

Take Care MRSA is in the Neighborhood

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Abstract Objective: The aim of the study was to: (i) evaluate the evidence for the emergence of MRSA in our community (ii) to evaluate the antibiotic profile of the isolated CA_MRSA strains .
Material and Design: The clinical specimens were purulent exudates from different forms of suppurative skin lesions that were processed for isolation of *S. aureus*./ **Setting:** The samples were obtained from 200 patients attending the outpatient clinic of the Dermatology Department in the Main University Hospital of Alexandria, Egypt./ **Subjects:** All samples were inoculated on to the surface of: blood agar, oxacillin supplemented CHROMagar Staph aureus (CSA+), and oxacillin-supplemented Mueller Hinton agar (MH+). Plates were examined after 24 hours and discarded as negative after 48 hours. All staphylococcal colonies isolated on each of CSA +and MH+ were subjected to antibiotic susceptibility testing by the single disc diffusion method using: oxacillin, ciprofloxacin, vancomycin, gentamicin, erythromycin, clindamycin trimethoprim, and sulfamethoxazole .**Results:** The most common bacteria isolated was *S. aureus*, isolated from 81.04% of the studied samples, where 45 (26.32 %) were CA-MRSA. The sensitivity to detect CA-MRSA after 24 h by CSA+ was 73.33%. Prolonging the incubation period to 48 h improved the sensitivity to 95.56 %. The sensitivity of MH+ after 24 h was 68.89%, increased to 80 % after 48 h incubation. Multi-drug resistant strains of the isolated CA-MRSA represented 17.78%. **Conclusions:** Further evaluation of CHROMagar Staph aureus with direct clinical specimens is needed before this medium can be used for routine direct screening for MRSA. Though the aim of selective and differential media for isolation of MRSA was to reduce the time and work load needed for its full identification when using ordinary media (which is 48 h ,(unfortunately 48 hours were required to increase the sensitivity of both CSA+ and MH+. So their use needs to be re-evaluated regarding cost, incubation time and performance. Empirical treatment should be guided by antibiotic susceptibility results due to the emergence of MRSA skin infection in the community.

KEY WORDS: Staphylococcus aureus; CA-MRSA; Oxacillin; CHROMagar Staph aureus; Oxacillin supplemented Mueller Hinton agar; Multidrug resistance

INTRODUCTION

Antimicrobial resistance has become such a growing global problem that, according to the Institute of Medicine, it may be a "paramount microbial threat of the twenty-first century."¹ Resistance has produced an increased burden of illness, longer hospitalization, excess deaths, and greater health care costs.²⁻³ Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the most important pathogens in terms of increasing prevalence and impact of nosocomial infection predominantly in

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immunocompromised patients. In recent years, however, infectious disease experts have noted an emergence of infections not associated with hospitalization, often referred to as community-acquired (CA) MRSA. Its incidence has risen dramatically in the past decade.⁴⁻⁵ In contrast to hospital- (or health-care-) acquired MRSA, CA-MRSA has a number of unique characteristics and may present an even greater threat to public health and a more significant challenge to clinicians.⁽⁶⁾

The majority of CA-MRSA cases are skin and soft tissue infections. Ragan⁽⁷⁾ mentioned that CA-MRSA skin infection is considered a rapidly emerging public health problem.

Gadage,⁽⁸⁾ reported that if the number of infections with CA-MRSA isolates significantly increased, it will force us to change our treatment of presumptive *S. aureus* infections, relying on clindamycin and vancomycin instead of β -lactams and that this necessitates a need to begin

surveillance for these strains.

Several classical methods have been used to detect the MRSA isolates including the 1 μ g/mL oxacillin disk diffusion, agar plate screen, agar dilution and the E'tests.^(9,10,11)

Now, chromogenic media incorporating chromogenic enzymatic substrates and a variety of antimicrobial agents are available for detection of *S. aureus*, including methicillin-resistant strains.^(12,13)

Some of the evaluations of these chromogenic media involved only stored collections of isolates, or included a relatively small number of clinical specimens⁽¹³⁾, while others found the adapted media to be effective for the growth of multidrug-resistant MRSA strains but less effective for the growth of community-acquired MRSA strains.^(14,15)

So this study aimed to estimate the presence of MRSA in the community, to compare the recovery of CA-MRSA strains on Chromagar Staph aureus with that on

oxacillin supplemented Mueller Hinton agar, and to evaluate the antibiotic profile of the isolated CA-MRSA strains.

Subjects and methods

The present study was conducted during a 6 months' period from July to December 2005. The study involved 200 pyoderma patients (104 females and 96 males) after obtaining informed consent, with ages ranging from 5 months to 60 years, attending the outpatient clinic of Dermatology Department in the Main University Hospital of Alexandria, Egypt.

All patients were clinically suffering bacterial skin infections with the following exclusion criteria: hospitalization, surgery, dialysis, indwelling line or catheter or admission to a long – term care facility in the 12 months before infection (to exclude any hospital acquired infection).

Sterile swabs were used for collecting all samples.⁽¹⁶⁾

- Folliculitis, cellulitis and erysipelas were vigorously rubbed with a sterile

swab.

- Exudates of crusty lesions (impetigo or small pustules) were collected from beneath the scab with a sterile wet swab.
- Furuncles and carbuncles were sampled by swabbing the purulent material from the deeper portions of the ulcers; if they were oozing or when incised.

Each swab was inoculated directly onto the surface of each of the following plates

1. Columbia agar plates with 5% blood and incubated for 24 h at 35°C.
2. CHROMagar Staph aureus (CHROMagar Company, Paris, France) with 4.0 mg/liter oxacillin (CSA+). The medium contained agar (15 g/liter), peptones (40 g/liter), NaCl (25 g/liter), and a proprietary chromogenic mix (3.5 g/liter). The medium was prepared as instructed by the manufacturer by avoiding heating at over 100°C. Oxacillin (4 µg/ml) was

added when the agar was cooled at 48°C. Each plate contained 20 ml of agar medium dispensed into 90-mm-diameter Petri dishes.

3. Mueller Hinton agar (Difco Laboratories, Detroit, Mich.) supplemented with 6.0mg/liter oxacillin and 4% NaCl (MH+).⁽⁹⁾ CSA+ and MH+ plates were aerobically incubated at 35°C, they were examined after 24 hours and discarded as negative after 48 hours.

Identification of colonies

Any bacterial growth obtained on the surface of blood agar plates was identified according to the method described by Forbes et al.,⁽¹⁷⁾

Regarding CSA+, according to the manufacturer instructions, the growth of colonies showing any pink or mauve coloration was considered to be positive (indicating MRSA). Regarding MH+, if any growth was detected, the isolate was considered oxacillin resistant.⁽⁹⁾ All isolated

colonies on CSA+ and MH+ were subjected to Gram stain, catalase, slide and tube coagulase tests to verify *Staph. aureus*. While methicillin or oxacillin resistance was confirmed by the detection of penicillin-binding protein 2a (PBP 2a) expressed by the *mecA* gene, using the oxacillin 1 µg disc diffusion test.⁽⁹⁾

4- Antibiotic susceptibility testing:

All staphylococcal colonies isolated on each of CSA+ and MH+ plates were subjected to antibiotic susceptibility testing by the single disc diffusion method,⁽⁹⁾ using the following antibiotic discs: ciprofloxacin, vancomycin, gentamicin, erythromycin, clindamycin, trimethoprim, and sulfamethoxazole.

Results:

The bacterial profile of pyoderma is summarised in Table 1. *Staph. Aureus* represented 171(81.04%) of the 197 (93.37%) Gram positive isolates. Gram negative isolates represented 14 (6.63%) of all isolates.

Table (1): The Bacterial Profile of the Studied 200 Pyoderma Patients.

Isolate	No.	%
<u>Gram +ve organisms</u>	197	93.37
<i>S.aureus</i>	171 [∞]	81.04
<i>S.saprophyticus</i>	10	4.74
<i>S.epidermidis</i>	7	3.32
<i>S.pyogenes</i>	7	3.32
<i>Bacillus spp.</i>	2	0.95
<u>Gram –ve organisms</u>	14	6.63
<i>P.mirabilis</i>	6	2.84
<i>E.coli</i>	6	2.84
<i>K.pneumoniae</i>	1	0.475
<i>P.aeruginosa</i>	1	0.475
Total	211*	100.00

*The number is not exclusive to cases, where 11 cases showed mixed infection.

[∞] 45 isolates proved to be MRSA on using 1µg oxacillin disc diffusion.

Total isolated CA-MRSA strains on both used culture media (CSA+, MH+) that were correctly identified by disk diffusion test using 1µg oxacillin disc were 45 strains. The sensitivities and specificities for both CSA+ and MH+ after 24 and 48-h incubations are summarized in Table 2 A,B

and 3 A,B. It is apparent that the sensitivity of both CSA+ and MH+ for detection of CA-MRSA increased from 73.33% and 68.89% to 95.5% and 80% respectively, with prolonging the incubation period to 48 hours while the specificity was not affected,

Table 2,3: Comparative efficiency of CSA+ and MH+ for isolation of CA-MRSA 2A- After 24 hours incubation

CSA+ Oxacillin disc	+	-	Total
+	33	12	45
-	0	126	126
Total	33	138	171

Sensitivity = 73.33%

Specificity =100%

2B- After 48 hours incubation

CSA+ Oxacillin disc	+	-	Total
+	43	2	45
-	0	126	126
Total	43	128	171

Sensitivity = 95.5%

Specificity =100%

Table 3 Isolation Rate of CA-MRSA on MH+ After: A-24, B-48 Incubation

Hours 3A- After 24 hours incubation

MH+ Oxacillin disc	+	-	Total
+	31	14	45
-	0	126	126
Total	31	140	171

Sensitivity =68. 89%

Specificity =100%

3B- After 48 hours incubation

MH+ Oxacillin disc	+	-	Total
+	36	9	45
-	0	126	126
Total	36	135	171

Sensitivity = 80%

Specificity =100%

Regarding antibiotic sensitivity, the isolated 45 CA-MRSA strains were susceptible to trimethoprim (75%), sulfamethoxazole (32%), clindamycin (75%), ciprofloxacin (76%), erythromycin (20%), and gentamycin (15%), while all were vancomycin sensitive (100%), Table 4. Moreover, 29 (64.44%) isolates were resistant to methicillin in addition to another antibiotic group, where 8 (17.78%) were resistant to two antibiotic groups in addition to methicillin, 5 (11, 11%) were resistant to three antibiotic groups other than methicillin, while 3 (6.67%) were resistant to methicillin and another four antibiotic groups. Table 5

Table 4: Antibiotic Susceptibility of 45 CA-MRSA Strains.

Antibiotic	Susceptible %
Trimethoprim	75
Sulfamethoxazole	32
Gentamicin	15
Erythromycin	20
Clindamycin	75
Vancomycin	100
Ciprofloxacin	76

Table 5: Multidrug Resistance Profile among the 45 CA-MRSA Strains

Resistance to antibiotic groups	No.	%
Methicillin+1group	29	64.44
Methicillin+2groups	8	17.78
Methicillin+3groups	5	11.11
Methicillin+4groups	3	6.67
Total	45	100.00

DISCUSSION

Dermatologists and other healthcare providers need to be aware of the epidemiology, clinical features, management, and prevention of CA-MRSA infection. Currently, infection caused by CA-MRSA is considered to represent a worldwide epidemic and infectious skin lesions are a frequent occurrence.⁽⁸⁾

As the emergence of MRSA in the community is a warning, it is imperative that MRSA be identified quickly and accurately.⁽¹⁸⁾

The accurate diagnosis of MRSA in microbiology laboratories is vital for patients' management. It is also essential

for meaningful interpretation of surveillance data. Currently surveillance data for MRSA are difficult to interpret, because there is no uniform testing method for detection of MRSA, and laboratories vary in their standard operating procedure and interpretation of breakpoint values.⁽¹⁹⁾ CHROMagar Staph aureus is a chromogenic medium designed to enable detection of colonies of *S. aureus* when 4.0 mg/liter of oxacillin are added to this medium, it can detect MRSA by their pink color.⁽²⁰⁾ The good visibility of pink colonies on CSA facilitates the recognition of potential *S. aureus* isolates and thus

increases the detection rate. So the use of chromogenic media can potentially reduce the number of confirmatory tests and achieve isolation and presumptive identification in a single step.⁽¹⁴⁾

In the present study CSA+ was tested for its capacity to screen for CA-MRSA obtained from patient suffered purulent skin infection, after 24 and 48 incubation hours. The sensitivity to detect CA-MRSA after 24 h was 73.33%. Prolonging the incubation period to 48 h improved the sensitivity to 95.56 %. The specificity was 100% and was not affected by a prolonged incubation period as no false positive colonies with mauve colour were obtained.

Han et al.,⁽²¹⁾evaluated CSA for detection of MRSA from nasal swabs, where they found its ability to detect MRSA at 24 h (89.72 %) and at 48 h (94.9%) and that it is a highly specific (100 %) media for detecting MRSA from nasal swab specimens and these results seems to be near to ours.

Kluytmans et al.,⁽¹⁵⁾ when used a well-defined collection consisting of 1,140 staphylococci, reported that the sensitivity of CSA was lower after 24 h (58.6%); and increased significantly after 48 h reaching up to 77.5%. But they discovered that, the specificity was high after 24 h (99.1%) and decreased significantly after 48 h of incubation (94.7%) due to marked increase in the false positive results due to coagulase negative staphylococci.

Merlino et al.,⁽¹⁴⁾ on there evaluation to CSA they found that multi-drug-resistant MRSA (HA-MRSA) strains were reliably detected on the medium (100%) with similar color changes, and all were positive for PBP 2a. However, non-multi-drug-resistant CA- MRSA grew inconsistently on the chromogenic medium where only 4 of 12 (30%) such isolates grew on the supplemented CHROMagar.

This seems to be consistent with Kluytmans et al., ⁽¹⁵⁾ findings that a substantial proportion of MRSA strains did

not grow on CSA supplemented with 4.0 mg of oxacillin or methicillin per litre. Merlino et al.,⁽¹⁴⁾ said that the cause of these organisms' growth anomaly on the test chromogenic medium remains unclear but may reflect active cotransportation of methicillin intracellularly with the chromogenic moiety. In the presence of methicillin or oxacillin, the chromogenically linked substrates may affect the cell membrane potential during permeation, leading to nonspecific membrane disorganization or induced cell death. In our study CSA+ failed to detect 12 CA-MRSA strains after 24 incubation hours and this high false negative rate was much reduced on prolonging the incubation to 48 h where only 2 MRSA strains were still not detected.

Mueller Hinton agar with 6 µg of oxacillin per ml supplemented with 4% NaCl (oxacillin agar screen) was recommended by Clinical Laboratory Standards Institute (CLSI) for the detection

of oxacillin resistant *S. aureus*, as literature had indicated that this method may be able to detect mecA-mediated oxacillin resistance in these strains.⁽¹⁰⁾

Accurate detection of methicillin resistance in *S. aureus* by routine methods such as Mueller Hinton agar is difficult due to the presence of two subpopulation of *S. aureus* (i.e. one susceptible and other resistant) which may coexist within a culture. All cells in culture may carry the genetic information for resistance but a small numbers can express this kind of resistance in routine susceptibility testing performed in the laboratory. This phenomenon is termed heterogeneous resistance and occurs in staphylococci resistant to penicillinase stable penicillin such as oxacillin.^(22,23)

The oxacillin agar screen test has been evaluated the most thoroughly. In studies performed since 1990 that used the presence of the mecA gene as the gold standard, the sensitivity of the agar screen

test for the detection of resistant strains was excellent.^(24,25)

Griethuysen et al.,⁽²⁶⁾ tested 267 MRSA strains that were all *mecA* gene-PCR positive, where 17 did not grow on oxacillin agar screen and this was associated with a sensitivity of 93.6%, and a specificity of 100%.

Sakoulas et al.,⁽²⁷⁾ mentioned that the oxacillin agar screen identified 201 of 203 *mecA*-positive isolates, corresponding to a sensitivity of 99%. It yielded 2 false-positive results for 107 methicillin sensitive *S. aureus* isolates tested for a specificity of 98.1%. While Yamazumi⁽²⁴⁾ found that oxacillin agar screen test had sensitivity and specificity both of 98.0%,

However, Cavassini et al.,⁽²⁸⁾ noted that when very heteroresistant strains were tested, the sensitivity decreased. In their study oxacillin-salt agar screening test showed a sensitivity of 82.5% and a specificity of 98.3%, respectively In the present study MH+ was tested for its

capacity to detect CA-MRSA from purulent skin infection, after 24 and 48 incubation hours. The sensitivity after 24 h was 68.89%. Though none of the literatures recommended prolonging the incubation period of MH+ to 48 h, we extended the incubation of MH+ plates to 48 h and this markedly raised the sensitivity to 80 % as this gave a chance to 5 more MRSA isolates to appear. The specificity was 100% and was not affected by prolonging the incubation period.

Although there is some over lap between HA and CA-MRSA strains, the current CA-MRSA strains generally remain more susceptible to classes of antimicrobials other than β -lactams, but CA-MRSA strains seen nowadays may be more virulent; patients may therefore present with more severe manifestations of infection. So culture and proper identification is important.^(29,30)

Vouillamoz et al.,⁽³¹⁾ stated that β -lactam drugs consisting of cephalosporins and

penicillins remained the most commonly prescribed therapy for skin and soft tissue infections and that the rate of use of cephalosporins increased over the 12- year study period although the infecting isolate was resistant to the agent prescribed in about 57% of patients.

Bogdanovich et al., (2005)⁽³²⁾ reported that because most skin and soft tissue infections were treated in outpatient settings with empiric antimicrobial therapy, few studies have attempted to estimate the number of *S. aureus* skin and soft tissue infections, and none have evaluated the antimicrobial drugs prescribed for these conditions. Now clinicians must take into account local and regional rates of CA-MRSA and consider the use of agents such as clindamycin or trimethoprim - sulfamethoxazole in the empiric treatment of skin and soft tissue infections.^(33,34)

This is why we investigated the antibiotic resistance profile of the isolated

45 CA-MRSA strains to find much variation in their response to the different antibiotics used. They were susceptible to ciprofloxacin (76%), trimethoprim and clindamycin (75 %), sulfamethoxazol (32%), erythromycin (20%), gentamycin (15%) and all isolates (100%) were vancomycin sensitive. It should be noticed that patients of the present study were empirically treated using erythromycin where 80% of the isolated CA-MRSA strains showed resistance.

A lot of literatures have tested for antibiotic resistance among CA-MRSA and marked variation in antibiotic profile was recorded and it should be worthy mentioned that none of the isolates proved to be vancomycin resistant.^(35,36,37) CA-MRSA carry virulence genes encoding a leukocyte-killing toxin called the Pantone-Valentine Leukocidin determinant which differentiate CA-MRSA from HA-MRSA by their susceptibility to most antimicrobial

drugs other than the β -lactam agents. These susceptibility patterns are dynamic and may vary markedly by region.^(38,39)

O'Brien et al.,⁽³⁷⁾ reported that MRSA that were resistant to ≥ 3 of antimicrobial drug groups of different classes were defined as multi-drug resistant MRSA (mMRSA) and those resistant to < 3 drug groups were defined as non-multi-drug resistant MRSA (nmMRSA). Sattler et al., (2002)⁽³⁸⁾ defined mMRSA as any MRSA which developed resistance to two or more antibiotics above the natural resistance profile. Moreno et al., (1995)⁽³⁹⁾ defined mMRSA as resistant to methicillin, cephalosporins, all β -lactams, occasionally gentamycin, erythromycin, and trimethoprim / sulphamethoxazole .

John (2003)⁽⁴⁰⁾ defined CA-MRSA as sensitive to all tested antibiotic groups except for methicillin. Regarding the 45 CA-MRSA of the present study, this definition was not applicable, where on 1y (64.44%) of these isolates were sensitive

to all other antibiotic categories tested. Indeed 8 (17.78%) isolates were resistant to ≥ 3 antibiotic categories, meaning that they were mMRSA.

Gorak et al., (1999)⁽⁴¹⁾ also recorded 22.5% of his CA-MRSA isolates as mMRSA. Even higher results were reported by Binh et al.,⁽⁴²⁾(88.5%), and Sook et al.,⁽⁴³⁾ 80% .

Chromagar Staph aureus medium could be used for isolation of CA-MRSA from clinical specimens. Though the aim of using selective and differential media for isolation of MRSA was to reduce the time and work load needed for its full identification when using ordinary media (which is 48 h).

Unfortunately in the present study we needed 48 hours to increase the sensitivity of both CSA+ and MH+. So their use needs to be re-evaluated regarding cost, incubation time and performance. Treatment of pyoderma should be guided by antibiotic susceptibility test results due

to the emergence of MRSA skin infection in the community.

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