

Screening of Nosocomial Methicillin-Resistant *Staphylococcus aureus* (MRSA) in The Intensive Care Units of Assiut University Hospital

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Background: *Staphylococcus aureus* is a major cause of nosocomial infections that uses numerous virulence factors, such as extracellular toxins and enzymes. Epidemic Methicillin-resistant *S. aureus* (MRSA) strains were described as multi-resistant strains with special capacity to colonize patients and staff and cause widespread outbreaks of infections. Rapid identification of MRSA from clinical specimens and screening of high risk patients for MRSA colonization have been found to be cost effective measures for limiting the spread of the organism in hospitals.

Objectives: The aim of this study was screening of MRSA infection in patients admitted to the Internal Medicine Department in Assiut University Hospital, using oxacillin resistance agar screen base (ORSAB) and MRSA screen test and to compare the antibiotic susceptibility pattern of MRSA infected patients with those of house-hold contacts and paramedical staff to evaluate their role in transmission of MRSA.

Methods: The present study included 455 patients with nosocomial infections admitted to the ICU of Neurology and Chest Departments and the Diabetic Foot Care Unit. 154 nurses and workers in these units as well as 110 healthy volunteers who were household contacts of the patients with *S. aureus* infections were also included. Swabs were collected from the anterior nares of all studied groups and from the bed sores of patients who developed nosocomial beds sores. Sputum samples and endotracheal aspirates were also collected from patients who developed nosocomial pneumonia. Pus samples were collected from post-operative infected wounds of diabetic foot patients. All samples were cultured on mannitol salts agar. Colonies were identified by growth characteristics, Gram staining, biochemical reactions and confirmed by coagulase tube test. Coagulase positive mannitol-fermenting colonies were subcultured on ORSAB medium then subjected to MRSA screen test to detect PBP2a. Sensitivity patterns of the isolated strains were detected by Kirby and Bauer technique.

Results: It was found that 24% of the patients (110/455) were infected nosocomially with *S. aureus*. 60 patients were infected with MRSA (55% of *S. aureus* infected patients and 13% of the whole patients). MRSA nasal colonization was reported in 17.5% of the patients (80/455), 5.2% of paramedical staff (8/154) and 18% of the household contacts (20/110). The results of antibiogram showed that MRSA strains remained sensitive to ciprofloxacin, rifampin, gentamycin and vancomycin with the highest sensitivity obtained by ciprofloxacin.

Conclusion: MRSA strains of patients and paramedical staff had the same antibiogram type while slightly different from those of their household contacts denoting that the paramedical staff had a major role in transmission of MRSA.

Clinical implications: Regular screening of patients gives an early warning of the presence of MRSA and assess the efficiency of barrier and application of basic infection control measures (standard precautions). Prevention is better than treatment, but for those who are already affected, control is the most likely achievable goal rather than eradication to prevent cross transmission. We recommend the use of molecular methods for accurate and rapid typing of MRSA.

INTRODUCTION

Staphylococcus aureus is one of the most important bacterial pathogens seriously contributing to the problem of nosocomial infections all over the world⁽¹⁾.

It was found that some strains of *S. aureus* quickly developed resistance to penicillin by producing B-lactamase enzyme, which could breakdown the penicillin molecule. A number of synthetic derivatives of penicillin, resistant to B-lactamase enzyme,

were developed. Methicillin became the standard treatment for penicillinase producing *S. aureus*⁽²⁾. In 1961, the first methicillin-resistant strains of *S. aureus* (MRSA) were isolated in Europe. Until the early 1980s MRSA reports consisted of isolated cases, later in 1982, epidemic MRSA strains were described as multi-resistant strains with special capacity to colonize patients and medical staff and cause widespread outbreaks of infections⁽³⁾.

Penicillin binding proteins (PbPs) in the bacterial cell wall have an enzymatic role in the synthesis of peptidoglycan. B-lactam antibiotics such as methicillin inactivate PBP1,2 and 3 by acylation of the catalytic site of PBP. Normally, PBPs possess a high affinity for B-lactam antibiotics but in MRSA this affinity is reduced resulting in antibiotic resistance. MRSA carry the *mec A* gene which encodes an additional low-antibiotic affinity PBP, known as PBP2a⁽⁴⁾.

Rapid identification of MRSA from clinical specimens and screening of high risk patients for MRSA colonization have been found to be cost effective measures for limiting the spread of the organism in hospitals⁽⁵⁾.

The outburst of infections caused by MRSA created obstacles for physicians and infection control personnel. The main problem was failure of treatment due to multiple resistances of these strains. MRSA infections are usually treated with vancomycin, a toxic and expensive antibiotic. It is, however, feared that other gram positive cocci groups, which have developed vancomycin resistance, such as the enterococci, may transmit the gene(s) responsible for this property to *S. aureus*, leaving few, if any, options for antimicrobial chemotherapy⁽⁶⁾. In fact, *S. aureus* isolates that exhibit increased resistance to vancomycin has been reported in the USA and Japan^(7,8).

Epidemiologically, MRSA is introduced to hospital settings through different routes, most commonly through a patient infected with MRSA who serves as a singular *S. aureus* infection reservoir⁽⁹⁾. Cross infection involving hospital staff is another mode of transmission⁽¹⁰⁾.

Due to difficulties created by inability to control the spread of the organism in an outbreak and to treat the patients infected by it, efforts must be made to detect MRSA eruptions early and to promptly implement infection control measures that may limit the consequences of a flare-up⁽¹¹⁾.

The aim of this study was screening for MRSA infection in patients admitted to the Internal Medicine Department in Assiut University Hospital, using ORSAB and MRSA screen test and to compare the antibiotic susceptibility pattern of MRSA infected patients with those of house-hold

contacts and paramedical staff to evaluate their role in transmission of MRSA.

SUBJECTS, MATERIAL AND METHODS

Subjects:

This study included 455 patients admitted to 3 units, the ICU of Neurology and Chest Dept. and the unit of diabetic foot care of Assiut University Hospital from Jan. 2004 to Jan. 2005. The patients enrolled in this study were monitored daily until discharge for subsequent development of nosocomial infection (bed sores, pneumonia, wound infection, etc.). 154 nurses and workers in these three units as well as 110 healthy volunteers in contact to the patients were also included.

Material and Methods:

- **Specimen collection:** Swabs were collected from the anterior nares of patients, paramedical staff and control group, and from the bed sores of patients who developed bed sores more than 72 hours after admission. Sputum samples and endotracheal aspirates were collected from patients who developed nosocomial pneumonia. Pus samples were collected from post-operative infected wounds of diabetic foot patients.
- **Identification of MRSA:** All samples were inoculated on mannitol salt agar (MSA) (BioMerieux) and incubated at 37°C for 48 hrs. *S. aureus* colonies were identified by growth characteristic on MSA (yellow colonies), and Gram staining, then confirmed by coagulase tube test⁽¹²⁾. Coagulase positive mannitol fermenter colonies were inoculated on oxacillin resistant agar screen base (ORSAB) (Oxoid, England). The medium contained 5.5% NaCl, Oxacillin at 2 mg/L, polymyxin B 50,000 IU/L, mannitol sugar and aniline blue dye as indicator. The plates were incubated at 37°C and examined at 24 and 48 hrs for mannitol fermenting colonies which change the colour from colourless to intense blue⁽¹³⁾. MRSA screen test (Denka Seiken Co.) was done on all positive growth (intense

blue colonies) on ORSAB to detect PBP2a associated with oxacillin resistance in staphylococci. It is a rapid (20 min) slide agglutination test based on the reaction of latex particles sensitized with monoclonal antibodies against PBP2a of *S. aureus* and PBP2a extracted from tested colonies. To extract PBP2a from the tested colonies, a loopful of cells was suspended in 4 drops of extraction reagent 1 and the suspension was placed in a heating block (>95°C) for 3 min. After allowing the suspension to cool (±10 min), 1 drop of extraction reagent 2 was added and the mixture was vortexed then centrifuged at 1.500 xg for 5 min. Then 50 ul of supernatant was mixed with 1 drop of latex for 3 min with a shaker⁽¹⁴⁾.

- **Antibiogram typing:** The isolated Staphylococcal strains were tested for resistance to antimicrobial agents by performing disc diffusion method using commercial discs (BioMerieux) according to the guidelines of the National Committee for Clinical Laboratory standard⁽¹⁵⁾. The following antimicrobial

discs and concentrations were used; oxacillin (1 ug), clindamycin (2 ug), erythromycin (15 ug), tetracycline (30 ug), vancomycin (30 ug), ciprofloxacin (5 ug), gentamycin (10 ug), and rifampicin (30 ug)^(11,16). The test was done by using diluted pur broth cultures (10⁵ cell/ml) Spread on the surface of Muller Hinton agar with 4 mm depth by a sterile swab (Kirby-Bauer Method)⁽¹⁷⁾.

RESULTS

This study included 455 patients with nosocomial infection admitted to the ICUs of Neurology and Chest Departments, and the unit of diabetic foot care at Assiut University Hospital. Out of the 455 patients, 110 patients had nosocomial *S. aureus* infection (24%), 63 of them were males and 47 were females and the mean age was 54.3±10.6 yrs. Nosocomial MRSA infection represented 55% of *S. aureus* infections (60/110 of patients) and 13% of the total nosocomial infections (60/455 of patients) (Table 1 and Fig. 1).

Table 1: Distribution of organisms causing nosocomial infections.

<i>S. aureus</i> infections						Other organisms		Total	
MSSA		MRSA		Total		No.	%	No.	%
No.	%	No.	%	No.	%				
50	11	60	13	110	24	345	76	455	100

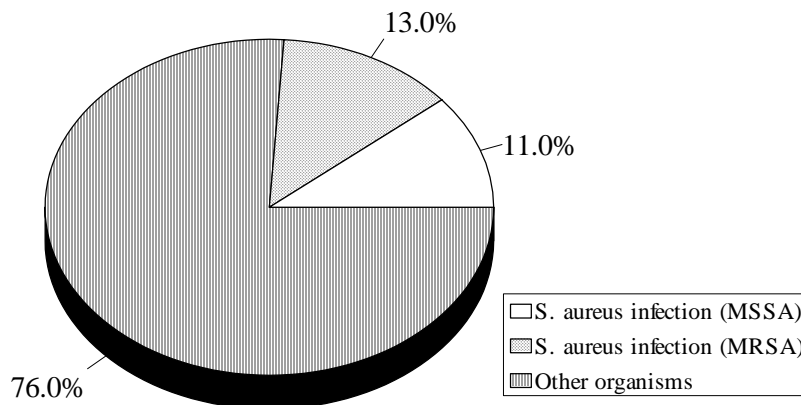


Fig. 1: Distribution of organisms causing infections

MRSA nasal colonization represented 73% of *S. aureus* infected patients (80/110), 17.5% of the whole patients (80/455), 5.2% of paramedical staff members group (8/154) and 18% of the control group (20/110).

The sensitivity rates of MRSA to other antibiotics among *S. aureus* infected patients, paramedical staff and the control group, respectively, were as follows: vancomycin (76.7%, 100% and 80%), tetracycline (43.3%, 37.5% and 75%), clindamycin (83.3%, 87.5%

and 75%), erythromycin (83.3%, 87.5% and 75%), rifampin (86.7%, 87.5% and 100%), ciprofloxacin (86.7%, 100% and 100%) and gentamycin (83.3%, 100% and 75%). Fig. (2) shows that the highest sensitivity rates were obtained by ciprofloxacin, while the lowest sensitivity rates were obtained by tetracycline. The antibiotic resistance rates among *S. aureus* infected patients, paramedical staff and the control group are shown in Table (2).

Table 2: Antibiotic resistance rates of MRSA among patients, paramedical staff and control group.

	Vancomycin		Tetracyclin		Clindamycin		Erythromycin		Rifampin		Ciprofloxacin		Gentamycin		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Patients	14	23.3	34	56.7	10	16.7	10	16.7	14	23.3	8	13.3	10	16.7	60	100
Paramedical staff	0	0	5	62.5	1	12.5	1	12.5	1	12.5	0	0	0	0	8	100
Control	4	20	5	25	5	25	5	25	0	0	0	0	5	25	20	100

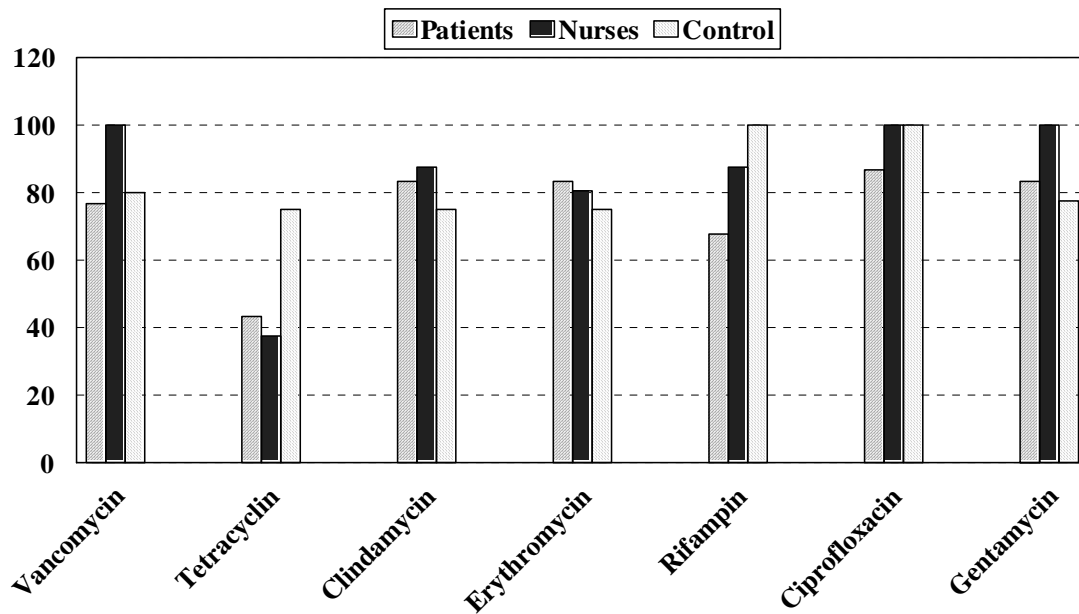


Fig. 2: Antibiotic sensitivity rates of MRSA among patients, nurses and control groups.

There was no significant difference in susceptibility patterns for the different types of antibiotics between the patients and the paramedical staff ($P > 0.05$), while there was a significant difference between the patients and their household contacts (control group) in the susceptibility patterns for tetracyclin and rifampin ($P < 0.05$) denoting that the strains of the patients had the same antibiogram type of those of the paramedical staff, therefore, the paramedical staff had a major role in transmission of MRSA to the patients.

DISCUSSION

MRSA is one of the most important causative organisms of nosocomial infections⁽¹⁸⁾. In this study the rate of *S. aureus* infections was 24% of the total nosocomial infections (110/455) and MRSA accounted for 55% of these *S. aureus* infections (60/110). High rates of MRSA infections were also demonstrated in several studies; in the United States, MRSA became endemic in many hospitals throughout the 1980s and early 1990s, with rates as high as 40% of all *S. aureus* isolates⁽¹⁹⁾. In 1992, MRSA accounted for 57% of all ICU acquired *S. aureus* infections recorded in the European prevalence of infection in intensive Care (EPIC) study⁽²⁰⁾. In ICUs of a medical center in Taiwan, *S. aureus* infection rate was 26.6% of which 88.2% were MRSA⁽²¹⁾. In Korea, MRSA accounted for 64% of *S. aureus* isolates from tertiary hospitals⁽²²⁾. Drinka *et al.*⁽²³⁾ reported that the rate of MRSA at the Wisconsin Veterans Home had increased from 18% of *S. aureus* clinical isolates in 1997 to 51% by 2002.

Regarding MRSA nasal colonization rates, MRSA was isolated from 17.5% of patients in the present study. Similar result was obtained in a study done in the Burn Unit in Mansoura University, which revealed that MRSA was present in 16.3% of patients nostrils⁽¹¹⁾.

In the present study MRSA nasal carriage rates of paramedical staff was 5.2%. Similar results were also found in several studies; In Nagoya Red Cross Hospital, 25 out of 448 nurses (5.6%) were found to be MRSA nasal carriers⁽²⁴⁾. In a study conducted in

Turkey, MRSA nasal carriage in hospital staff was 6%⁽²⁵⁾. Another study revealed that in most epidemics of MRSA, a relatively low level of nasal carriage (3%) has been found in hospital personnel. However, it is very common to find a high rate of MRSA nasal carriage in health care workers, where MRSA is endemic⁽²⁶⁾. Higher rates of MRSA nasal carriage among hospital staff were found in other studies. John *et al.*⁽²⁷⁾ revealed that MRSA carrier rate among hospital personnel in an ICU was 25%. In Japan 44% of isolates from hospital staff were identified as MRSA in a study conducted by Kakinohana *et al.*⁽²⁸⁾. Another study done by Ishikawa *et al.*⁽²⁹⁾ revealed that MRSA was isolated from 6 out of 34 (17.6%) medical workers in urology ward in Fujita Health University.

The rate of MRSA nasal colonization in the contacts of patients was 18%. Similar results were also obtained by Calfee *et al.*⁽³⁰⁾ who revealed that MRSA was isolated from 25 out of 172 contacts of patients who had MRSA infections (14.5%). On the other hand MRSA nasal carriage rate of the control group was low (2.6%) in a study done in Turkey by Cesur and Cokca⁽²⁵⁾.

The antibiotic resistance rates of MRSA among *S. aureus* infected patients showed that the lowest resistance rate was obtained by ciprofloxacin (13.3%) followed by clindamycin, erythromycin and gentamycin (16.7%) and then comes vancomycin and rifampin (23.3%), while the highest resistance rate was obtained by tetracycline (56.7%). On the other hand, the following studies show that the lowest resistance rates of MRSA were obtained by vancomycin and rifampin; Leski *et al.*⁽¹⁾, revealed that the lowest resistance rates were obtained by vancomycin (0%) and rifampin (2.3%), followed by ciprofloxacin (12.5%), while the highest resistance rates were obtained by clindamycin and erythromycin (94%), followed by ciprofloxacin (89%).

In a study conducted in the burn unit in Mansoura Hospital, the lowest resistance rates were obtained by vancomycin (0%) and rifampin (16.7%), while the highest resistance rate were obtained by tetracycline (100%) and gentamycin (58%)⁽¹¹⁾. Another study in Korean Hospitals revealed that the lowest resistance rates were obtained also by vancomycin (0%) and rifampin (18%), while

the highest resistance rates were obtained by erythromycin (98%), gentamycin (95%), tetracycline (89.5%) and clindamycin (84.3%)⁽³¹⁾.

Concerning the resistance of MRSA to other antibiotics among paramedical staff, our results showed that no strains were resistant to vancomycin, ciprofloxacin and gentamycin (0%), while the highest resistance rates were observed by tetracycline (62.5%).

On the contrary, a study conducted by Aoki and Kashiwagi⁽³²⁾ revealed that all strains were resistant to gentamycin and ciprofloxacin but remained sensitive to vancomycin. Kawashima⁽²⁴⁾ had found that all strains of MRSA were resistant to ciprofloxacin while remained sensitive to vancomycin.

In conclusions, vancomycin remains the main stay of treatment of MRSA, but with more extensive use of this antibiotic the likelihood of resistance emerging increases. Alternative antibiotic regimens can be used to treat MRSA infections, in particular ciprofloxacin, gentamycin and rifampin. MRSA strains of patients and paramedical staff had the same antibiogram type denoting that they had a major role in transmission of MRSA.

Regular screening of patients gives an early warning of the presence of MRSA and assess the efficiency of barrier and infection control techniques. Prevention is better than treatment, but for those who are already affected, control is the most likely achievable goal rather than eradication. We recommend the use of molecular methods for accurate and rapid typing of MRSA.

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