DETECTION OF METHICILLIN RESISTANT \textit{Staphylococcus aureus} ISOLATED FROM NASAL CARRIAGE OF HEALTH CARE WORKERS BY POLYMERASE CHAIN REACTION

Seham O. Alsulami $^1$, Huda A. Al Doghaither $^{1,2}$, Archana P. Iyer$^{1,2,*}$

$^1$Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
$^2$Vitamin D Pharmacogenomics Research Group, King Abdulaziz University, Jeddah, Saudi Arabia

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ABSTRACT

Methicillin resistant \textit{Staphylococcus aureus} (MRSA) and coagulase negative \textit{Staphylococci} (MRCoNS) are major health problems worldwide. There is a growing concern about the rapid rise in resistance of nosocomial infections to antimicrobial agents. The aim of the present study was to determine nasal colonization of MRSA and MRCoNS among healthcare workers (HCWs) in King Abdulaziz University hospital. A total of 100 respondents were selected for this study and were classified into two groups: HCWs group which included 50 technicians and nurses, and the control group which included 50 university students. Nasal swabs from anterior nares were cultured on selective media for the presence of mecA gene that is common to these bacteria. Polymerase chain reaction (PCR) and specific primers were used to determine mecA and coagulase gene (coa). The identified isolates were tested by restriction fragment length polymorphism (RFLP). Results of current study revealed that among 50 HCWs, 21 (42%) were nasal carriers for MRCoNS and 12 (24%) for MRSA. The control group was negative for MRSA and MRCoNS. The results of the current study will help in making decision to prevent the spread of infections among workers in the field of health care.

KEYWORDS

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* Corresponding author
E-mail: arch729@gmail.com(Archana P. Iyer)

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1 Introduction

Nosocomial or hospital acquired infections (HAIs) are one of the most common problems in hospitals throughout the world, these are the infections that develop during hospitalization and are not present nor incubating at the time of the patients’ admission (Revelas, 2012). The source of nosocomial infections can be either endogenous (e.g. from the patient’s own flora) or exogenous (e.g. from a source other than the patients). HAI is an infection whose development is favored by environment, such as one acquired by a patient during hospital visit or one developing among hospital staff (Shrestha et al., 2009). About 20% of patients undergoing surgery acquire at least one nosocomial infection, leading to increased morbidity, mortality, hospital stay and costs. *Staphylococci* and *Enterococci* are major causes of nosocomial infections (Boyce et al., 1992).

Methicillin resistant *S. aureus* (MRSA) and coagulase negative *Staphylococci* (MRCoNS) which by definition are resistant to the semi-synthetic penicillin (i.e. methicillin) (Van Hal et al., 2007). The prevalence of MRSA infections has increased dramatically over the past two decades (Williamson et al., 2014). According to Biau & AL-Abdli (2015), due to antibiotics resistance, MRSA infections required more complicated treatment. Resistance due to mecA gene, which encodes an alternative *Penicillin Binding Protein* (PBP2a), prevents the action of beta lactam antibiotics (Jousselin et al., 2016). In addition, coagulase gene (Coa) is usually used to identify and compare *S. aureus* because it consists of 81-bp tandem repeats region at the 3′ ends (Hookey et al., 1998). Although, most of the MRSA infections do not take a serious clinical course in healthcare workers, some infections tend to become chronic and can cause severe health problems, depending upon the strength of the individual’s immune system. For healthcare facilities, surveillance is an important and accepted method to assess the incidence of infection due to multidrug-resistant bacteria and if necessary, to improve infection control measures (Deurenberg et al., 2007). The aim of the current study was to determine the frequency of MRSA and MRCoNS among HCWs using PCR-RFLP analysis.

2 Materials and Methods

2.1 Sample collection

Samples were collected from King Abdulaziz University Hospital (KAUH) and King Abdulaziz University (KAU) students. HCWs samples included nurses and technicians from different wards: Medical Intensive Care Unit (MICU), Surgical Intensive Care Unit (SICU), Cardiac Care Unit (CCU), and Outpatient Departments (OPD) of the same hospital. The control samples included the students. A total of 100 respondents were selected for this study, among this 50 were from HCWs while the other 50 were control students. The positive subjects were grouped according to place of work, age and gender.

2.2 Isolation, growth and identification of bacteria

Each swab was immediately placed in an enrichment broth and incubated at 37°C for 24 h; these collected samples were processed in the microbiology laboratory on the same day of sampling. The enrichment broth shows the presence of the mecA gene of *Staphylococci* was MeReSa Agar Base. The selective broth was prepared by suspending 40.06 g of the medium into 500 ml of the distilled water and boiling. The medium was cooled (45-50°C) and MeReSa selective supplement (FD299) was added with 5 ml distilled water and mixed well. The final volume was 2 mg of methicillin in each vial.

Soon after, the medium was poured into sterile Petri plates and cooled to check for sterility by keeping at 37°C overnight. In this study, the detection of MRSA and MRCoNS were determined by direct culture of each swab on chromogenic medium. All cultures which were showing light pink colored growth selected and confirmed to be mecA positive isolates following coagulase, and were finally confirmed by PCR using specific primers as shown below, and all others are recorded as MSSA isolates (HiMedia Labs. Products, India).

2.3 PCR amplification

Bacterial DNA was extracted and stored at -20°C using GeneJet Genomic DNA Purification Kit #K0721 (Thermo Scientific, USA) according to the manufacturer's instructions. For PCR, mecA gene primers were used to amplify mecA gene and MecA-positive strains were screened for coa gene using specific primers. The primers sequences are shown in Table 1. PCR products were detected using 2% agarose gel electrophoresis and visualized with ethidium bromide dye. Coa gene products were subjected to restriction digestion by AluI restriction enzyme (Hookey et al., 1998).

| Gene | Primer sequence | PCR conditions | Number of cycles | Product size |
|------|----------------|----------------|-----------------|-------------|
| **mecA** | FP: 5′AAATCGATGTTAAAGGTTGGC 3′ | 94°C – 30 s | 40 | 533 bp |
| | | 55°C – 30 s | | |
| | | 72°C – 1 min | | |
| | | 72°C – 5 min (final extension) | | |
| **Coa** | FP: 5′ATAGAG ATGCTGGTACAGG3′ | 94°C – 1 min | 30 | 350 bp |
| | | 60°C – 1 min | | |
| | | 72°C – 1 min | | |
| | | 72°C – 5 min (final extension) | | |
2.4 Statistical analysis

Statistical analysis was performed using SPSS for windows v. 20.0. Chi square test was used to compare frequency distribution of MRSA and MRCoNS among HCWs. P values of < 0.05 were considered to be statistically significant.

3 Results

Out of 50 HCWs participating in the study, 21 (42%) were nasal carriers of MRCoNS and 12 (24%) for MRSA in the anterior nares based on culture results and coagulase test. Table 2 summarizes the organization of the positive subjects according to the unit, age, and gender. The results showed that none of the students, who are participating as controls, were positive for MRSA and MRCoNS nasal carriage. The absence of MRCoNS and MRSA nasal carriage in students was an actual important result when compared to the HCWs.

As previously described, all MRSA isolates (n=12; 24%) and MRCoNS (n=21; 42%) were obtained from 50 HCWs from four different units at KAUH (MICU, SICU, CICU and OPD). The highest incidence of MRSA (12%) and MRCoNS (26%) was recorded in MICU from 28 HCWs. Furthermore, 8% of MRSA and 14% of MRCoNS isolates in SICU were from 16 HCWs. While in CCU, 2% of MRSA and MRCoNS were from two HCWs, respectively. The lowest nasal carriage was isolated from 4 HCWs OPD by 2% in MRSA and (0%) of MRCoNS. Results of this assay demonstrated that all MRSA and MRCoNS isolates detected by chromogenic method showed the presence of amplified bands of mecA gene (533 bp) (Figure 1).

Statistical analysis of results revealed that there was no significant difference between the units and MRSA and MRCoNS using chi-square test (P>0.05). The age of HCWs infection ranged from 23 to 51 years (mean age 33 ± 5.82 years). Among these, 31 females were (62%) and two were males (4%). With respect to nasal carriage of MRSA, 11 females and one male were colonized. While in case of MRCoNS, 20 females and one male were colonized. No significant difference was reported between age and MRSA or MRCoNS, individually. Moreover, there were no significant differences between gender and MRSA or MRCoNS separately, as shown in table 3.

Coa gene is important for the identification of S. aureus, with sizes ranging from 400 bp to 900 bp. Alu I restriction

Table 2

| Parameters |单元 | MICU | 28 | 19 | 6 | 12 | 13 | 26 |
|------------|-----|-----|----|----|---|----|----|----|
|            | SICU | 16  | 11 | 4  | 8 | 7  | 14 |
|            | CCU  | 2   | 2  | 1  | 2 | 1  | 2  |
|            | OPD  | 4   | 1  | 1  | 2 | 0  | 0  |

| Parameters | 年龄 | 20-29 | 12 | 8 | 2 | 4 | 6 | 12 |
|------------|-----|-------|----|---|---|---|---|---|
|            | 30-39| 33    | 21 | 8 | 16 | 13 | 26 |
|            | 40-49| 3     | 2  | 1 | 2  | 2  | 4  |
|            | 50-59| 2     | 1  | 1 | 2  | 0  | 0  |

| Parameters | 性别 | 女 | 48 | 31 | 11 | 22 | 20 | 40 |
|------------|-----|---|----|----|----|----|----|----|
|            | 男   | 2  | 2  | 1  | 2  | 1  | 2  |
|            | 总计 | 50 | 33 | 12 | 24 | 21 | 42 |

Figure 1 Agarose gel electrophoresis (2%) of mecA gene PCR products where lane 1: 100 bp DNA ladder and lane 2-9: PCR product.

Table 3 Chi-square test for MRSA and MRCoNS colonization disaggregated by age group, gender, and units

| Parameters | χ2  | df | P value |
|------------|-----|----|---------|
| MRSA       |     |    |         |
| Age        | 0.756 | 3 | 0.685   |
| Unit       | 0.854 | 3 | 0.837   |
| Gender     | 0.772 | 1 | 0.38    |
| MRCoNS     |     |    |         |
| Age        | 3.256 | 3 | 0.815   |
| Unit       | 3.195 | 3 | 0.363   |
| Gender     | 0.055 | 1 | 0.169   |

P value is not significant (P > 0.05)
enzyme digestion of the PCR-amplified of coa gene yielded one distinct PCR-RFLP pattern. The PCR products of coa gene (Figure 2) of all MRSA strains and controls were cut by Alu I enzyme and gave one fragment as shown in Figure 3.

![Figure 3 Agarose gel electrophoresis (2%) of coa gene Alu I restriction enzyme digestion PCR products, where lane 1: 100 bp DNA ladder, lane 2: control sample and lanes 3-10: Alu I restriction digestion pattern.](image)

4 Discussion

*Staphylococci* are the major causes of nosocomial infections. MRSA and MRCoNS are resistant to all β-lactam antibiotics and are considered as the most important causes of nosocomial infections around the world. One of the most effective methods for preventing the spread of MRSA and MRCoNS, requires detection of colonized HCWs and measuring the associated risk factors of colonization.

Results of this study revealed low prevalence rate of MRSA nasal carriage (24%) among HCWs but high prevalence rate of MRCoNS nasal carriage (42%). Although, these rates are lower than those reported in other areas of the world (Lu et al., 2005; Shibabaw et al., 2013; Hussein et al., 2017). The prevalence has decreased gradually from 38% in 2002 to the current rate of 24% in same hospital (Madani, 2002). There were considerable differences among main regions for the rate of MRSA nasal carriage in Makkah, Dahran and Riyadh cities (Yousef et al., 2013). Therefore, it seems that the difference in the incidence of MRSA reflects host or environmental factors. Additionally, high variation may be due to the epidemiology of MRSA in transition period and infection control rules may be most effective. Comparing the results of this study with other regional countries, it appears that Saudi Arabia has a higher MRSA prevalence rate compared to other neighbor counties (Aly & Balkhy, 2012). These different rates among MRSA from different countries may be attributed to variations in patient populations, the biological characteristics of the *S. aureus* strains, and/or infection control practices (Orrett & Land, 2006).

In this study, the frequency of MRSA carriage varied between different departments. The prevalence was highest in MICU and it was followed by SICU, which was close to the results that were obtained by Alunbas et al. (2013). In a study accomplished in Libya, Zorgani et al. (2009) reported that the incidence of MRSA were 42% and 27% within MICU and SICU, respectively. This is markedly higher than those reported from other countries (Goyal et al., 2002). On the other hand, the least carriage rate in HCWs was reported in CCU and OPD (Akhtar, 2010).

MRSA nasal carrier rate was high (20%) in age group of 20 to 40 years and less (4%) in age groups above 40 years. These finding was contrary to that observed in the study done in Saudi Arabia which showed infection in the 'extremes of age' group (younger than one or older than 60 years) (Madani, 2002). Another study by Iyer et al. (2014) from Jeddah found the double rates of MRSA colonization in age group of 40-50 years, which documented that MRSA infection, occur at a higher incidence in older people owing to a weaker immune system.

MRCoNS nasal carriage was seen in 42% of the HCWs in the present study. Results of this study are in agreement with the findings of Mahesh et al. (2015) those who reported that overall nosocomial infection rates were 42 (41.17%) of the HCWs. In this study, prevalence of MRCoNS rate was lower than other previous reports done in other countries such as France, Germany, Greece, Italy and Turkey (Sader et al., 2007; Mehdinejad et al., 2008). The variations in nasal carriage prevalence of MRCoNS might be due to the variations in geographical distributions of bacterial strains or due to the infection control practices.

There was a remarkable difference in MRCoNS carriage rate in HCWs of MICU (26%), SICU (14%), CCU (2%) and OPD (0%). In accordance with the results of these studies, length of ICU stay was a significant risk factor for ICU-acquired infection both in univariate and multivariate analyses in this study. Each invasive procedure performed poses a risk of infection; it is in agreement with the results of other studies (Ak et al., 2011).

In case of gender study, no significant differences were reported in the rates of nasal carriage in males and females. This finding was contrary to that observed in the study done in Nigerian where females harbored bacteria significantly more often than males (Lamikanra et al., 1985). Further, findings of present study are in agreement in the findings of Majumda et al. (2009).

In this study, none of the screened students (50) were found positive for MRSA or MRCoNS colonization. Similar to results of this study, a study carried out by Iyer et al. (2014) on nasal colonization, suggesting that people who are not exposed to the pathogen have a very low risk of nasal carriage. On the other hand, Mollaghan et al. (2010) reported regarding the globally increased prevalence of MRSA nasal colonization in the community. Risk factors that increase the prevalence of MRSA nasal colonization in Asia may be misuse of antibiotic and lower socioeconomic status.
These findings indicate the need to develop rapid and regular screening programs for transmission of MRSA in HCWs settings. This study clearly showed that rapid identification of MRSA isolates can be done using the PCR specific for the meca gene.

A study conducted by Tiwari et al. (2008) suggested that coa PCR was sensitive and specific in confirming the fact that this gene is present in all S. aureus isolates. Alu I restriction enzymes was used for coa gene PCR-RFLP, aiming at a more detailed characterization of MRSA isolates, since it generated one RFLP band pattern. Our results are in accordance with Lawrence et al. (1996) who isolated MRSA strains from various hospitals and showed that the strains were closely related and had a unique RFLP pattern when analyzed by coa gene typing. In contrast to the findings of these results, Kobayashi et al. (1995), who found that MRSA was classified into 6 RFLP patterns, with 5 patterns detected frequently in MRSA. Eed et al. (2015) showed that Alu I digestion of the coa gene PCR products of 58 MRSA strains yielded nine different RFLP patterns. In conclusion, the results of the current study showed that 42% of the HCWs were nasal carriers for MRCoNS and 24% for MRSA. The results of the current study might help in decision making to prevent the spread of infections among workers in the field of health care.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

References

Ak O, Batrel A, Ozer S, Colakoglu S (2011) Nosocomial infections and risk factors in the intensive care unit of a teaching and research hospital: A prospective cohort study. Medical Science Monitor 17: PH29-PH34. DOI: 10.12659/MSM.881750.

Akhtar N (2010) Staphylococcal nasal carriage of health care workers. Journal of the College of Physicians and Surgeons--Pakistan 20: 439-443. DOI: 07.2010/JCPSP.439443.

Altunbas A, Shorbagi A, Ascioglu S, Zarakolu P, Cetinkaya-Sardan Y (2013) Risk factors for intensive care unit acquired nasal colonization of MRSA and its impact on MRSA infection. Journal of Clinical Laboratory Analysis 27: 412-417. DOI: 10.1002/jcla.21620.

Aly M, Balkhy HH (2012) The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. Antimicrobial Resistance and Infection Control 1: 26. DOI: 10.1186/2047-2994-1-26.

Baiu SH, AL-Abdli NE (2015) Screening of MRSA in and outside Benghazi hospitals. American Journal of Microbiological Research 3: 144-147. DOI: 10.12691/ajmrr-3-4-4.

Boyce JM (1992) Methicillin-resistant Staphylococcus aureus in hospitals and long-term care facilities: microbiology, epidemiology, and preventive measures. Infection Control and Hospital Epidemiology 13: 725-737.

Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE (2007) The molecular evolution of methicillin-resistant Staphylococcus aureus. Clinical Microbiology and Infection 13: 222-235. DOI:10.1111/j.1469-0691.2006.01573.x.

Eed EM, Ghonaim MM, Hussein YM, Saber TM, Khalifa AS (2015) Phenotypic and molecular characterization of HA-MRSA in Taif hospitals, Saudi Arabia. The Journal of Infection in Developing Countries 9: 298-303. DOI:10.3855/jidc.5954.

Goyal R, Das S, Mathur M (2002) Colonisation of methicillin resistant Staphylococcus aureus among health care workers in a tertiary care hospital of Delhi. Indian journal of medical sciences 56: 321-324.

Hookey JV, Richardson JF, Cookson BD (1998) Molecular typing of Staphylococcus aureus based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. Journal of Clinical Microbiology 36: 1083-1089.

Hussein NR, Assafi MS, Ijaz T (2017) Methicillin-resistant Staphylococcus aureus nasal colonisation amongst healthcare workers in Kurdistan Region, Iraq. Journal of Global Antimicrobial Resistance 9: 78-81. DOI: 10.1016/j.jgar.2017.01.010.

Iyer A, Kumasani T, Azhar E, Barbour E, Harakeh S (2014) High incidence rate of methicillin-resistant Staphylococcus aureus (MRSA) among healthcare workers in Saudi Arabia The Journal Of Infection In Developing Countries 8: 372-378. DOI:10.3855/jidc.3589.

Jousselin A, Manzano C, Biette A, Reed P, Pinho MG, Rosato AE, Kelley WL, Renzoni A (2016) The Staphylococcus aureus chaperone PrsA is a new auxiliary factor of oxacillin resistance affecting penicillin-binding protein 2A. Antimicrobial Agents and Chemotherapy 60: 1656-1666. DOI:10.1128/AAC.02333-15.

Kobayashi N, Taniguchi K, Kojima K, Urasawa S, Uehara N, Omizu Y, Kishi Y, Yagihashi A, Kurokawa I (1995) Analysis of methicillin-resistant and methicillin-susceptible Staphylococcus aureus by a molecular typing method based on coagulase gene polymorphisms. Epidemiology and Infection 115: 419-426. DOI: https://doi.org/10.1017/S095026880005857X.

Lamikanra A, Paul BD, Akinwole OB, Paul MO (1985) Nasal carriage of Staphylococcus aureus in a population of healthy
Nigerian students. Journal of Medical Microbiology 19: 211-216. DOI:10.1099/00221615-19-2-211.

Lawrence C, Cosseron M, Minoz O, Brun-Buisson C, Costa Y, Samii K, Duval J, Leclercq R (1996) Use of the coagulase gene typing method for detection of carriers of methicillin-resistant Staphylococcus aureus. Journal of Antimicrobial Chemotherapy 37: 687-696. DOI: https://doi.org/10.1093/jac/37.4.687.

Lu PL, Chin LC, Peng CF, Chiang YH, Chen TP, Ma L, Siu LK (2005) Risk factors and molecular analysis of community methicillin-resistant Staphylococcus aureus carriage. Journal of Clinical Microbiology 43: 132-139. DOI: 10.1128/JCM.43.1.132-139.2005.

Madani TA (2002) Epidemiology and clinical features of methicillin-resistant Staphylococcus aureus in the University Hospital, Jeddah, Saudi Arabia. The Canadian Journal of Infectious Diseases 13: 245-250.

Mahesh CB, Badami VS, Ramakant BK, Kullkarni K (2015) Mupirocin resistance in nasal isolates of Staphylococci from health care workers. National Journal of Basic Medical Sciences 5: 78-81.

Majumda D, Barua A, Paul B (2009) Nasal carriage of methicillin resistant Staphylococcus in healthy population of East Sikkim. Indian Journal of Community Medicine 34: 364-365. DOI: 10.4103/0970-0218.58403.

Mehdinejad M, Sheik AF, Jolodar A (2008) Study of methicillin resistance in Staphylococcus aureus and species of coagulase negative staphylococci isolated from various clinical specimens. Pakistan Journal of Medical Sciences 24: 719-724.

Mollaghan AM, Lucey B, Coffey A, Cotter L (2010) Emergence of MRSA clone ST22 in healthy young adults in the community in the absence of risk factors. Epidemiology and Infection 138: 673-676. DOI: 10.1017/S0950268810000191.

Orrett FA, Land M (2006) Methicillin-resistant Staphylococcus aureus prevalence: current susceptibility patterns in Trinidad. BMC Infectious Diseases 6: 83. DOI: 10.1186/1471-2334-6-83.Revelas A (2012) Healthcare – associated infections: A public health problem. Nigerian Medical Journal: Journal of the Nigeria Medical Association 53: 59-64. DOI: 10.4103/0300-1652.103543.

Sader HS, Watters AA, Fritsche TR, Jones RN (2007) Daptomycin antimicrobial activity tested against methicillin-resistant staphylococci and vancomycin-resistant enterococci isolated in European medical centers (2005). BMC Infectious Diseases 7: 29. DOI: 10.1186/1471-2334-7-29.

Shibabaw A, Abebe T, Mihret A (2013) Nasal carriage rate of methicillin resistant Staphylococcus aureus among Dessie Referral Hospital Health Care Workers; Dessie, Northeast Ethiopia. Antimicrobial Resistance and Infection Control 2: 25. DOI: 10.1186/2047-2994-2-25.

Shrestha B, Pokhrel B, Mohapatra T (2009) Study of nosocomial isolates of Staphylococcus aureus with special reference to methicillin resistant S. aureus in a tertiary care hospital in Nepal. Nepal Medical College journal 11: 123-126.

Tiwari HK, Sapkota D, Gaur A, Mathuria JP, Singh A, Sen MR (2008) Molecular typing of clinical Staphylococcus aureus isolates from northern India using coagulase gene PCR-RFLP. The Southeast Asian Journal of Tropical Medicine and Public Health 39: 467-473.

Van Hal SJ, Stark D, Lockwood B, Marriott D, Harkness J (2007) Methicillin-resistant Staphylococcus aureus (MRSA) detection: comparison of two molecular methods (IDI-MRSA PCR assay and GenoType MRSA Direct PCR assay) with three selective MRSA agar (MRSA ID, MRSASelect, and CHROMagar MRSA) for use with infection-control swabs. Journal of Clinical Microbiology 45: 2486-2490. DOI: 10.1128/JCM.00139-07.

Williamson DA, Coombs GW, Nimmo GR (2014) Staphylococcus aureus ‘down under’: Contemporary epidemiology of S. aureus in Australia, Nw Zealand, and the South West Pacific. Clinical Microbiology and Infection 20: 597-604. DOI: 10.1111/1469-0691.12702.

Yousef SA, Mahmoud SY, Eihab MT (2013) Prevalence of methicillin-resistant Staphylococcus aureus in Saudi Arabia: Systemic review and meta-analysis. African Journal of Clinical and Experimental Microbiology 14: 146-154. DOI: 10.4314/ajcem.v14i3.5.

Zorgani A, Elahmier O, Franka E, Grera A, Abudher A, Ghenghesh KS (2009) Detection of meticillin-resistant Staphylococcus aureus among healthcare workers in Libyan hospitals. Journal of Hospital Infection 73: 91-92. DOI: 10.1016/j.jhin.2009.06.019.