30 mg), cefoxitin (FOX 30 mg), Ciprofloxacin (CIP 5 mg), clindamycin (DA 2 mg), erythromycin (E 15 mg), gentamicin (CN 10 mg), methicillin (MET 5 mg), penicillin (P 10 IU), tetracycline (TE 30 mg), trimethoprim (TMP 5 mg), vancomycin (VA 30 mg), the results were interpreted according to the standard zone diameter recommended by other research.[17]

Statistical Analysis

Statistical analyses were done using the Statistical Package for Social Sciences (SPSS) Version 18 computer software. *P* values < 0.05 were considered significant.

Results

The results revealed that among the total 200 different specimens, 62(31%) isolates were MRSA. The resistant rate to methicillin was significantly higher ($P = 0.004$) among those isolates recovered from inpatients compared to other specimens with a prevalence ratio 2.4 (Table 1).

The antimicrobial susceptibility patterns of MRSA isolates revealed that all MRSA isolates were found to be multi-drug resistant since (17.7%), (30.6%) of them were resistant to vancomycin and ciprofloxacin, respectively. (82.3% were resistant to each of gentamicin and clindamycin, and all of them were resistant to methicillin and cefoxitin (Tables 2 and 3).

Regarding the source of MRSA, the results showed that 22 (44%) isolates were recovered from the 50 specimens of inpatients, and 17 (34%) isolates were recovered from the 50 specimens of outpatients. Although it was higher among inpatients, however, the difference was statistically insignificant ($P = 0.068$) (Table 4).

Discussion

Undoubtedly, the importance of the present study is arising from several facts, probably the up most of these is the increasing incidence of the MRSA worldwide coupled with its propensity to cause nosocomial as well as community-associated outbreaks.[18,19] Additionally, such studies are urgently demanded for adoption of strategies that efficiently

Table 2. **Sensitivity rate of *S. aureus* isolates to different antimicrobials.**

Antimicrobial	Sensitive		Resistant	
	No.	%	No.	%
Vancomycin	51	82.3	11	17.7
Ciprofloxacin	43	69.4	19	30.6
Trimethoprim	26	41.9	36	58.1
Erythromycin	16	25.8	46	74.2
Tetracycline	16	25.8	46	74.2
Gentamicin	11	17.7	51	82.3
Clindamycin	11	17.7	51	82.3
Penicillin	2	3.2	60	96.8
Ceftriaxone	2	3.2	60	96.8
Methicillin	0	0	62	100
Cefoxitin	0	0	62	100

Table 3. **Multi-drug resistant *S. aureus* isolates.**

No. of antimicrobials	No. resistant (%)
11	7 (11.3%)
10	8 (14.5%)
9	12 (21.8%)
8	14 (25.4%)
7	11 (20%)
6	3 (5.4%)
5	7 (12.7%)

Table 4. **Hospital versus community source of MRSA.**

Source of specimen	Total No.	No. MRSA (%)	*P* value
Outpatient (Community-associated)	50	17 (34%)	$P = 0.068$
Inpatients (hospital-associated)	50	22 (44%)	

Table 1. **MRSA among different study groups by culture method.**

Study groups	Total no.	Methicillin resistant			Prevalence ratio	95% CI for PR	*P* value
		No.	%	95% CI for RF			
Environmental swabs	50	9	18	(9.1–31.9)	Reference		
Healthcare workers	50	14	28	(16.7–42.7)	1.6	(0.7–3.3)	0.23 [NS]
Outpatients	50	17	34	(21.6–48.9)	1.8	(0.9–3.8)	0.068 [NS]
Inpatients	50	22	44	(30.3–58.7)	2.4	(1.3–4.8)	0.004 [S]

combat MRSA infections and decrease the enormous burden caused by these multi-virulence pathogen and may significantly enhance the efficiency of MRSA management.[20]

In the present study, cefoxitin was incorporated with Mueller-Hinton broth and used as an MRSA screening broth. Cefoxitin screening broth has been used by several previous studies which affirmed that cefoxitin was more efficient than Oxacillin for detection of MRSA from different pathological specimens.[14,21] Moreover, it has been suggested that cefoxitin screening broth can be used as an alternative to PCR for detection of MRSA in resource constraint settings.[15] The results of culture method revealed that the majority of MRSA isolates were recovered from hospitalized patients, which was significantly higher as compared to other study groups ($P = 0.004$). Deeply in this context, it was found that the relative risk to acquire MRSA infection among inpatients was 2.4 times more than that in other groups. Actually, these results were not unusual since similar results have been reported by previous studies.[13,22]

The infection rate by MRSA among outpatients was come in the second priority with a prevalence ratio of 1.8. Although the infection rate was high compared to the reference, it was failed to reach the levels of statistical significance ($P = 0.06$). Furthermore, recent interest has focused on the changing epidemiology of CA-MRSA, since it is now seen outside of the initial specific population groups, and the community strains were beginning to spread back into hospitals.[5,18] However, several researchers have suggested that the distinction between the HA-MRSA and CA-MRSA is going to fade, and each of them may serve as a "reservoir" for another.[23] The healthcare workers are forming another ring in the chain of MRSA spread. Several studies have documented that HCWs play a prominent role in the dissemination of MRSA in and out the healthcare settings.[24] In the present study, the carrier rate of MRSA among the HCWs was found to be 28%. Of note, previous studies conducted in Iraq have reported a rate of *S. aureus* carriage among HCWs was around 30%.[25]

The current results also revealed that 18% of the MRSA were originated from the hospital environment. These results are consistent with previous reports documented the detection of MRSA stains from various environmental surfaces and fomites in healthcare facilities, including the stethoscopes.[26] Actually, if we considered the three former sources as mobile reservoirs for MRSA, the hospital environment constitute the constant source, since MRSA strains were capable of surviving for days to weeks on environmental surfaces in healthcare settings.[27] In our hospital environment, an effective control measures to reduce the rate of contamination by MRSA are recommended. These measures should include implementation of efficient screening program for patients at admission, active surveillance system for detection and management of HCWs carriers, and an effective and continual program for cleaning and disinfection of hospital's environment.

Twenty-two (44%) of MRSA isolates were hospital-associated, while, 17 (34%) were community-associated. Although the rate of detection of MRSA was higher from inpatients compared to those isolated from outpatients, the difference between the two groups was insignificant. These results are consistent with most of the previous studies conducted in this field that documented a higher rate of MRSA among inpatients.[5,18,23] However, on the contrary few studies have reported a higher detection rate of MRSA from community compared to hospitals.[28] The elevated rate of MRSA among inpatients seems more logic as hospitalized patients are under high risk for acquisition of MRSA infection during their hospital admission. In this context, the risk factors for MRSA colonization at admission included: hospital admission in the past year, more than 2 admissions, a hospital stay of 5 days or more chronic underlying diseases, and isolation of MRSA in the past 6 months.[29]

Regarding the antimicrobial susceptibility patterns of MRSA isolates, it was found that 82.3% of these isolates were sensitive to vancomycin also showed multiple drug resistance; therefore, they may be considered as VRSA. This result is consistent with previous Iraqi study, which found that 90% of MRSA were sensitive to vancomycin.[30] Several studies have documented a reduced sensitivity of MRSA to vancomycin.[8,22,31] On the contrary, other studies have reported that vancomycin has retained its activity against MRSA.32 The relatively high sensitivity rate of local strains of MRSA to vancomycin obtained in the present study may be due to the limited use of vancomycin in our clinical practice and healthcare settings. The results also showed that the second most effective antimicrobial agent against MRSA was Ciprofloxacin (sensitivity rate 69.4% and the resistant rate 30.6%). The present result is in agreement with most previous studies which documented low levels of resistance of MRSA to Ciprofloxacin; on the contrary other studies have reported a high resistance rate against Ciprofloxacin.[28,33]

The results also showed that all MRSA isolates were resistant to cefoxitin. These results are not unusual since cefoxitin is often grouped with the second generation cephalosporins, and that staphylococci resistant to methicillin/oxacillin should be considered resistant to cefoxitin.[13,28] On the other hand, two isolates were appeared sensitive to penicillin. These probably related to those isolates which are non-genetically determined, i.e. those lack the *mecA* gene. However, they should be considered as resistant to all β-lactams even if they showed susceptibility *in vitro*, because the mechanism, PBP2a production, results in cross-resistance for the class.[34] It is clearly obvious that all MRSA isolates enrolled in the present study are multi-drug resistant, and this is one of the fascinating results obtained.

Conflict of Interest

None.

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