Original Article

Early Detection and Control of Methicillin Resistant *Staphylococcus Aureus* (MRSA) Outbreak in an Intensive Care Unit

Banu Bayraktar[1], Alper Gündüz[2], Erman Oryaşın[3], Duygu Erdemir[4], Leyla Teke[1], Elif Aktaş[1], Rıza Durmaz[5], Selma Şen[6], Nuray Uzun[2], Bülent Bozdoğan[7]
[1]Clinical Microbiology Laboratory, Şişli Etfal Training and Research Hospital, Istanbul
[2]Infectious disease and Clinical Microbiology University of Health Sciences Şişli Etfal Training and Research Hospital
[3]Aydın Adnan Menderes University, Aydın, Turkey Recombinant DNA and Recombinant Protein Research Center (REDPROM)
[4]Becton, Dickinson and Company Becton, Dickinson and Company
[5]Microbiol. Reference Lab., Ankara, Turkey Clinical Microbiology Laboratory
[6]Sisli Hamidiye Etfal Training and Research Hospital Sisli Hamidiye Etfal Training and Research Hospital
[7]Merkez Kampüs Aydin Menderes University, Faculty of Medicine, Department of Medical Microbiology

Address for Correspondence: Banu Bayraktar, Clinical Microbiology Laboratory, Şişli Etfal Training and Research Hospital, Istanbul
banu_bayraktar@yahoo.com
https://orcid.org/0000-0002-3128-0581
+90 505 832 55 23

Received: 27 February 2020
Accepted: 08 September 2020

DOI: 10.4274/balkanmedj.galenos.2020.

Cite this article as: Bayraktar B, Gündüz A, Oryaşın E, Erdemir D, Teke L, Aktaş E, Durmaz R, Şen S, Uzun N, Bozdoğan B. Early Detection and Control of Methicillin Resistant Staphylococcus Aureus (MRSA) Outbreak in an Intensive Care Unit. Balkan Med J

Background: Although Methicillin resistant ***Staphylococcus aureus* (MRSA)** is one of the major pathogens of healthcare associated infections, we had only sporadic cases in our **intensive** care unit (ICU) for years.
Aims: This study aims to investigate the sudden increase in the number of MRSA cases in ICU.
Study design: A descriptive study to investigate MRSA outbreak in an ICU and signify following standard precautions.
Methods: From the 5th of December 2016 to 26th January 2017, we detected 11 new MRSA cases in ICU. Screening of 73 ICU healthcare workers (HCWs) and screening of 13 patients was performed for outbreak investigation. Nine clinical isolates available in stocks and eight screening MRSA isolates were included in molecular studies. PFGE, Spa-mecA-mecC-pvl in-house multiplex PCR assay and spa typing, SCCmec typing were performed for all isolates. ST **(Sequence type) clone** type of representative strain was determined by Multi-Locus Sequence typing.

Results: All strains were positive for mecA gene, and have the same AST **(Antimicrobial susceptibility testing)** pattern. None of the strains were carrying pvl gene. All clinical, two patient screening and three nasal isolates of HCWs showed the same pulsotype (PT), named as **clone** A. Sequence type of these isolates is t030 and SCCmec type is SCCmecIII. Unrelated three isolates were having PFGE PT B-SCCmecI-t030, PFGE PT C-SCCmecIII-t459, **PFGE PT** D-SCCmecIII. MLST result showed that the outbreak strain belonged to ST239 **clone**.
Conclusion: The outbreak clone (PFGE pulsotype A, ST239-SCCmecIII-t030) was determined by using various molecular techniques. Infection control measures were started in ICU unit. Patients who had already been discharged to other services were put on contact isolation. No further cases occurred after 26th January and the outbreak declared over.
Keywords: MLST, MRSA, Outbreak

Methicillin resistant *Staphylococus aureus* (MRSA) has been described for the first time in1961, following the use of methicillin in 1959. MRSA is a causative agent of a broad spectrum of infectious diseases and considered as a frequent pathogen of healthcare associated infections [1,2]. Methicillin resistance in *S.aureus* is encoded by *mec*A or *mec*C genes which are carried on mobile genetic element known as staphylococcal cassette chromosome (SCC) [1,3]. MRSA outbreaks are serious situations that can result in significant mortality and morbidity [4]. Rapid diagnostic tests are needed to detect infections caused by MRSA strains and to limit the spread of those strains [3]. Genotyping of outbreak strains to detect the possible source of the outbreak and immediate **implementation** of control measures are necessary. Frequently used molecular methods for typing purposes are pulsed-field gel electrophoresis (PFGE), *spa* typing, SCC***mec*** typing, multilocus sequence typing (MLST).
In this study, we aimed to investigate new MRSA cases in the ICU. We screened patients and healthcare workers, and performed spa typing, PFGE, SCC*mec* typing and MLST on MRSA strains in order to decide whether any transmission was occuring and infection preventive measures are mandatory.

METHODS

Outbreak description and bacterial isolates: From 5th of December 2016 to 26th January 2017, we detected 11 new MRSA cases in adult ICU. On 20th, 23rd, 24thof December 2016, Clinical Microbiology Department noticed three consecutive MRSA isolates, two from blood and one from tracheal aspirate culture, having the same antibiotic susceptibility pattern belonging to three different patients in the adult ICU. We searched the Laboratory Information System for MRSA growths starting from October; we found three more isolates and two more cases who have already been discharged from ICU to neurology and general surgery wards. The first isolate is from a blood culture dated 5th of December and belongs to a male patient who had a positive blood culture on the 20th of December. We determine him as the index case. The index case was a morbidly obese patient hospitalized in ICU because of the respiratory failure due to pneumonia and left ventricular dysfunction.
Nasal screening of 74 ICU healthcare workers (HCWs) and nasal, throat, axilla, groin and rectum screening of 13 patients were performed for outbreak investigation. Identification and antibiotic susceptibility test (AST) were done by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and Phoenix100 ID/AST system (Becton **Dickinson**, Sparks, USA) respectively.
Nine clinical isolates available in stocks (7 blood, 1 tracheal aspirate, 1 abscess; one isolate per patient) and eight screening MRSA isolates (5 HCWs and 3 patients) were included in molecular studies.

Evaluation of *mec*A, *mec*C, *spa* and *PVL* Genes by Multiplex PCR: In house multiplex PCR assay was performed as described by Stegger et al. [5].Instagene Matrix (Biorad) was used for DNA extraction. Confirmation of methicillin resistance and identification of isolates was done by amplification of *mec*A/ *mec*C genes and *spa* gene respectively. We also searched for Panton Valentin Leukocidin (*PVL*) gene.

***spa*typing:** s*pa*gene amplicons were purified and sequenced. The open-source spa typing programme at http://spatyper.fortinbras.us/ is used for *spa sequence analysis.*

Pulse Field Gel Electrophoresis (PFGE): PFGE typing of *Staphylococcus aureus* strains was carried out following the protocol of Yetkin et al. [6].Briefly, bacterial cells were embedded into low melting agarose including lysostaphin (5 U/mL). Cells in plugs were digested with proteinase K. After washing the plugs, genomic DNA in the plugs was restricted by 30 U of ***SmaI*** (Promega Corporation, USA) for 24 h at 25 °C in a water bath. DNA fragments were separated on 1% agarose gels run in 0.5× Tris-borate-EDTA buffer using a CHEF-DR III system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14 °C at 6 V/cm^2 for 20 h. The initial and final switch times were 5.3 sec and 34.9 sec, respectively. The gel was stained with ethidium bromide (5 µg/mL) for 20 min and destained with distilled water for 30 min. The

DNA band profiles were visualized under UV light, photographed using a gel logic 2200 imaging system (Kodak Co., Rochester, NY, USA), and analyzed by GelCompar software (version 7.0; Applied Maths, Sint-Martens-Latem, Belgium). A 1% band tolerance was used for comparison of DNA profiles. The clonal relationship among isolates was evaluated by using the criteria of Tenover et al. [7].

SCC*mec* typing: SCC*mec*typing was carried out by using in-house multiplex PCR according to the procedure described by McClure-Warnier et al. [8]. Nine primer pairs were used for the screening of major SCC***mec*** types and subtypes I to V.

Multi-Locus Sequence typing (MLST): The allelic profile of the outbreak MRSA strain was obtained by sequencing internal fragments of seven house-keeping genes as described athttp://saureus.mlst.net/misc/info.asp. [9].Carbamate kinase **(arc)**, Shikimate dehydrogenase (**aro**), Glycerol kinase (**glp**), Guanylate kinase (**gmk**), Phosphate acetyltransferase (**pta**), Triosephosphate isomerase (**tpi**), Acetyl coenzyme A acetyltransferase (**yqi**). The DNA sequence of each **allele** analyzed by using data analysis software at the http://saureus.mlst.net/sql/singlelocus.asp web **site,** and allele numbers of seven amplicons were found out. By entering all allelic numbers of the strain in a program at the http://saureus.mlst.net/sql/allelicprofilechoice.as web site sequence type of the outbreak strain was determined

Results

Patient and HCWs demographics; clonal characteristics and antibiotic susceptibilities of MRSA isolates and molecular typing test results were summarized in Table.1.

Screening Cultures: Screening was performed in ICU on 24th of December. Five out of 13 patients were carrying MRSA strains, in multiple sites of their body; two out of five sharing the same antibiotic **susceptibility** profile was shown to be the outbreak strain by molecular typing methods (15 %). Of those two colonized patients, the one carried the outbreak strain at nasal and groin sites, the other one at the throat, nasal, groin and rectum sites. Rectum colonized patient was 86 years old man with a Fournier's gangrene. 18 HCWs out of 73 were *S.aureus* positive for their nasal swab cultures(10 MSSA; 8 MRSA) and molecular typing was done for **five** MRSA sharing the same or **similar** antibiotic susceptibility pattern with outbreak strain **(Table.1).** Three of HCW's nasal isolates were the outbreak strain (4%).

Antibiotic Susceptibilities: We performed the AST using EUCAST recommended methods and interpretation. The outbreak strain was resistant to **ciprofloxacin** (CIP), levofloxacin (LEV), clindamycin (DA), **erythromycin** (E), **tetracycline** (TE), gentamicin (GN) and tobramycin (TOB) and susceptible to trimethoprim-sulfamethoxazole (TMP-SXT), daptomycin (DPC), fusidic acid (FD), **linezolid** (LZD), and vancomycin (VA) except CI-8 and CI-9. CI-8 and CI-9 were susceptible to DA, E, GN and TOB.

Decolonization of HCWs: All of the HCWs, except one pregnant nurse, were decolonized by TMP-SXT tablets (800/160mg BID for 10 days). We checked the colonization status of HCWs one week after the completion of treatment. They all cleared from the MRSA.

Evaluation of *mec*A-*mec*C-*SPAPVL* Multiplex PCR: All strains were positive for *mec*A gene and negative for PVL gene.

Molecular typing: All clinical isolates, and two patient screening and three nasal isolates of HCW's showed the same PFGE pulsotype (PT) and named as **clone** A **(Fig. 1).** The *spa* sequence type of these isolates is t030, the SCC*mec* type is SCC*mec*III. MLST result is ST239. Clinical isolate 8 (CI-8) and HCW-3 have different SCC*mec* types, SCC*mec*I and SCC*mec*V respectively. Unrelated three isolates were having PFGE PT B-SCC*mec*I-t030, PFGE PT C-SCC*mec*III-t459, PFGE PT D-SCC*mec*III **(Fig. 2).**

Discussion

In this study, we report our experience about the MRSA outbreak in an adult ICU. The outbreak was noticed at the middle of December 2016, by that time four patients have already been discharged from ICU to other clinical wards. We assume that our index case is a male patient who stayed in ICU for over a month. Few healthcare workers were colonized with the outbreak strain and we assume HCWs might have transmitted the outbreak strain to other patients during the medical care as reported in the literature before [10].

To interrupt the MRSA transmission, various infection control measures were established immediately. Nasal screening of HCWs and nasal, throat, axilla, groin and rectal screening of ICU patients were performed [11]. All of HCWs carrying the MRSA strains except one pregnant employee were treated with co-trimoxazole as decolonization procedure since **mupirocin** ointment for nasal usage is not available on the market in our country [12]. We put our infected and colonized patients in contact isolation, including those who have already been discharged to other wards [13]. Infection control nurses gave education sessions to HCWs promoting hand hygiene and enhancing cleaning procedures. We screened patients a month later and there were still patients colonized with the outbreak strain, but no new MRSA cases occurred. So, we declared the outbreak was over and decided to follow up on the situation. Identification of an outbreak is usually based on molecular typing which is a significant tool in infection control. Molecular typing methods show the nosocomial transmission of the successful clone and **elucidate** its transmission routes and sources in an outbreak setting [14].

Different molecular methods are available for typing of *Staphylococcus aureus* isolates, including PFGE, multilocus variable number of tandem repeat analysis (MLVA), SCC*mec* typing, *spa* typing, MLST and full-genome sequencing [1,3,15]. PFGE is still excepted as the "gold standard" for molecular typing of MRSA and ***SmaI*** is the enzyme of the choice for macrorestriction [16]. The major drawback of the PFGE is the insufficient **comparability** of results between laboratories which might be overcome by using standardized protocols nationally [2,16]. On the other hand, results of MLST are comparable between laboratories but its routine use in infection control is restricted by high cost, labour intensity and **availability** of DNA sequencer [16]. Recently, it has been reported that *spa* typing and MLST typing have comparable performance in a macroepidemiologic **study** [15]. Moreover, *spa* typing has higher discriminatory power over MLST, so one ST type can harbour several *spa* types, but they remain within the same clonal complex [17]. In this study, we used several typing methods, PFGE, SCC*mec* typing, *spa* typing and MLST, simultaneously, for the characterization of the outbreak strain. The outbreak isolates showed the same PFGE pulsotype named as cloneA and were ST239-SCC*mec*III-t030 strains.ST239-SCC*mec*III-t030 has previously been reported the most frequent hospital associated (HA) MRSA clone in Turkey [18] and named as TR09. Other studies from Turkey also reported the dominance of MLST ST239 and spa type t030[19,20,21] characterized MRSA **clones. "Oksuz et al. found Vienna/Hungarian/Brazillian clone (ST239-MRSA-III) as the most prevalent clone (53.9%) in a university hospital over 5 years, and rest of the MRSA clones were largely diverse [21].** Geographic distribution of major HA-MRSA clones are known worldwide, but sometimes shifts of these MRSA clones have been occured over time in countries, in a region or even in a single hospital [22]. MRSA was evolved from MSSA in 1961 by the acquisition of a staphylococcal casette chromosome *mec*(SCC*mec*) [1]. Then, a limited number of clones with a certain genetic background and SCC*mec*type have spread worldwide [22]. MRSA ST239 lineage is a globally disseminated HA-MRSA consisted of more than five clades, such as Asia, North America, South America, Europe andAustralia [23]. ST239 is a successful clone in terms of infiltration and adaptation in hospital settings[24]. Several studies are looking for the reasons behind this success. Recently, Hong et al. [24] reported that higher expression of staphylococcal protein A (SpA) in HA-MRSA ST239 helps to durable colonization and immune evasion of the clone. Furthermore, they stipulated that SpA may be a significant factor for the adaptation and persistance of the ST239 clone in hospital **environments.** Moreover, two colonization factors sasX protein, first described in ST239 clone during an outbreak in London and arginine catabolic mobile element (ACME) **are found to be** associated with the **epidemiological** success of the MRSA. ACME is first described in CA-MRSA USA300 and has spread horizontally to HA sequence types such as ST5 and ST239[25,26]. Lastly, enteric carriage of HA-MRSA has been shown for ST228 and was taken as **responsible for maintaining a long-term** outbreak in a tertiary hospital [27]. In our patient group, we found out that one patient has enteric colonization with the outbreak strain. But he was not the index case.
The outbreak strain was resistant to CIP, LEV, DA, E, TE, GN and TOB and susceptible to TMP-SXT, DPC, FD, LZD and VA. This resistance pattern was very similar with the previous reports [21,23,28]. PVL was not detected on the outbreak strain. In conclusion, we described an MRSA outbreak in the ICU due to the clonal spread of the specific strain. Infection control measures put in practice immediately upon the detection of clustering. Through the microbiologic typing methods, outbreak strain was described in detail and index case was identified. Universal contact precautions and treatment of infected patients and decolonization of HCW for nasal carriage controlled the outbreak.

Disclosures

Ethics Committee Approval: Local Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital (date: 30.04.2019; number: 2377).
Financial Disclosure: No relevant financial relationship exits.

References:

[1] Liu, J., Chen, D., Peters, B. M., Li, L., Li, B., Xu, Z., & Shirliff, M. E. (2016). Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant *Staphylococcus aureus*. *Microb Pathog, 101*, 56-67. doi:10.1016/j.micpath.2016.10.028

[2] Simor, A. E., Ofner-Agostini, M., Bryce, E., McGeer, A., Paton, S., Mulvey, M. R.,Canadian Nosocomial Infection Surveillance Program, H. C. (2002).

Laboratory characterization of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: results of 5 years of National Surveillance, 1995-1999. *J Infect Dis, 186*(5), 652-660. doi:10.1086/342292

[3] te Witt, R., van Belkum, A., & van Leeuwen, W. B. (2010). Molecular diagnostics and genotyping of MRSA: an update. *Expert Rev Mol Diagn, 10*(4), 375-380. doi:10.1586/erm.10.34

[4] Khan, A., Lampitoc, M., Slaripour, M., McKernan, P., Devli, R., Muller, M.P. (2009).Rapid control of amethicillin reistant *Staphylococcus aureus*(MRSA)outbreak in a medical surgical intensive care unit (ICU). The *Canadian Journal of Infection Control,* Spring, 12-16.

[5] Stegger, M., Andersen, P. S., Kearns, A., Pichon, B., Holmes, M. A., Edwards, G., Larsen, A. R. (2012). Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either mecA or the new mecA homologue mecA(LGA251). *Clin Microbiol Infect, 18*(4), 395-400. doi:10.1111/j.1469-0691.2011.03715.x

[6] Yetkin G, K. Ç., Durmaz B, Durmaz R, Çizmeci Z, İşeri L. (2009). Molecular typing of methicillin-resistant *Staphylococcus aureus* isolated from bloodstream infections in a university hospital. *Turk J Med Sci, 39*(6), 959-968.

[7] Tenover, F. C., Arbeit, R. D., & Goering, R. V. (1997). How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. *Infect Control Hosp Epidemiol, 18*(6), 426-439.

[8] McClure-Warnier, J. A., Conly, J. M., & Zhang, K. (2013). Multiplex PCR assay for typing of staphylococcal cassette chromosome mec types I to V in methicillin resistant *Staphylococcus aureus*. *J Vis Exp*(79). doi:10.3791/50779

[9] Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J., & Spratt, B. G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol, 38*(3), 1008-1015.

[10] Hegde, N. R., Stock, N. K., Petráš, P., Melter, O., Kapounová, G., Vopalková, P., Jindrák, V. (2016). Importance of Multifaceted Approaches in Infection Control: A Practical Experience from an Outbreak Investigation. *PLoS One, 11*(6), e0157981. doi:10.1371/journal.pone.0157981

[11] Chipolombwe, J., Torok, M. E., Mbelle, N., & Nyasulu, P. (2016). Methicillin resistant *Staphylococcus aureus* multiple sites surveillance: a systemic review of the literature. *Infect Drug Resist, 9*, 35-42. doi:10.2147/IDR.S95372

[12] Song, X., Cheung, S., Klontz, K., Short, B., Campos, J., & Singh, N. (2010). A stepwise approach to control an outbreak and ongoing transmission of

methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Am J Infect Control, 38*(8), 607-611. doi:10.1016/j.ajic.2010.02.017

[13] Jernigan, J. A., Titus, M. G., Groschel, D. H., Getchell-White, S., & Farr, B. M. (1996). Effectiveness of contact isolation during a hospital outbreak of methicillin-resistant *Staphylococcus aureus*. *Am J Epidemiol, 143*(5), 496-504.

[14] Mutters, N. T., Heeg, K., Spath, I., Henny, N., & Gunther, F. (2017).Improvement of infection control management by routine molecular evaluation of pathogen clusters. *Diagn Microbiol Infect Dis, 88*(1), 82-87. doi:10.1016/j.diagmicrobio.2017.01.013

[15] O'Hara, F. P., Suaya, J. A., Ray, G. T., Baxter, R., Brown, M. L., Mera, R. M., Amrine-Madsen, H. (2016). spa Typing and Multilocus Sequence Typing Show Comparable Performance in a Macroepidemiologic Study of *Staphylococcus aureus* in the United States. *Microb Drug Resist, 22*(1), 88-96. doi:10.1089/mdr.2014.0238

[16] Strommenger, B., Kettlitz, C., Weniger, T., Harmsen, D., Friedrich, A. W., & Witte, W. (2006). Assignment of Staphylococcus Isolates to Groups by spa Typing, SmaI Macrorestriction Analysis, and Multilocus Sequence Typing. *J Clin Microbiol, 44*(7), 2533-2540. doi:10.1128/jcm.00420-06

[17] Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A., & Stobberingh, E. E. (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect, 13*(3), 222-235. doi:10.1111/j.1469-0691.2006.01573.x

[18] Bozdogan, B., Yildiz, O., Oryasin, E., Kirdar, S., Gulcu, B., Aktepe, O., Sener, A. G. (2013). [t030 is the most common spa type among methicillin-resistant *Staphylococcus aureus* strains isolated from Turkish hospitals]. *Mikrobiyol Bul, 47*(4), 571-581.

[19] Alp, E., Klaassen, C. H. W., Doganay, M., Altoparlak, U., Aydin, K., Engin, A., Voss, A. (2009). MRSA genotypes in Turkey: Persistence over 10 years of a single clone of ST239. *Journal of Infection, 58*(6), 433-438. doi:10.1016/j.jinf.2009.04.006

[20] Kirca Yilmaz, S., Acuner, I. C., Strommenger, B., Bek, Y., & Witte, W. (2014). [Infectivity-resistotype-genotype clustering of methicillin-resistant *Staphylococcus aureus* strains in the Central Blacksea Region of Turkey]. *Mikrobiyol Bul, 48*(1), 14-27

[21] Oksuz, L., Dupieux, C., Tristan, A., Bes, M., Etienne, J., & Gurler, N. (2013). The high diversity of MRSA clones detected in a university hospital in istanbul. *Int J Med Sci, 10*(12), 1740-1745. doi:10.7150/ijms.6438

[22] Deurenberg, R. H., & Stobberingh, E. E. (2009). The molecular evolution of hospital- and community-associated methicillin-resistant *Staphylococcus aureus*. *Curr Mol Med, 9*(2), 100-115.

[23] Yamamoto, T., Takano, T., Higuchi, W., Iwao, Y., Singur, O., Reva, I., Potapov, V. (2012). Comparative genomics and drug resistance of a geographic variant of ST239 methicillin-resistant *Staphylococcus aureus* emerged in Russia. *PLoS One, 7*(1), e29187. doi:10.1371/journal.pone.0029187
[24] Hong, X., Qin, J., Li, T., Dai, Y., Wang, Y., Liu, Q., Li, M. (2016). Staphylococcal Protein A Promotes Colonization and Immune Evasion of the Epidemic Healthcare-Associated MRSA ST239. *Front Microbiol, 7*, 951. doi:10.3389/fmicb.2016.00951
[25] Otto, M. (2013). How colonization factors are linked to outbreaks of methicillin-resistant *Staphylococcus aureus*: the roles of SasX and ACME. *Biomol Concepts, 4*(5), 533-537. doi:10.1515/bmc-2013-0025
[26] Sabat, A. J., Kock, R., Akkerboom, V., Hendrix, R., Skov, R. L., Becker, K., & Friedrich, A. W. (2013). Novel organization of the arginine catabolic mobile element and staphylococcal cassette chromosome mec composite island and its horizontal transfer between distinct *Staphylococcus aureus* genotypes. *Antimicrob Agents Chemother, 57*(11), 5774-5777. doi:10.1128/AAC.01321-13
[27] Senn, L., Clerc, O., Zanetti, G., Basset, P., Prod'hom, G., Gordon, N. C., Blanc, D. S. (2016). The Stealthy Superbug: the Role of Asymptomatic Enteric Carriage in Maintaining a Long-Term Hospital Outbreak of ST228 Methicillin-Resistant *Staphylococcus aureus*. *mBio, 7*(1), e02039-02015. doi:10.1128/mBio.02039-15
[28] Teo, J., Tan, T. Y., Hon, P. Y., Lee, W., Koh, T. H., Krishnan, P., Network for Antimicrobial Resistance, S. (2013). ST22 and ST239 MRSA duopoly in Singaporean hospitals: 2006-2010. *Epidemiol Infect, 141*(1), 153-157. doi:10.1017/S0950268812000337

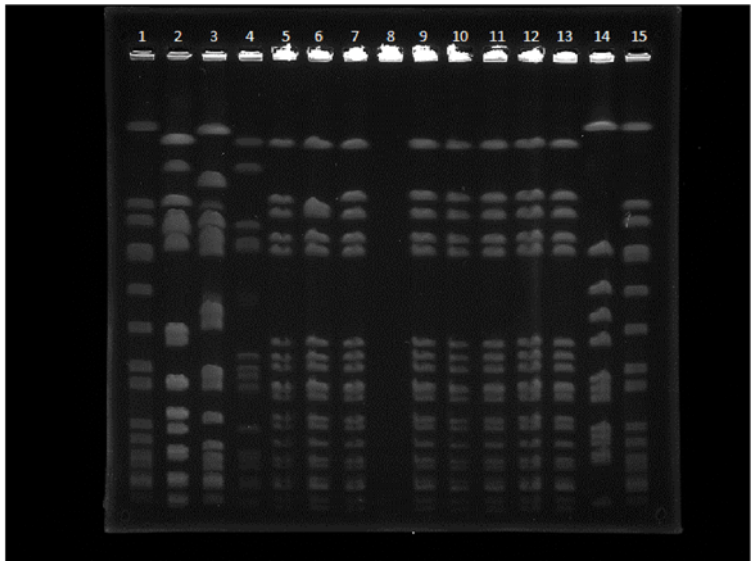

FIG. 1 PFGE *sma*I restriction patterns of MRSA clinical isolates(CI) clone A. Lanes 1, 15, Salmonella Braenderup molecular size marker; lanes 4 to 7 CI-1, CI-2, Cl-3, CI-4 and lanes 9 to13 CI-5, CI-6, CI-8, CI-7, CI-9 respectively; lanes 2 to 4 and 14 clinical isolates that were unrelated to the outbreak; Lane 8 empty.

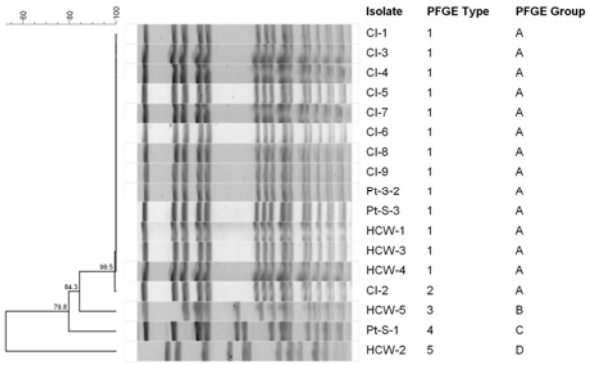

FIG. 2 PFGE dendogram showing different PTs seen over the duration of outbreak (A to D). PT A belongs to outbreak strain.

Table 1. Patient and HCWs demographics; clonal characteristics and antibiotic susceptibilities of MRSA isolates

Isolate Identifier	Age	Gender	Diagnosis	Isolation site	Theraphy	Outcome	PFGE	Spa type	SCC*mec* type	Cefoxitin	Ciprofloxac	Clindamyci	Daptomyci	Erythromy	Fusidic	Gentamicin	Levofloxaci	Linezolid	Rifampicin	Tetracyclin	TMP-SXT	Tobramyci	
			Patients' isolates																				
I -1	48	M	Pneumonia	Blood	PIP-TZP 4,5gr IV TID Daptomycin 500mg IV daily	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
I -2	79	F	Cerebrovascular disease	Trachea	Linezolid 600mg IV BID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
I -3	74	F	Meninx benign neoplasm	Trachea	Vancomycin 1gr IV daily	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
I -4	68	M	Colon cancer	Blood	Daptomycin 350mg IV daily	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
I -5	46	F	Falling from high	Trachea	Tigecycline 50mg IV BID; Meropenem 1g IV TID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
I-6	49	F	Over cancer	Blood	Vancomycin 1gr IV BID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
I-7	88	M	Colon cancer	Abcess	Tigecycline 50mg IV BID; Meropenem 1g IV TID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
I-8	23	M	Drug user, toxication	Blood	Tigecycline 50mg IV BID; Meropenem 1g IV TID	Discharged	A	t030	Type I	R	R	S	S	S	S	S	R	S	R	R	S	S	S
I-9	86	M	Fournier's gangrene	Blood	Linezolid 600mg BID	Died	A	t030	Type III	R	R	S	S	S	S	S	R	S	R	R	S	S	S
			Screening cultures isolates																				
t-S-1	63	K	Crebellar tumor, epilepsy	Throat, nasal	Vancomycin 1gr IV BID	Died	C	t459	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
t-S-2*	74	F	Meninx benign neoplasm	Nasal, groin	Vancomycin 1gr IV daily	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
t-S-3**	86	M	Fournier's gangrene	Throat, nasal,groin, rectum	Linezolid 600mg IV BID	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
CW-1	44	E		Nasal	TMP-SXT 160/800 mg PO BID		A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S

CW-2	23	E		Nasal	TMP-SXT 160/800 mg PO BID		D	ND	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
CW-3	28	E		Nasal	TMP-SXT 160/800 mg PO BID		A	t030	Type V	R	R	R	S	R	S	R	R	S	R	R	S	R	S
CW-4	30	K		Nasal	Not decolonizied		A	t030	Type III	R	R	S	S	S	S	S	R	S	R	R	S	S	S
CW-5	24	K		Nasal	TMP-SXT 160/800 mg PO BID		B	t030	Type I	R	R	S	S	S	S	S	R	S	R	R	S	S	S

I: clinical isolate; Pt-S: Patient screning; HCW: healthcare worker; F: female; M: male; IV: intravenous; PO: peroral; BID: two times a day; TID: Three times a day; S: susceptible; R: resistant; PIP-TZP: *iperacillin/Tazobactam;* TMP-SXT: Trimethoprim/sulfamethoxazole *Pt-S-2and CI-3 are belonged to same patient, **Pt-S-3 and CI-9 are belonged to same patient.