

Risk factors of nasal carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* among health care staff in a teaching hospital in central Saudi Arabia

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ABSTRACT

الأهداف: التحقق في عوامل الخطر المحتملة من العوامل الديموغرافية المختلفة على نسبة استيطان البكتيريا (المكورات) العنقودية والبكتيريا العنقودية المقاومة للمضادات الحيوية بواسطة مسحات من الأنف والمرتبطة بالمشاكل الصحية بين موظفي الرعاية الصحية بمستشفى الملك خالد الجامعي.

الطريقة: أُجريت الدراسة خلال الفترة ما بين مايو 2012 إلى يناير 2013 في مستشفى الملك خالد الجامعي بالرياض، المملكة العربية السعودية. تم جمع مسحات مأخوذة من 200 موظف وموظفة بالرعاية الصحية. وتم التعرف على نوع البكتيريا بناءً على كيفية تواجدها، صبغة Gram، إنزيم catalase واختبار coagulase، واختبار Staphaurex PlusH، والوسيط الكروموجيني، واختبار أوكساسيلين وسيفوكيتين باستخدام طريقة الإنبات القرضي. وجرى التصنيف باستخدام طريقة النشر القرضي واختبار E. تم اعتماد البلمرة المتسلسلة لتأكيد استخدام نظام GeneXpert® Dx System (Cepheid) للكشف عن جين mecA.

النتائج: كان من بين 200 من الموظفين 80 (40%) حامل للبكتيريا العنقودية و36 (18%) منهم كان لديهم استيطان للبكتيريا العنقودية المقاومة للمضادات الحيوية. كان هنالك اختلاف كبير بين حاملي البكتيريا العنقودية وفقاً للجنس حيث كانت نسبتهم من الذكور (p=0.012) غالبيتهم من المرضين (p=0.006)، ومدة عملهم بالمستشفى تتراوح ما بين 4 إلى 6 أعوام (p=0.002). وأيضاً، لم يكن هنالك أي علاقة مهمة إحصائية بين عوامل الخطر من العوامل الديموغرافية ومعدل المصابين بالبكتيريا العنقودية المقاومة للمضادات الحيوية (p>0.05).

الخاتمة: أظهرت الدراسة الحالية أن طاقم التمريض هم الأكثر عرضة لاستيطان البكتيريا العنقودية والبكتيريا العنقودية المقاومة للمضادات الحيوية مقارنة بموظفي الرعاية الصحية الآخرين. يتطلب منع تفشي وانتشار هذه العدوى الكشف المخبري الدوري على الموظفين في مجال الرعاية الصحية.

Objectives: To investigate possible risk factors of *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA) nasal carriage associated with various health troubles among healthcare workers (HCWs) at King Khalid University Hospital (KKUH).

Method: This prospective study was conducted between May 2012 and January 2013 in KKHU, Riyadh, Saudi Arabia. A total of 200 nasal swabs were collected from HCWs. Identification was carried out based on morphology, Gram stain, catalase and coagulase test, Staphaurex PlusH test, chromogenic medium, oxacillin, and cefoxitin test using disc diffusion method. Characterization was carried out using disk diffusion method and E-test. Polymerase chain reaction was carried out to confirm using GeneXpert® Dx System (Cepheid) to detect mecA gene.

Results: Among the 200 isolates, 80 (40%) were *S. aureus* carriers, and 36 (18%) of all HCWs were identified as MRSA carriers. There was a significant difference of *S. aureus* according to gender with male carriers (p=0.012), occupation particularly among nurses (p=0.006), and duration of working years in the hospital among 4-6 years group (p=0.002). Moreover, none of the risk factors assessed were significantly associated with the carriage rate of MRSA (p>0.05).

Conclusion: The current study revealed that nursing staff was the potential colonizers of *S. aureus* and MRSA compared with other HCWs. Regular screening of carriers is required for prevention of nosocomial infections.

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Staphylococcus aureus (*S. aureus*) are commensal Gram-positive cocci (GPC), which colonizes in 20-30% of the human population,¹ as well as livestock and domestic animals.^{2,3} A human pathogen, *S. aureus* is known to cause skin and soft tissue infections from mild to life-threatening sepsis, pneumonia, and toxic shock syndrome. *Staphylococcus aureus* colonizes the skin and mucosa of human beings and in several animal species; although multiple body sites can be colonized in human beings, the anterior nares of the nose is the most frequent carriage site for *S. aureus*.⁴ *Staphylococcus aureus*' resistance is not only for methicillin and other B-lactam antibacterial drugs, but it is causing a high mortality rate among patients again ending with the high cost of prescription medications, which may cause severe consequences, and may extend to other antibacterial drugs.⁵ Methicillin-resistant *S. aureus* (MRSA) causing nosocomial infection is a versatile and dangerous pathogen emerging rapidly in hospitals, usually infecting the skin causing boils, pustules, and impetigo.⁶ A recent study from 7 hospitals in Riyadh indicated that the prevalence of MRSA among *S. aureus* isolates ranges from 12-49% with most of the tested hospitals giving a prevalence of 27-33%.⁷ The first report in the international literature in 1992 was a comparative study undertaken to investigate the sensitivity of MRSA strains isolated from the Kingdom of Saudi Arabia (KSA) and Great Britain to antibiotics and biocides.⁸ In 1993, Haddad et al⁹ documented the first outbreak of MRSA in the neonatal intensive care unit at a tertiary care hospital in Riyadh, KSA. In the year 2000, Alghaithy et al¹⁰ investigated the carrier status and antibiotic resistance among hospital and non-hospital personnel in Abha, KSA, and isolated MRSA from 5.1% of the hospital and 18.3% from the non-hospital carriers. Hospital staff can act as a source for transmission of MRSA when transient, or permanent colonization of the nares or oropharynx occurs. The MRSA carrier rates among healthcare workers (HCWs) vary between 0.4% and 0.18%.¹¹ Mean nasal

MRSA carriage in HCW was 4.1% in 104 studies.¹² In KSA, very few studies have been conducted so far to investigate the incidence or prevalence of MRSA, and identify the risk factors associated with MRSA infection in the community and/or hospital patients. Any effective strategy to prevent the spread of the pathogen, which is a key part of the detected speed with MRSA carriage is reported as having an important role to play. The end results are available for rapid detection methods for increasing the number of MRSA before it is too late for 2-3 days, so the most recent, rapid methods have been developed to increase the use of polymerase chain reaction (PCR) techniques, and rapid tests run reduces the time to detection from 48-72 to 2-5 hours for MRSA carriers. Taking in consideration the above mentioned facts, the main aim of this study was to investigate, and assess possible risk factors of *S. aureus* and MRSA carriage among HCWs in KCUH, Riyadh, KSA to compare different methods for MRSA isolation and detection, as well as to determine its susceptibility patterns to commonly used antibiotics.

Methods. Selection and collection of samples. This was a prospective study conducted from May 2012 to January 2013 at KCUH, Riyadh, KSA. A total of 200 randomly obtained nasal swabs from different categories of HCWs were collected. Health care workers include doctors, nurses, laboratory technologist, pharmacists, cleaners, and administrative staff working in different wards at KCUH. A moist cotton swab of AMIES (a special medium to keep the organism alive until testing day) medium was inserted into each nostril in turn to a depth approximately one cm and rotated 5 times, and both nostrils were sampled using the same swab.¹³ Informed consent was obtained from each subject, after receiving the approval of the study by the Institutional Review Board, College of Medicine, King Saud University, Riyadh, KSA. The study was conducted according to the Helsinki declaration. Electronic database was used as a source to find related articles and research.

Microbiological methods. All specimens were taken to the Microbiology laboratory within a maximum of 24 hours (h). Culturing of the specimens was initiated with inoculation onto the oxacillin blood agar medium and Mannitol salt agar medium with oxacillin.¹⁴ Meanwhile, the swabs were also broken off into brain heart infusion broth for enrichment and incubated at 37°C aerobically for 24 h.¹⁵ After incubation the growth in broth was tested with Staphaurex Plus kit (Remel

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Products, Lenexa, KS, USA), and all broth were then subculture into blood agar and mannitol salt agar with, and without 4 mg/L oxacillin for recovery of MRSA and methicillin sensitive *S. aureus* (MSSA) strains. All the plates were incubated aerobically at 35°C for 24-48 h for typical colonies of *S. aureus*. Species identification was confirmed by morphological, Gram stain, catalase, slide coagulase, tube coagulase tests and serologically positive with Staphaurex Plus^H test (Remel Products, Lenexa, KS, USA). All the isolates were screened for MRSA by MRSA Chromogenic agar (bioMérieux UK Limited, Basingstoke Hampshire, UK) 1 µg Oxacillin and 30 µg Cefoxitin disk diffusion to detect *mecA*-mediated resistance in *S. aureus*. Isolates were considered resistant when the diameter of inhibition for Oxacillin was ≤10 mm, and for Cefoxitin was ≤21 mm (Clinical and Laboratory Standards Institute [CLSI] 2012). The antimicrobial susceptibility test was

carried out using Kirby-Bauer's disc diffusion method to Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Erythromycin (15 µg), Gentamycin (10 µg), Imipenem (10 µg), Linezolid (30 µg), Mupirocin (20 µg), Trimethoprim-sulfamethoxazole (23.75 µg), Tetracycline (30 µg), Penicillin (10 µg), and Amoxicillin (20 µg). Moreover, the E-test method was performed to determine the susceptibility of all isolates of staphylococci for Vancomycin. The zone sizes were measured and interpreted according to CLSI 2012.

Genotyping. The Cepheid Xpert MRSA/SA Skin and Soft Tissue Infection Assay (Xpert MRSA/SA SSTI Assay) performed in the GeneXpert® Dx System, (Cepheid, Sunnyvale, CA, USA) is a qualitative in vitro diagnostic test intended for detection of *S. aureus* and MRSA from skin and soft tissue infection swabs.

Statistical analysis. For data analysis, the findings were statistically analyzed using the Statistical Package

Table 1 - Potential risk factors for nasal carriage of *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA) among health care workers in Riyadh, Saudi Arabia.

Characteristics	N	<i>S. aureus</i> carriage		MRSA carriage	
		n (%)	P-value	n (%)	P-value
<i>Gender</i>			0.021		0.253
Female	95	30 (32.0)		14 (15.0)	
Male	105	50 (48.0)		22 (21.0)	
<i>Age, years</i>			0.486		0.252
<30	95	36 (38.0)		16 (17.0)	
30-50	95	42 (44.2)		19 (20.0)	
>50	10	2 (20.0)		1 (10.0)	
<i>Year of working</i>			0.002		0.073
1-3	70	22 (31.4)		10 (14.3)	
4-6	66	39 (59.0)		17 (26.0)	
7-10	33	9 (27.2)		2 (6.1)	
>10	31	10 (32.2)		7 (23.0)	
<i>Level of education</i>			0.149		0.497
University graduate	180	75 (42.0)		33 (18.3)	
High graduate	20	5 (25.0)		3 (15.0)	
<i>Nasal abnormalities</i>			0.058		0.410
Present	35	19 (54.2)		8 (23.0)	
Absent	165	61 (37.0)		28 (17.0)	
<i>Antibiotic use in previous 3 months</i>			0.052		0.630
Present	12	8 (67.0)		2 (17.0)	
Absent	188	72 (38.2)		34 (18.1)	
<i>Occupation</i>			0.006		0.140
Doctors	64	26 (41.0)		14 (22.0)	
Nurses	78	40 (51.3)		18 (23.1)	
Cleaners	8	2 (25.0)		1 (13.0)	
Laboratory technologist	16	8 (50.0)		3 (19.0)	
Food handlers	11	2 (18.2)		0 (0.0)	
Administrative staff	11	2 (18.2)		0 (0.0)	
Pharmacists	12	0 (0.0)		0 (0.0)	

for Social Sciences version 19.0 (SPSS Inc., Chicago, IL, USA). However, Fisher's exact test and Chi-square test were used to compare between MRSA and MSSA groups with respect to different antibiotics sensitivity (nominal variables), such as, c30 (Chloramphenicol 30 µg), cip5 (Ciprofloxacin 5 µg), DA2 (Clindamycin 2 µg), P10 (Penicillin 10 µg), and CN10 (Gentamycin 10 µg). Student t-test was used for independent groups to compare between the age of HCW for MRSA and MSSA group. A $p < 0.05$ was considered statistically significant.

Results. The distribution of nasal carriers of *S. aureus* and MRSA in different categories of hospital staff is shown in Table 1. In this study, out of 136 GPC tested, 78 isolates gave positive slide clumping, 80 isolates gave positive tube clumping, and was therefore identified as *S. aureus*. As expected all positive tube coagulase strains (80/200) gave also positive Staphaurex. Moreover, out of the examined 200 nasal swabs specimens, 40% (80/200) carried *S. aureus*. Out of the 200 studied specimens, 36 *S. aureus* strains gave the characteristic blue green colonies color in MRSA chromogenic agar and therefore assigned as MRSA strains. Oxacillin disk (1 µg) and cefoxitin disc (30 µg) diffusion method were used to detect MRSA and cefoxitin disk was more sensitive (100%) to detect MRSA than oxacillin (97%). Cefoxitin disc diffusion test is in concordance with PCR for *mecA* gene. The overall nasal carriage rate of MRSA among examined 200 nasal swabs specimens was 18% (36/200) regardless of several studied potential risk factors as shown in Table 1. According to gender, the results obtained showed that there was a high prevalence

rate of *S. aureus* among males (48%[50/105]) in comparison with females (32% [30/95]). Statistical analysis of such data revealed that this difference is significant ($p=0.021$). Our results also exposed statically significant and competing high prevalence rate of *S. aureus* colonization among nurses 51.3% (40/78) compared with other HCWs ($p=0.006$). Regarding the duration of health care year services, the present study revealed that *S. aureus* carriage rate varied in relation to HCWs year duration. There was a significant difference in subjects who worked for 4-6 years services when compared with 7-10 years services ($p=0.002$). However, the highest rate of 59% was observed among those subjects who worked for 4-6 years services, whereas the lowest carriage rate was detected among those 7-10 years services, and this difference was significant ($p=0.002$). Our present study also identified that other demographic characterizations for *S. aureus* carriage did not show significant variation in relation to the level of age groups ($p=0.486$), education ($p=0.149$), nasal abnormalities ($p=0.058$), and insistent antibiotic used ($p=0.052$). As for nasal carriage of MRSA on gender, just like the general prevalence carriage of *S. aureus*, results revealed that there was a high prevalence rate among males (21%, 22/105) in comparison with females (15%, 14/95). However, statistical analysis of such data showed that this difference is not significant ($p=0.253$). Also, like the general *S. aureus* carriage rate, results also showed as expected a relatively high prevalence rate of MRSA among nurses (23%), doctors (22%), and laboratory technicians (19%) in comparison with other HCWs (ranged 13-0%). However this differences proved statistically non-significant ($p=0.140$). Based

Table 2 - Antibiotic resistance pattern of methicillin sensitive *Staphylococcus aureus* (*S. aureus*) (MSSA) and methicillin-resistant *S. aureus* (MRSA).

Antibiotics	MSSA (n=44)			(n=36)		
	Sensitive	Intermediate	Resistant n (%)	Sensitive	Intermediate	Resistant
Penicillin	0 (0.0)	0 (0.0)	44 (100.0)	0 (0.0)	0 (0.0)	36 (100.0)
Tetracycline	35 (79.5)	4 (9.1)	5 (11.4)	25 (69.4)	7 (19.4)	4 (11.1)
Trimethoprim-sulfamethoxazole	41 (93.2)	3 (6.8)	0 (0.0)	30 (83.3)	6 (16.7)	0 (0.0)
Oxacillin	44 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	36 (100.0)
Mupirocin	44 (100.0)	0 (0.0)	0 (0.0)	36 (100.0)	0 (0.0)	0 (0.0)
Gentamycin	41 (93.2)	3 (6.8)	0 (0.0)	33 (91.7)	2 (5.6)	1 (2.8)
Erythromycin	37 (84.1)	2 (4.5)	5 (11.4)	1 (2.8)	9 (25.0)	26 (72.2)
Clindamycin	41 (93.2)	0 (0.0)	3 (6.8)	1 (2.8)	10 (27.8)	25 (69.4)
Chloramphenicol	44 (100.0)	0 (0.0)	0 (0.0)	31 (86.1)	4 (11.1)	1 (2.8)
Ciprofloxacin	41 (93.2)	3 (6.8)	0 (0.0)	30 (83.3)	6 (16.7)	0 (0.0)
Amoxicillin	44 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	36 (100.0)

on years of health care services, the highest rate of MRSA carriage rate was observed among those subjects who worked for 4-6 years services (26%), whereas the lowest carriage rate was detected among those 7-10 years services, but this variation was not significant ($p=0.037$). The MRSA carriage rate result of the study revealed that it was higher in HCWs (7.6%) with equal or less than 5 years service or practice experience ($p>0.05$). All recovered *S. aureus* isolates (80) were tested against 14 antibiotics using conventional disk diffusion test and E-test for Vancomycin is shown in Table 2. All recovered *S. aureus* isolates were completely susceptible (100%) to Trimethoprim-sulfamethoxazole, Mupirocin, Ciprofloxacin, Linezolid, and Vancomycin. Furthermore, the present results indicated that out of 80 *S. aureus*, the carriage rate for MSSA was 22% (44/200), which exhibited full sensitivity to Oxacillin (100%), Amoxicillin (100%), and high sensitivity to other non B-lactam antibiotics like Trimethoprim-sulfamethoxazole, Mupirocin, Gentamycin, Imipenem Chloramphenicol, Ciprofloxacin and Linezolid (ranged between 100-79.5%). Moreover, antimicrobial resistance patterns of MRSA strains tested by disk diffusion were as follows: Erythromycin (72.2%), Clindamycin (69.4%), and Tetracycline (11.1%).

In this study, MRSA and MSSA strains in parallel with the quality control (QC) analyzed by PCR using the technology of GeneXpert® Dx System (Cepheid, USA) at a Military Hospital, Asser, Saudi Arabia. The examined MRSA strains conform qualitatively and quantitatively to QC MRSA strain ATCC 23591. Likewise, the MSSA strain (ATCC 25923) proved negative for the *mec* and staphylococcal cassette chromosome (SCC) targets.

Discussion. In agreement with Abu Hujier and Sharif,¹⁶ tube coagulase test is regarded as the gold standard agglutination method to detect *S. aureus* because the tube coagulase test remains a simple, cheap, rapid, and reliable method available to all diagnostic laboratories. The findings of this study revealed an excellent result with Staphaurex Plus^H test to detect all *S. aureus*, and is also consistent with those previously reported.¹⁷ Our result for nasal carriage rate of *S. aureus* is remarkably lower than that reported in Yemen¹⁸ and Nigeria.¹⁹ These authors reported an overall nasal carriage of *S. aureus* among HCWs was 85% (60/70),¹⁸ and 52.5% (104/198).¹⁹ However, the carrier rate of *S. aureus* in the present study is quite high as compared with others, such as Rongpharpi et al,²⁰ and Vinodhkumaradithyaa et al²¹ in which both

studies were conducted in India, and carriage rate of nasal *S. aureus* among HCWs was 22% (70/315),²⁰ and 13% (13/100).²¹ On the other hand, a similar study conducted in KSA²² on 352 HCWs detected *S. aureus* in 112 (31.8%). These discrepancies in such studies may be attributed to the sample size, culture methods, and abuse of rational antibiotic policy. The sensitivity of MRSA Chromogenic agar to detect MRSA was 100%, and this result is in agreement with Rahbar et al²³ who reported that CHROMagar MRSA is highly sensitive and specific in differentiating between MSSA and MRSA. Regarding the cefoxitin disk, the result is consistent with Anand et al²⁴ who compared cefoxitin disc diffusion test with oxacillin agar screening and detection of *mecA* gene by PCR. In these study, results of cefoxitin disc diffusion test is in concordance with the PCR for *mecA* gene. Thus, this test can be an alternative to PCR for detection of MRSA in resource constraint settings.²⁵

Regarding the frequency of MRSA; the result was in agreement with Shakya et al²⁶ who reported that the international range of MRSA carriage is approximately 6-18% among the HCWs in the hospital setting. This carriage rate of MRSA is remarkably lower than that reported in Egypt,²⁷ Yemen,¹⁸ and Libya.²⁸ However, carrier rate of MRSA in the present study is quite high compared with others, such as in Ethiopia,²⁹ India,³⁰ and Iran.¹³ Regarding the impact of MRSA on demographic characters; the results were in agreement with previous studies where they came out with same results.²⁹ The prevalence rate in various population is diverse with each report, the reason may be due to social economic status, race, and ethnicity. A study from India by Rongpharpi et al²⁰ found that the prevalence of *S. aureus* nasal carriage was higher among males HCWs (54.28% [38/70]) compared with that of females HCWs (45.71% [32/70]), where our results were far different. Likewise, Ahmad²² reported from KSA that the prevalence of *S. aureus* among females HCWs was higher (44.6% [66/148]) than males (22.5% [46/204]). These contradictory results may also be due to sample size and methods adopted used for the screening process. In a similar study conducted in Ethiopia by Shibabaw et al²⁹ who studied the prevalence of *S. aureus* among 118 HCWs, they found 36.8% (21/57) in males and 21.3% (13/61) in females. Likewise in India, Rongpharpi et al²⁰ found the prevalence of the *S. aureus* nasal carriage was higher among males HCWs (54.28% [38/70]) compared with that of females HCWs (45.71% [32/70]). In contrast, Ahmad²² reported from KSA that

prevalence of *S. aureus* among females HCWs was higher (44.6% [66/148]) than males (22.5% [46/204]). The reason of high *S. aureus* carriage among males compared with females reported from different countries are not known. This difference might be attributed to the fact that men's maneuvers and their circle size of social contacts, especially in Arab countries and Middle East, is much higher than those of females. Our results also exposed statistically significant and competing high prevalence rate of *S. aureus* colonization among nurses and doctors compare to other HCWs, presumably due to frequent contact with patients infected with *S. aureus* and/or carriers. A similar finding was previously reported by Ahmad²² from KSA.

In the present study analyzed results help to know the impact on the prevalence rate of *S. aureus* with duration of health care year services, and the results are consistent with those previously reported by Shibabaw et al²⁹ who claimed that the younger and less practiced HCWs could be due to their lack of knowledge with regard to infection control policies and their missing experience in taking care of patients. However, other demographic characterizations for *S. aureus* carriage that shows no significance are compatible with those previously reported from Iran.¹³ The obtained results of the sensitivity show that MSSA exhibited low MDR pattern to only 4R markers. These findings are consistent with Rahimi et al.³¹ As expected, obtained data of MRSA isolates susceptibility testing showed 100% resistance to Penicillin, Oxacillin, Amoxicillin, Cefoxitin, and Imipenem. These findings were in agreement with those of Abdelmonem¹⁸ who found that MRSA resistance against Penicillin (97.77%) and Amoxicillin (100%) was high among HCWs, and with Farzana et al³² who found more than 80% of MRSA strains were resistant to Penicillin and Ampicillin. Moreover, antimicrobial resistance patterns of MRSA strains tested by disk diffusion were as follows: Erythromycin (72.2%) (this was consistent with Ahmed et al's study²⁸ when he found that out of the 109 confirmed MRSA of HCWs isolates, 74% were resistant to Erythromycin). Finally, in agreement with several workers,³³ the PCR provides a sensitive and specific method to identify MRSA carrier status, although it is more expensive and not easily accommodated in most laboratories.

In conclusion, the continued emergence of *S. aureus* and MRSA in the community is a public health problem, and therefore warrants increased vigilance in the diagnosis and management of suspected and confirmed staphylococcal infections. The current study revealed that nursing staffs were the potential colonizers of *S. aureus* and MRSA when compared

to HCWs. These carriers may serve as a reservoir and disseminator of MRSA, and should be treated. Regular screening of carriers is required for the prevention of nosocomial infection. The inappropriate or unnecessary use of antibiotics should be avoided, and this will also reduce the likelihood of the emergence and spread of vancomycin-resistant *S. aureus*. Taking in consideration the MDR patterns among recovered MRSA, it is concluded that none B-lactam antibiotics, namely, Clindamycin and the macrolide Erythromycin, both drugs were associated with all MRSA strains reflecting their great abuse and/or irrational use in our country.

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