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Full Length Research Paper

Investigation of nasal colonization of health care workers by methicillin-resistant *Staphylococcus aureus* with using new generation real-time PCR assay: Discussing of risks

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nasal infectious pathogen which is becoming of significant importance year by year. Mortality, morbidity and treatment costs of MRSA infections have all increased. The most effective preventative tool is rapid confirmation of MRSA existence, followed by efficient execution of the required infection control measures. This study was performed with the aim of evaluating MRSA colonization in health care staff from intensive care units (internal and surgical intensive care units) (ICUs) and how certain risk factors affect their colonization status. The study was conducted prospectively using samples obtained from nasal swabs of health-care staffs working in different missions in the intensive care unit of Gaziantep University Training Hospital in southeast of Turkey. The nasal swab samples were processed using a real-time PCR method platform called GeneXpert (Cepheid). Our PCR screen revealed the presence of MRSA in 14 of 98 health-care staffs. Of these 14 health-care staffs carrying nasal MRSA, 10 were male, 8 were assistant health-care personnel and 11 have been working for over one year in the intensive care unit. Our data showed that male gender and an employment during of more than one year served as significant risk factors for nasal MRSA colonization.

Key words: Methicillin-resistant *Staphylococcus aureus* (MRSA), health care staff, nasal colonization.

INRTODUCTION

Methicillin is an antibiotic derived from penicillin, which has been used as a drug clinically since 1960. Shortly after use clinically in 1960, the *Staphylococcus aureus* (*S. aureus)* strain of bacteria acquired an improved resistance to methicillin (Ulusoy et al., 2003). Methicillin-resistant *S. aureus* (MRSA) strains have become prevalent in extremely hospitalized patients due to the over use of common cephalosporins since 1980 (Ulusoy et al., 2003; Daum and Seal, 2001).

Methicillin-resistance of *S. aureus* strains is caused by expression of the *mecA* gene. Beta-lactam antibiotics, such as penicillin and methicillin, act by preventing murein transpeptidation during bacterial cell wall synthesis by mediation of penicillin binding protein (PBP) enzyme. In the presence of the mecA gene product, PBP-2a, (a variant penicillin-binding protein) which has low affinity to beta-lactam antibiotics, is synthesized; its expression is responsible for the resultant resistance to staphylococci bacteria (Ünal 2007). Natural heterogeneity within potentially resistant populations, with typically 99.9% or greater being initially susceptible (depending on test conditions), means that detection *mec A* by PCR has become the gold standard in the diagnostic laboratory (Ünal, 2007; Thomas et al., 2007). To diagnose MRSA, the Clinical and Laboratory Standards Institute (CLSI)

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recommends certain techniques, including the oxacillin-salt agar method, disc diffusion tests, along with other automated systems. However, the biggest disadvantage of these techniques is the increased time required for their execution (48-72 h) (Velasco et al., 2005). A little part of isolated same MRSA population expresses higher level resistance (hetero-resistance). An additional disadvantage of such automated systems is that hetero-resistant MRSA strains may elicit false negative results (Swenson et al., 2001).

Nosocomial infections represent a common global problem, causing high mortality and morbidity and exponential costs. MRSA is a frequent agent of nosocomial infection. Transmission of MRSA could be prevented through the use of early diagnosis and the execution of exposure precautions. This study was designed to analyze the rates of MRSA colonization in the nasal cavities of health care workers from ICUs and the risk factors for such colonization.

MATERIALS AND METHODS

Healthcare workers from an 18-bed surgical intensive care unit and a 28-bed internal intensive care unit within Gaziantep University Training Hospital in southeast of Turkey were included in this study. Subjects in this prospective study were enrolled during July-August, 2008. Patients related to the study were informed and written consents were obtained. Demographic information of all participants including age, gender, job (staff or consultant), working period and antibiotic intake (during sampling or a week before sampling) was obtained and recorded.

We detected MRSA with GeneXpert (Cepheid, Sunnyvale, CA)'s cartridges by RT-PCR. This system is detected staphylococcal chromosomal cassette (SCC), staphylococcal protein A (*SPA*) and *mecA* gene region. From type I to V of SCCmec cassette could be detected by GeneXpert. Analytical sensitivitiness of this system is 10-100 CFU/swab (SCC*mec* type I, II, III, V are 10 CFU/ swab, SCC*mec* type IV is 50 CFU/swab and SCC*mec* type IVa is 100 CFU/swab). Samples were collected from the anterior nares with two tips and joint swabs specialized to MRSA test. Swabs were inserted into tubes filled with dilution liquid (guanidinium tiosianat and surfactant), broken at the joint, thus releasing the cotton swab portion into the tube and then vortexed for 10 s. Three wells were present on each MRSA cartridge; S (sample) well, 1st well and 2nd well. Elution liquid, containing the collected DNA sample, was added to the S well. This well contained lyophilized Taq polymerase, dNTPs and cattle serum albumin. The 1st well contained lyophilized primers, probes and cattle serum albumin; reagent 1 (3 ml sodium hydroxide) was then added. The 2nd well contained lyophilized sample processing control (SPC) and reagent 2 (2.75 mL tris buffer, EDTA and surfactant mixture) was then added. The SPC included *Bacillus globi* spores and was able to detect internal inhibition of RT-PCR analyses related with sample. Refinement processes such as decontamination or extraction were not performed. RT-PCR results from the samples were obtained in approximately 70 min.

All reagents were stored at 2-28°C. After samples were collected, swabs were stored at 15-30°C for up to 24 h. The tip of one swab was used in the RT-PCR test and the tip of the second swab was stored at 2 - 8°C for up to 5 days. Whenever the sample isolated from the first swab was insufficient, the second tip was used. Xpert cartridges are disposable, thus we were able to prove PCR safety and prevent spread of MRSA. Insufficient quantity of target bacteria, presence of PCR inhibitors in collected samples detected with SPC, reagent level, cartridge loading, prob safety and fluorescent stability with PCC, were all evaluated. Statistical analysis was performed using a chi-square test.

RESULTS

A total of 98 samples from health care workers of ICUs were collected. Demographic information of samples is shown in Table 1. A total of 14 out of 98 (14.3%) samples showed MRSA colonization. This is depicted in Table 2. Ten male (71.4%) and four (28.6%) female health care workers displayed MRSA colonization. The prevalence of nasal carriage of male health care workers was statistically high ($p = 0.02$).

Eight of the nasal MRSA carriers (57.1%) were trained nurses; this observation was not statistically significant when compared to all the other groups. Twelve of the health care workers in this study testing positive for MRSA colonization (85.7%) were staff in ICUs and the rest of them (14.3%) were consultant. The difference between these groups was not statistically significant.

Forty-nine samples were collected from each ICU and same rates of MRSA colonization (7 persons) were detected. MRSA colonization rates of two ICUs were equal. Eleven of the MRSA carriers (78.6 %) have been working for over one year at the time of screening. A working period of over one year posed a significant risk factor to MRSA colonization, showing statistically significance ($p = 0.008$).

DISCUSSION

MRSA is of the most significant pathogens responsible for nosocomial infections. Mortality and morbidity rates in MRSA infections is roughly twice the level of those seen in cases of methicillin-sensitive *Staphylococcus epidermidis* (MSSE) infections (Engemann et al., 2003) and the cost of treatment for MRSA is 1.5 - 3 fold that of MSSE (Engemann et al., 2003; Capitano et al., 2003). MRSA infections are widespread in Turkey and worldwide. According to a study which is aimed to screen antibiotics surveillance in Turkey and Mediterranean countries, MRSA rates among *S. aureus* strains isolated from blood cultures are 43, 40 and 35% in 2003, 2004 and 2005, respectively (Borg et al., 2007). According to a sentry study, MRSA rate of 30.9% was reported in Turkey (Sader et al., 2007). Prevalence rate of MRSA strains is higher (84%) especially in ICUs (Rosenthal et al., 2006). Sentry surveillance program during 1997 - 1999 years reported that MRSA prevalence is 22.4% in Australia, 66.8% in Japanese, 34.9% in Latin America countries, 32.4% in USA and 26% in European Countries (Gulay, 2008).

In this respect, diagnosis and control measures for MRSA infections are of the essence (Vriens et al., 2002).

Table 1. Distribution of samples collected from health care workers.

Working period	Internal Intensive Care Unit					Surgery Intensive Care Unit					Total
	Doctor		Nurse	Trained nurse		Doctor		Nurse	Trained nurse		
	Male	Female	Female	Male	Female	Male	Female	Female	Male	Female	
0 – 1 Month	3	3	-	2	1	-	-	4	2	-	15
2 - 6 months	1	-	7	2	-	-	-	8	-	-	18
7 - 12 months	-	-	6	-	-	2	-	2	2	1	13
>12 months	-	2	9	11	2	5	-	13	9	1	52
Total	9		22	18		7		27	15		98

Eight of 15 doctors served as consultants and the rest serve as staffs.

Table 2. Distribution of healthcare workers in whom MRSA colonization was detected.

No	Gender	Age	Job	Department	Staff and consultant	Working period
1	M[1]	25	T[3]	IICU[5]	S[7]	2 years
2	M	25	T	IICU	S	5 years
3	M	29	T	IICU	S	1 year
4	M	26	T	IICU	S	9 months
5	F[2]	25	N[4]	IICU	S	1 month
6	M	28	Dr	IICU	Co[8]	1 year
7	M	27	T	IICU	S	7 years
8	M	37	T	SICU[6]	S	10 years
9	F	25	N	SICU	S	4 years
10	M	30	T	SICU	S	1.5 year
11	M	31	Dr	SICU	Co	5 years
12	F	25	N	SICU	S	5 year
13	F	21	N	SICU	S	2 months
14	M	34	T	SICU	S	12 years

M[1]: Male, F[2]: Female, T[3]: Trained nurse, N[4]: Nurse, IICU[5]: Internal Intensive Care Unit, SICU[6]: Surgery Intensive Care Unit, S[7]: Staff, Co[8]: Consultant.

Health care workers with colonized MRSA infections present a significant reservoir for transmission of the bacteria to the other workers and to patients (Wenzel et al., 1991; Coello et al., 1994). Previous studies support the notion that the spread of MRSA could be prevented or even eliminated through the use of precautions in conjunction with routine diagnostic tests (Huletsky et al., 2004).

Methicillin resistance of *S. aureus* could be diagnosed by various techniques. The conventional culture technique for MRSA diagnosis is common, rapid and inexpensive. However, such techniques are time-consuming, taking 48-72 h and standardization is difficult (Unal, 2007).

MRSA diagnosis with molecular techniques has been performed since 1980 (Kumar Malhotra et al., 2008). These techniques display the highest level of sensitivity and allow simultaneous detection of *S. aureus* and the *mecA* gene (Holferder et al., 2006). Also, because molecular techniques can utilize collected samples directly, the time required is also much shorter than for standard diagnostic techniques (Jones et al., 2002). The GeneXpert MRSA diagnosis system (Cepheid, Sunnyvale, CA) is an FDA-certified molecular technique for the diagnosis of MRSA infection (Kumar Malhotra et al., 2008). It is a fully automatic RT-PCR platform, taking approximately 70 min. Metha et al. (2007) compared the GeneXpert MRSA test with the conventional culture test using nose swab samples and reported that sensitivity was 98.5% and specificity was 90.4% for GeneXpert MRSA test. This study was designed to emphasize the increased rates of MRSA diagnosis using this test. The ICUs of a hospital is a department that hospitalized risky patients with a higher tendency for infection. We proposed that using rapid diagnosis tests in the ICU is a vital tool for control of MRSA spread and diagnosis.

MRSA carriage by health care workers is important for transmission to patient. Healthcare workers serve as a potential reservoir of MRSA for hospital patients and such MRSA carriers can lead to epidemic spread (Herwaldt, 2003). MRSA diagnosis rates differ among hospitals and communities. The most frequent diagnoses come from

ICU (Gould, 2005). Screening studies among healthcare workers in our country reported that the MRSA diagnosis rate in ICU is 1.5 -35.1% (Kocazeybek et al., 2003; Yetkin et al., 2006; Cesur and Cokca, 2004; Senol and Ozkurk, 2003; Celik et al., 2005). In other countries, colonization rates were reported to be 1.12-19.7% (Scudeller et al., 2000; Gupta et al., 1999; Cespedes et al., 2002). In this study, the portion of nasal carriers of MRSA in our ICU is 14.3%.

Previous studies have shown that working as a health care worker have significant risk factors for MRSA colonization. Hızel et al. (2005) and Shopsin et al. (2000) reported that MRSA colonization is higher in male healthcare workers. In this study, we observed the same, with higher nasal colonization rates found in our male healthcare workers.

Trained nurses had 57% MRSA colonization. This situation may be related with both excessive work load and poor sanitation measures within this group. Another risk factor related to colonization has been duration of employment of healthcare workers. We propose that in-service training for hospital workers should be updated and porters should be screened periodically. Nasal carriage of MRSA in healthcare workers is asymptomatic, and early diagnosis of this carriage is extremely advantageous to preventing transmission.

If preventions are taken to control transmission are accurately applied, infection rate could be decreased. It is necessary that regular investigation of the microorganism colonization in health care workers as well as the surveillance of patients should be conducted. Contact separation is a major prevention tool to control infection in patients with detected MRSA. All hospitals should choose screening program due to their surveillance data and hospitals whose MRSA incidence is high should choose fast diagnosis technique used in this article. These comments will be helpful to diagnose patients quickly and take precautions.

REFERENCES

Borg MA, Kraker M, Scicluna E, Van de SB V, Tiemersma E, Monen J, Grudmann H, Armed Project Members and colloborators (2007). Prevalance of methicillin-resistant *Staphylococcus aureus* (MRSA) in invasive isolates from southern and eastern Mediterranean countries. J. Antimicrob. Chemother. 60(6): 1310-1315.

Capitano B, Pharm D, Leshem OA, Nightingale CH, Nicolau DP (2003). Cost effect of managing methicillin resistance *Staphylococcus aureus* in a long-term care facility. J. Am. Geriatr. Soc. 51: 10-16.

Celik I, Cihangiroglu M, Sevim E, Cabalak M, Akbulut A (2005). Sağlık çalışanlarının burunlarından izole edilen koagülaz pozitif ve negatif stafilokoklarda metisilin direnci ve slime pozitifliği. Fırat Tıp Dergisi 10(3): 123-126.

Cespedes C, Miller M, Quagliarello B, Vavagiakis P, Klein RS, Lowy FD (2002). Differences between *Staphylococcus aureus* isolates from medical and nonmedical hospital personel. J. Clin. Microbiol. 46: 2594-2597.

Cesur S, Cokca F (2004). Nasal carriage of methicillin resistant *Staphylococcus aureus* among hospital staff and outpatients. Infect. Control Hosp. Epidemiol. 25: 169-171.

Coello R, Jimenez J, Garcia M, Arroyo P, Minguez D, Fernandez C, Cruzet F, Gasper C (1994). Prospective study of infection, colonisation and carriage of methicillin resistant *Staphylococcus aureus* in an outbreak affecting 990 patients. Eur. J. Clin. Microbiol. Infect. Dis. 13(1): 74-81.

Daum RS, Seal JB (2001). Evolving antimicrobial chemotherapy for *Staphylococcus aureus* infections: Our backs to the wall. Crit. Care Med. 4: 92-96.

Engemann J, Carmeli Y, Cosgrove SE, Fowler VG, Bronstein MZ, Trivette SL, Briggs JP, Sexton DJ, Kaye KS (2003). Adverse clinical and ecomomic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. Clin. Infect. Dis. 36: 592-598.

Gould IM (2005). The clinical significance of methicillin resistant *Staphylococcus aureus*. J. Hosp. Infect. 61: 277-282.

Gupta N, Prakash SK, Malik VK, Methndiratta PL, Mathur MD (1999). Community acquired methicillin resistant *Staphylococcus aureus*: a new threat for hospital outbreaks. Indian J. Pathol. Microbiol. 42: 421-426.

Gulay Z (2008). Gram pozitif bakteri infeksiyonları: Direnç ve Epidemiyoloji. Ankem Derg. 22(2): 276-286.

Herwaldt LA (2003). *Staphylococcus aureus* nasal carriage and surgical site infections. Surgery, 134: 2-8.

Hizel S, Sanlı C, Kaygusuz S, Tunc A (2005). Kırıkkale Üniversitesi hastane personeli ile hasta ziyaretçilerinde nazal *Staphylococcus aureus* taşıyıcılığı. Van Tıp Dergisi 12(2): 140-144.

Holferder M, Eigner U, Turnwald AM, Witte W, Weizenegger M, Fahr A (2006). Direct detection of methicillin resistance *Staphylococcus aureus* in clinical specimens by a nucleic asid-based hybridisation assay. Clin. Microbiol. Infect. 12: 1163-1167.

Huletsky A, Giroux R, Rossbach V, Gagnon M, Vaillan-court M, Bernier M., Gagnon P, Truchon K, Bastien M., Picard FJ., Belkum A, Quelette M, Ray PH, Bergeron MG (2004). New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of Staphylococci. J. Clin. Microbiol. 42(5): 1875-1884.

Jones D, Speck M, Daschner FD, Grundmann H (2002). Rapid PCR-based identification of methicillin-resistant *Staphylococcus aureus* from screening swabs. J. Clin. Microbiol. 40: 1821-1823.

Kocazeybek B, Ayyildiz A, Gulsoy O, Bayindir O, Sonmez B, Demiroglu C (2003). Hastane personelinde burun portörlüğü ve izole edilen mikroorganizmaların antibiyotiklere duyarlılıkları. Turk Mikrobiol Cem. Derg. 33: 52-56.

Kumar Malhotra S, Haccuria K, Michiels M, Ieven M, Poyart C, Hryniewicz W, Goossen H, MOSAR WP2 Study Team (2008). Current trends in rapid diagnostics for methicillin-resistant *Staphyloccus aureus* and glycopeptide-resistant *Enterococcus* species. J. Clin. Microbiol. 46(5): 1577-1587.

Metha M, Gonzales T, Hacek D, Burdsall D, NG C, Peterson L (2007). Performance of two FDA-cleared PCR tests for MRSA compared to microbiological cultures, abstr. 47th Intersci. Conf. Antimicrob Agents Chemother. 17-20 September 2007, D-880.

Rosenthal VD, Maki DG, Salamao R, Moreno CA, Mehta Y, Higuera F, Cuellar LE, Arikan OA, Aboungal R, Leblebicioglu H, International Nasocomial Infection Control Consortium (2006). Deviceassociated nosocomial infections in 55 intensive care units of 8 developing countries. Ann. Int. Med. 145(8): 582-591.

Sader HS, Watters AA, Fritsche TR, Jones RN (2007). Daptomycin antimicrobial activity tested against 285 methicillin-resistant staphylococci and vancomycin resistant enterococci isolates in European Medical Centers (2005). BMC Infect Dis. 7(1): 29-38.

Scudeller L, Leoncini O, Boni S, Navvara A, Rezzani A, Verdirosi S, Maserati R (2000). MRSA cariage: the relationship between community and healthcare setting. A study in an Italian hospital. J. Hosp. Infect. 46: 222-229.

Senol G, Ozturk T (2003). Bir eğitim hastanesinin cerrahi ve ameliyathane personelinde *Staphylococcus aureus* taşıyıcılığı. Turk Mikrobiol. Cem. Derg. 33: 47-51.

Shopsin B, Mathema B, Martinez J (2000). Prevalance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in the community. JID, 182: 359-362.

Swenson JM, Williams PP, Killgore G, O'Hara CM, Tenever FC (2001). Performance of eight methods, including two rapid methods, for

detection of oxacillin resistance in a challenge set of *Staphylococcus aureus* organism. J. Clin. Microbiol. 39: 3785-3789.
Thomas LC, Gidding HF, Ginn AN, Olmat T, Iredell J (2007). Development of real-time *Staphylococcus aureus* and MRSA (SAM-) PCR for routine blood culture. J. Microbiol. Methods, 68: 296-302.
Ulusoy S (2003). Yoğun bakım ünitesinde Gram-pozitif mikro-organizmalar ve direnç sorunu. Yoğun bakım Derg. 3(2): 118-128.
Unal S (2007). Stafilokoklarda metisilin ve enterokoklarda vankomisin direncinin belirlenmesi. Ankem Derg. 21(2): 166-170.
Velasco D, Delmar Tomas M, Cartelle M, Beceiro A, Perez A, Molina F (2005). Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. J. Antimicrob. Chemother. 55: 379-382.
Vriens M, Blok H, Fluit A, Troelstra A, Werken C, Verhoef J (2002). Costs associated with a strict policy to eradicate methicillin-resistant *Staphylococcus aureus* in a Dutch university medical center: a 10-year survey. Eur. J. Clin. Microbiol. Infect. Dis. 21: 782-786.
Wenzel RP, Nettlemen MD, Jones RN, Pfaller MA (1991). Methicillin resistant *Staphylococcus aureus*: implications for the 1990 and effective control measures. Am. J. Med. 91: 221-227.
Yetkin G, Kuzucu Ç, Bayraktar M, Iraz M (2006). İnönü Üniversitesi Tıp Fakültesinde yoğun bakımlarda yatan hastalarda ve hastane personelinde *Staphylococcus aureus* ve MRSA taşıyıcılığı. İnönü Üniversitesi Tıp Fakültesi Dergisi. 13(2): 91-93.