Molecular Detection of Methicillin-Resistant Staphylococcus aureus by Non-Protein Coding RNA-Mediated Monoplex Polymerase Chain Reaction

Cheryl Yeap Soo Yean¹, Kishanraj Selva Raju¹, Rathinam Xavier¹, Sreeramanan Subramaniam², Subash C. B. Gopinath³,⁴, Suresh V. Chinni¹*  

¹ Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Bedong, Malaysia, ² School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia, ³ Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, Kangar, Perlis, Malaysia, ⁴ School of Bioprocess Engineering, Universiti Malaysia Perlis, Arau, Perlis, Malaysia  

* v_suresh@aimst.edu.my

Abstract

Non-protein coding RNA (npcRNA) is a functional RNA molecule that is not translated into a protein. Bacterial npcRNAs are structurally diversified molecules, typically 50–200 nucleotides in length. They play a crucial physiological role in cellular networking, including stress responses, replication and bacterial virulence. In this study, by using an identified npcRNA gene (Sau-02) in Methicillin-resistant Staphylococcus aureus (MRSA), we identified the Gram-positive bacteria S. aureus. A Sau-02-mediated monoplex Polymerase Chain Reaction (PCR) assay was designed that displayed high sensitivity and specificity. Fourteen different bacteria and 18 S. aureus strains were tested, and the results showed that the Sau-02 gene is specific to S. aureus. The detection limit was tested against genomic DNA from MRSA and was found to be ~10 genome copies. Further, the detection was extended to whole-cell MRSA detection, and we reached the detection limit with two bacteria. The monoplex PCR assay demonstrated in this study is a novel detection method that can replicate other npcRNA-mediated detection assays.

Introduction

Over the last several years, there have been dramatic enhancements to Staphylococcus aureus strains to confer resistance against the antibiotic methicillin. The number of Methicillin-resistant Staphylococcus aureus (MRSA) infections is a worldwide concern, particularly in nosocomial settings, as MRSA accounts for 10 to 40% of the overall S. aureus isolates in the United States and European countries [1,2]. MRSA infection is serious and difficult to treat, and only a few antimicrobial agents are available for treating MRSA [3,4]. Therefore, it is important to generate a rapid and accurate detection of MRSA by using a novel molecule as the probe, which can yield higher sensitivity and specificity.
The classical methods of MRSA detection include biochemical tests, the agar dilution technique, and antibiotic susceptibility tests such as the Epsilometer test, Kirby-Bauer disc diffusion method, and immuno-diffusion technique. These techniques often provide ambiguous results and are time-consuming, usually requiring 5 to 7 days. Though, many molecular tests exist; PCR detection of MRSA are currently based on the femA gene [5–7], the meca gene [6–9], or staphylococcal toxin genes such as eta, etb, sea, seb, sec-1, sed, see, and tst [10]. However, there are limitations when using toxin genes because they are present within the coding region [10,11] and are prone to mutation. Further, the meca gene is used predominantly for detecting MRSA; it is conserved in MRSA but often yields unspecific result because it is specific not only to MRSA but also to other methicillin-resistant Staphylococci such as Methicillin-resistant Staphylococcus epidermidis [8]. The same issue occurs when using the femA gene for the molecular diagnosis of MRSA [12–14]. In contrast, detection by DNA hybridization has been found to be a sensitive method for identifying MRSA. However, DNA hybridization suffers from a few disadvantages, particularly in that more cells are required. Moreover, DNA extraction and immobilization on a membrane are tedious processes.

Importance of npcRNA-mediated analysis

The importance of non-protein coding RNA (npcRNA) has been attested in past with functional evidence of its cellular milieu [15–17]. Further, there are evidences to support the use of npcRNA and other short nucleic acids in downstream analytical applications [16,18–20]. To generate a nucleic acid-mediated monoplex PCR, we selected an npcRNA gene as a tool for the detection of MRSA because npcRNA genes are more resistant to mutation than protein-coding genes. Point mutations tend to appear at the non-synonymous regions of genes that code for proteins [21], thus making the detection of a bacterium via PCR using a protein coding gene disadvantageous. In the case of an npcRNA mutation, the functional and secondary structure of the npcRNA would be altered [22]; hence, the bacterium may no longer survive, and detection would not be required. The current study presents a new approach to detecting MRSA by amplifying an npcRNA through PCR as a monoplex. The specificity and sensitivity of the monoplex PCR were studied. The results showed that the designed npcRNA primers are highly specific only to the selective bacteria with the Sau-02 gene and are expressed in Staphylococcus aureus and MRSA.

Recent research has revealed that RNAs are key regulators in pathogens. Bacterial small npcRNAs are structurally diverse molecules that are 50–200 nucleotides long and belong to different classes [23]. The functions of npcRNA include the regulation of stress responses, plasmid and viral replication, bacterial virulence and quorum sensing. In general, npcRNA includes all RNAs except mRNA. Regulatory npcRNAs can base-pair to mRNAs that are acting in trans- or cis- [23] and can thereby either repress or activate translation efficiency by affecting the mRNA target stability [23] for genes that encode virulence proteins [24]. Further, some npcRNAs can bind and modulate the activity of proteins [25].

A recent study achieved the attomolar detection of multiple pathogens by using a npcRNA-mediated genosensor [26], and npcRNA proved to be a novel diagnostic marker for effectively discriminating Salmonella species [18]. Herein, we have improved on the detection of MRSA by developing npcRNA-mediated monoplex PCR using the Sau-02 gene. A total of 142 npcRNAs were identified in S. aureus [27]. Sau-02 is present only in S. aureus including MRSA, even other genus with Methicillin resistance are not possessing Sau-02 gene and hence it is specific to Staphylococcus aureus. Thus it can facilitate downstream analyses such as molecular detection.
Materials and Methods

Bacterial strains

The bacterial isolates in this study used were acquired from Universiti Sains Malaysia (USM); and AIMST University, Malaysia. All bacterial isolates were maintained at -80°C in the recommended storage solution and were revived by inoculation into LB media at 37°C with shaking condition prior to monoplex PCR.

Polymerase chain reaction (PCR)

The specific primers that recognize only *Staphylococcus aureus*/*MRSA* were detected based on the *Sau-02* gene. The forward and reverse primers were *Sau-02*-F: 5′ - GTAAAAAGACGACATGCAGGAA-3′ and *Sau-02*-R: 5′ - CCATCATTTCAAATCTTTGACA -3′. The PCR master reagent mix contained 20 pmol of primers for the *Sau-02* gene, 1X PCR buffer, 2.5 mM MgCl₂, 1 mM dNTPs, 1U DNA Polymerase and DNA template. PCR grade water was used as a negative control by replacing the DNA to evaluate the occurrence of contaminated DNA. The mixture was vortexed briefly. PCR amplifications were carried out using a Bio-Rad DNA Engine thermal cycler with single initial denaturation step (95°C for 300 s), 34 cycles of denaturation (95°C for 30 s), annealing (61°C for 30 s), and extension (72°C for 30 s), followed by a final extension step (72°C for 600 s). The amplified products were resolved using agarose gel (1.5%) electrophoresis, followed by staining with ethidium bromide, and were visualized under appropriate UV illumination.

Optimization by gradient PCR

Gradient PCR is one potential strategy to reduce non-specific annealing and amplification. In the present study, using the gradient function of the universal block, a gradient of 54°C to 62°C was set for the MRSA primers. By varying the annealing temperature in each row, we created 8 discrete annealing temperatures and found that 61°C was the optimal annealing temperature to minimize unspecific annealing of the primers. Thus, further analyses were carried out using constant annealing at 61°C.

Determination of the analytical specificity of the *Sau-02* gene

Specificity test was performed using 18 *S. aureus* strains and 14 non-*Staphylococcus aureus* strains to determine the specificity of the *Sau-02* gene. The other steps were followed as mentioned above.

Determination of the analytical sensitivity of the *Sau-02* gene

To determine the detection sensitivity of the *Sau-02* gene, genomic DNA or whole cells from the test strains were used. To test the detection limit of the npcRNA-mediated monoplex PCR using genomic DNA, different amounts of genomic DNA were extracted from cultured cells, and the templates were serially diluted from 350 ng to 35 ag per PCR. Similarly, the limit of detection of the monoplex PCR with whole bacterial cells was determined by using 2 μl of template from cultured broth of 10-fold serially diluted bacterial culture [10⁻¹ to 10⁻¹⁰] suspensions ranging from TNTC (Too Numerous To Count) to 7 cells/100μl.
Results and Discussion

Analysis of the location of the Sau-02 gene

*Staphylococcus aureus* is an opportunistic bacterium considered as a pathogen for significant infection to human and infects skin and respiratory system. Increasing researches on *S. aureus* in the past attested the importance of *S. aureus* strains with studies on metabolic pathways and analysis on genes [28,29]. Sau-02 is one of the recently identifiednpcRNAs exclusively present in *S. aureus* determined through blastn search. Before being utilized for analysis by monoplex PCR, the location of the Sau-02 gene was analyzed. Based on this preliminary analysis, it was found that the Sau-02 gene is located in two regions of the *S. aureus* genome. These two copies are located in the regions of 1006365–1006481 and 1006734–1006849. Moreover, these Sau-02 genes are located between two hypothetical protein genes: SAS028 (1006203–1006364) and SAS029 (1007021–1007293) in the opposite orientation (Fig 1).

Determination of the Sau-02 gene’s specificity using different bacterial species

The reliability of analytical technique developed is determined by its specificity and sensitivity. Specificity is a prime step for analytical development and can enhance the sensitivity of a given system [30]. In the current study, the specificity of the PCR assay was evaluated using 12 non-*Staphylococcus aureus* species and 2 *Staphylococcus aureus* strains. These bacterial species included *Bacillus subtilis*, *Salmonella typhi*, *Shigella flexneri*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Acinetobacter baumanii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Staphylococcus aureus* and MRSA. Based on monoplex PCR analysis using specific primers for the Sau-02 gene, the PCR amplified products were resolved on agarose gel. From this analysis, it was obvious that the Sau-02 gene was highly specific to both *S. aureus* and MRSA (lanes 12 and 13 in Fig 2). The other bacterial species showed no amplifications (lanes 1 to 11 and 14 in Fig 1).

Fig 1. Location of preferred non-protein coding RNA. Location of the Sau-02 gene is shown. Other genes responsible for hypothetical proteins are also shown.

doi:10.1371/journal.pone.0158736.g001
Determination of the Sau-02 gene’s specificity using MRSA strains

Similar to the above analysis, we also performed monoplex PCR using 18 Staphylococcus aureus strains. An agarose gel displaying the results of the specific PCR using 8 non S. aureus species, 17 strains of MRSA and 1 S. aureus is shown in Fig 3. The analytical specificity testing was positively identified in all MRSA strains and Staphylococcus aureus, where amplicons of 110 bp were detected, thus indicating the occurrence of the target Sau-02 gene in Staphylococcus aureus and MRSA only.

Determination of the sensitivity of Sau-02 detection using genomic DNA

The analytical sensitivity (limits of detection, LOD) of the Sau-02 gene was determined by testing serially diluted genomic DNA stock extracted from MRSA. The sensitivity test was performed to establish the MRSA detection limit. This test was conducted to check the minimal concentration of template that is required to detect MRSA. The genomic DNA was serially diluted to $10^{-10}$ from the stock, representing concentrations of 339.52 ng/μl to 33.952 ag/μl.
From these results, it was noted that MRSA is detectable until the concentration of $10^{-7}$ dilution (33.95 fg/μl) calculated to be ~10 genomic copies of MRSA (Fig 4). The 50% of the highest intensity was observed with 339.52 pg/μl of genomic DNA (Fig 4).

Determination of the sensitivity of Sau-02 detection using whole bacterial cell

Whole cell detection has been an interesting and preferred strategy to detect an organism using the appropriate probe [32]. To give weight to the detection strategy shown here with monoplex PCR, we performed npcRNA-mediated whole cell detection. The bacterial culture was serially diluted as shown in the Fig 5. The numbers of colonies were estimated in all the dilutions per 100 μl of bacterial culture as shown in Table 1. From the results, it is clear that the current npcRNA-mediated monoplex PCR could detect bacterial dilutions up to $10^{-9}$ [~2 bacteria/ reaction] (Fig 6). The 50% of the highest intensity was observed with $10^{-8}$ dilution having ~7 bacteria (Fig 6). The scanning profile obtained using ImageJ software has also shown a clear trend line for the amplification of the Sau-02 gene from MRSA.

S. aureus and MRSA infections are the worldwide challenge in healthcare. The current need in clinical microbiology is to develop an accurate detection method that is highly sensitive and
specific. The World Health Organization recently structured an action plan against antimicrobial resistance which involves a series of approaches including a novel strategy for the diagnosis of pathogens [33]. A DNA microarray was developed representing genes coding housekeeping proteins, virulence factors and antibiotic determinants to detect bacteremia causing *S. aureus*, *E. coli*, *P. aeruginosa* [34]. Despite the accuracy of this method, it suffers from reduced sensitivity due to single or point mutation in the long probes used in microarrays. Application of PCR based on 16S rRNA has replaced time consuming culture based detection until the availability of genome sequences [35]. Currently, detecting meca gene by PCR is the gold standard to identify methicillin resistant *S. aureus* [36]. This detection method is limiting because of the presence of meca gene in non-*Staphylococcus aureus* species [37] and non-*Staphylococcus* strains [38]. The two copies of *Sau-02* npcRNA gene present in *Staphylococcus aureus* and MRSA are highly specific and conserved. Hence, we developed the npcRNA based detection using *Sau-02* gene. Most of the detection studies carried out quantitatively to show the presence

---

**Fig 4. Detection limits of MRSA using genomic DNA.** Lane M: Fermentas 100 bp DNA ladder; Lane C: Negative control (339.515 ng/μl); Lane1 to Lane 11 are sequential dilutions with dilution factors from 1 to 10⁻¹⁰ respectively; Lane 1: 1 (339.52 ng/μl); Lane 2: 10⁻¹ (33.95 ng/μl); Lane 3: 10⁻² (3.39 ng/μl); Lane 4: 10⁻³ (339.52 pg/μl); Lane 5: 10⁻⁴ (33.952 pg/μl); Lane 6: 10⁻⁵ (3.39 pg/μl); Lane 7: 10⁻⁶ (339.52 fg/μl); Lane 8: 10⁻⁷ (33.95 fg/μl); Lane 9: 10⁻⁸ (3.39 fg/μl); Lane 10: 10⁻⁹ (339.52 ag/μl); Lane 11: 10⁻¹⁰ (33.952 ag/μl). + indicates a positive result. Star shows the sensitivity limit. Triangle arrowhead displays the band position. Lower panel is the scanned image from ImageJ software. The trend line for expression is drawn.

doi:10.1371/journal.pone.0158736.g004
Fig 5. Schematic for the preparation of culture dilutions. Preparations of initial dilutions are shown. Other dilutions were prepared in similar way.

doi:10.1371/journal.pone.0158736.g005

| Dilution factor | Number of bacterial colonies (x 10^2) |
|-----------------|--------------------------------------|
| 1               | TNTC                                  |
| -1              | TNTC                                  |
| -2              | TNTC                                  |
| -3              | TNTC                                  |
| -4              | TNTC                                  |
| -5              | TNTC                                  |
| -6              | TNTC                                  |
| -7              | TNTC                                  |
| -8              | 705                                   |
| -9              | 88                                    |
| -10             | 7                                     |

TNTC—too numerous to count

doi:10.1371/journal.pone.0158736.t001
or absence of MRSA [36,39,40]. However, in our study we showed the detection level of genomic DNA equivalent to genome copies and whole cell MRSA as ~10 and 2 respectively. From the present investigation, we recommend that the \textit{Sau}-02 gene shall be exclusively used for the detection of \textit{S. aureus}. Similarly, the \textit{Sau}-02 and \textit{mecA} genes shall be used to detect MRSA. This approach will enhance the accuracy, sensitivity, specificity, speed, and cost effectiveness in the detection of \textit{S. aureus} and MRSA.

**Conclusion**

Non-protein coding RNA (npcRNA) is a widely accepted functional molecule (50–200 nucleotides long) that displays various vital functions. Herein, we used an npcRNA gene (\textit{Sau}-02), to detect \textit{S. aureus} and MRSA. A monoplex PCR assay was designed that demonstrated high sensitivity and specificity. The specificity of \textit{Sau}-02 gene was determined by testing 14 non-\textit{S. aureus} samples.
*Staphylococcus aureus* bacterial species (10 Gram negative and 4 Gram positive) and 18 *S. aureus* including 17 MRSA strains. This study proved the detection of MRSA to the level of 34 fg genomic DNA equivalent to ~10 genomic copies of MRSA. The high sensitivity of the detection using whole cells of MRSA is experimentally shown to detect to the level of $10^{-9}$ dilution i.e., ~2 bacterial cells/reaction. The npcRNA-mediated monoplex PCR assay shown in this study demonstrates a novel and sensitive detection method that can be implemented in other systems.

**Author Contributions**
Conceived and designed the experiments: CYSY SVC RX. Performed the experiments: CYSY KSR SVC. Analyzed the data: CYSY KSR SVC SS SCBG. Contributed reagents/materials/analysis tools: SVC. Wrote the paper: CYSY KSR SVC SCBG.

**References**
1. Tiemersma EW, Bronzwaer SLAM, Lyytikäinen O, Degener JE, Schrijnemakers P, Bruinsma N, et al. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. *Emerg Infect Dis*. 2004 Sep; 10 (9):1627–34. PMID: 15488166
2. Panillio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, et al. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975–1991. *Infect Control Hosp Epidemiol*. 1992 Oct; 13 (10):582–6. PMID: 1469266
3. Hodgson JE, Curnock SP, Dyke KG, Morris R, Sylvester DR, Gross MS. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. *Antimicrob Agents Chemother*. 1994 May; 38(5):1205–6. PMID: 8067768
4. Rasmussen AKI, Skov RL, Venezia RA, Johnson JK, Stender H. Evaluation of mupA EVIGENE Assay for Determination of High-Level Mupirocin Resistance in *Staphylococcus aureus*. *Journal of Clinical Microbiology*. 2010 Sep 1; 48(11):4253–5. doi: 10.1128/JCM.00088-10. PMID: 20810775
5. Mehrota M, Wang G, Johnson WM. Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Meticillin Resistance. *Journal of clinical microbiology*. 2000; 38(3):1032–1035. PMID: 10698991
6. Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, Wauters G, et al. Specific detection of meticillin-resistant *Staphylococcus aureus* species by multiplex PCR. *J Clin Microbiol*. 1995 Nov; 33(11):2864–7. PMID: 8576335
7. Vannuffel P, Laterre P-F, Bouyer M, Gigi J, Vandercam B, Reynaert M, et al. Rapid and specific molecular identification of meticillin-resistant *Staphylococcus aureus* in endotracheal aspirates from mechanically ventilated patients. *Journal of Clinical Microbiology*. 1998; 36(8):2366–7. PMID: 9666026
8. Unal S, Hoskins J, Flokowitsch JE, Wu CY, Preston DA, Skatrud PL. Detection of meticillin-resistant *staphylococci* by using the polymerase chain reaction. *J Clin Microbiol*. 1992 Jul; 30(7):1685–91. PMID: 1629321
9. Krishnan PU, Miles K, Shetty N. Detection of meticillin and mupirocin resistance in *Staphylococcus aureus* isolates using conventional and molecular methods: a descriptive study from a burns unit with high prevalence of MRSA. *Journal of clinical pathology*. 2002; 55(10):745–748. PMID: 12354799
10. Johnson WM, Tyler SD, Ewan EP, Ashton FE, Pollard DR, Rozee KR. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *J Clin Microbiol*. 1991 Mar; 29(3):426–30. PMID: 2037659
11. Marrack P, Kappler J. The *staphylococcal* enterotoxins and their relatives. *Science*. 1990 May 11; 248 (4956):705–11. PMID: 2185544
12. Archer GL, Pennell E. Detection of meticillin resistance in *staphylococci* by using a DNA probe. *Antimicrob Agents Chemother*. 1990 Sep; 34(9):1720–4. PMID: 2285284
13. Ligozzi M, Rossolini GM, Tonin EA, Fontana R. Nonradioactive DNA probe for detection of gene for meticillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1991 Mar; 35(3):575–8. PMID: 2039210
14. de Lencastre H, Sá Figueiredo AM, Urban C, Rahal J, Tomasz A. Multiple mechanisms of meticillin resistance and improved methods for detection in clinical isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1991 Apr; 35(4):632–9. PMID: 2069369
15. Kumarevel TS, Gopinath SCB, Nishikawa S, Mizuno H, Kumar PKR. Identification of important chemi-
16. Gopinath SC, Balasundaresan D, Kumarevel T, Misono TS, Mizuno H, Kumar PK. Insights into anti-ter-
17. Gopinath SC, Wadhwa R, Kumar PK. Expression of noncoding vault RNA in human malignant cells 
18. Nithya R, Ahmed SA, Hoe C-H, Gopinath SC, Citartan M, Chinni SV, et al. Non-Protein Coding RNA 
19. Nadzirah S, Azizah N, Hashim U, Gopinath SC, Kashif M. Titanium Dioxide Nanoparticle-Based Inter-
20. Perumal V, Hashim U, Gopinath SC, Haarindraprasad R, Poopalan P, Liu W-W, et al. A new nano-
21. Haag-Liautard C, Coffey N, Houle D, Lynch M, Charlesworth B, Keightley PD. Direct estimation of the 
22. Hrdlickova B, de Almeida RC, Borek Z, Withoff S. Genetic variation in the non-coding genome: Involve-
23. Chinni SV, Raabe CA, Zakaria R, Randau G, Hoe CH, Zemann A, et al. Experimental identification and 
24. Romby P, Vandenesch F, Wagner EGH. The role of RNAs in the regulation of virulence-gene expres-
25. Repola F, Darfeuille F. Small regulatory non-coding RNAs in bacteria: physiology and mechanistic 
26. Vijian D, Chinni SV, Yin LS, Lertanantawong B, Surareungchai W. Non-protein coding RNA-based gen-
27. Abu-Qatouseh LF, Chinni SV, Seggewiss J, Proctor RA, Brosius J, Rozhdestvensky TS, et al. Identifi-
28. Proctor RA, Kriegeskorte A, Kahl BC, Becker K, Löffler B, Peters G. Staphylococcus aureus Small Col-
29. Chen C, Zhang X, Shang F, Sun H, Sun B, Xue T. The Staphylococcus aureus Protein-Coding Gene 
30. Lakshmipriya T, Fujimaki M, Gopinath SC, Awazu K, Horiguchi Y, Nagasaki Y. A high-performance 
31. ImageJ [Internet]. [cited 2016 Jun 14]. Available: https://imagej.nih.gov/ij/
32. Zhao W-H, Hu Z-Q. β-lactamases identified in clinical isolates of Pseudomonas aeruginosa. Critical 
33. Hoffman SJ, Outterson K, Rettingen J-A, Cars O, Clift C, Rizvi Z, et al. An international legal framework 
34. Cleven BEE, Palka-Santini M, Gielen J, Meembror S, Krönke M, Krut O. Identification and Characteriza-

PLOS ONE | DOI:10.1371/journal.pone.0158736 July 1, 2016 11 / 12
35. Fournier P-E, Dubourg G, Raoult D. Clinical detection and characterization of bacterial pathogens in the genomics era. Genome Med [Internet]. 2014 Nov 29 [cited 2016 Jun 16]; 6. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4295418/

36. Pillai MM, Latha R, Sarkar G. Detection of Methicillin Resistance in Staphylococcus Aureus by Polymerase Chain Reaction and Conventional Methods: A Comparative Study. J Lab Physicians. 2012; 4(2):83–8. doi: 10.4103/0974-2727.105587 PMID: 23441000

37. Xu Z, Mkrtchyan HV, Cutler RR. Antibiotic resistance and mecA characterization of coagulase-negative staphylococci isolated from three hotels in London, UK. Front Microbiol. 2015; 6:947. doi:10.3389/fmicb.2015.00947 PMID: 26441881

38. Seyedmonir E, Yilmaz F, Icgen B. mecA Gene Dissemination Among Staphylococcal and Non-staphylococcal Isolates Shed in Surface Waters. Bull Environ Contam Toxicol. 2015 Jul; 95(1):131–8. doi: 10.1007/s00128-015-1510-z PMID: 25733448

39. Delport JA, Mohorovic I, Burn S, McCormick JK, Schaus D, Lannigan R, et al. Rapid detection of methicillin resistant Staphylococcus aureus bacteremia using a combined three-hour short- incubation-MALDI-ToF identification and the Alere culture colony PBP2a detection test. J Med Microbiol. 2016 May 23;

40. Panda RK, Mahapatra A, Mallick B, Chayani N. Evaluation of Genotypic and Phenotypic Methods for Detection of Methicillin Resistant Staphylococcus aureus in a Tertiary Care Hospital of Eastern Odisha. J Clin Diagn Res. 2016 Feb; 10(2):DC19–DC21. doi: 10.7860/JCDR/2016/17476.7278 PMID: 27042463