Haryana Vet. (June, 2020) **59(1),** 19-21

Research Article

COMPARATIVE STUDY TO CHECK THE EFFICACY OF DIFFERENT METHODS OF DNA ISOLATION

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Received: 30.03.2019; Accepted:19.08.2019

ABSTRACT

Twenty-five confirmed *Staphylococcus aureus* isolates which showed phenotypic resistance to methicillin were subjected to genotypic testing by PCR to check the presence of 16S *r*DNA (genus specific), *fem* (species specific), *mec*A gene (methicillin resistance). DNA was isolated using boiling and snap chill method, Phenol Chloroform isoamylalcohol (PCI) method and kit method. PCR was run using the DNA to compare the efficacy of the three. Genus and *mec*A specific gene isolated from all the three techniques amplified but amplification was only seen in species specific gene isolated using kit method and PCI method. Thus, the study concluded that DNA isolation by *kit* and PCI method was more efficient than by boiling and snap chill method.

Keywords: Boiling and snap chill method, DNA isolation, Phenol Chloroform isoamyl-alcohol (PCI) method, *Staphylococcus aureus*

Extraction of DNA (Deoxyribonucleic acid) is a fundamental step to characterize microbiota associated with animals, environment or humans. The first isolation of DNA was done in 1869 by Friedrich Miescher. Currently, it is a routine procedure in molecular biology or forensic analyses. Selection of a particular technique for the isolation of a DNA from the samples has always been a challenge. DNA can be isolated using a combination of physical and chemical methods. The choice is dictated by the availability of materials, cost, time, safety, and risk of contamination. It is essential to find time and cost effective technique of DNA extraction without compromising its quality. PCR sensitivity detection is considered to show the variation between different DNA isolation methods. In order to check the sensitivity of PCR for the DNA extracted from the commonly used methods in the laboratory, this study was carried out.

MATERIAL AND METHOD

Twenty-Five Methicillin resistant *S. aureus* isolates isolated from different sources (meat, milk, community) were taken from School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University (SPH & Z, GADVASU) culture collection. The isolates were revived by taking a loopful of glycerol stock (stored at -20 °C) and inoculated in 10 mL Tryptose soy broth (TSB). Inoculated tubes were incubated at 37 °C overnight. Turbidity in the broth indicated presence of an organism in the sample. A loopful of inoculum from TSB was streaked on TSA (Tryptose soy agar) plates and incubated for 24 hours. A colony from it was taken and inoculated in TSB. From this broth, DNA was isolated using: i) boiling and snap chill method, ii) Phenol chloroform isoamyl alcohol (PCI) method and iii) HiPurATM bacterial genomic DNA purification kit (HiMedia Lab, Mumbai).

Boiling and snap chill Method: The DNA was isolated as per Arora *et al.* (2006) with slight modifications. In a microcentrifuge tube, 100 µl broth culture was centrifuged at 2,348× g for 5 minutes. Supernatant was discarded and the pellet was resuspended in 100 µl of nuclease free water and kept in boiling water bath for 10 min. After heat treatment, the cell lysate was immediately kept on ice for 10 min followed by centrifugation at 2,348×g for 5 min. The supernatant containing the DNA was aliquoted in a sterile tube and stored at -20 °C until further use.

HiPurATM bacterial genomic DNA purification kit: The isolation of genomic DNA from *S. aureus* was done using HiPurATM bacterial genomic DNA purification kit (HiMedia Lab, Mumbai). *S. aureus* test isolates were inoculated in TSB and incubated at 37 °C for 24 h. After incubation, 1.5 ml of TSB was centrifuged at 12,000 × g for 2 minutes. The supernatant was discarded and the pellet was resuspended in 200 µl of lysozyme solution (45 mg/ml solution, HiMedia lab. Mumbai) and incubated at 37 °C for 30 minutes. After this, 20 µl of protinease K solution (20 mg/ml stock solution, HiMedia lab, Mumbai) and 200 µl of lysis solution was added followed by thorough vortexing for a few seconds and incubation at 55 °C for 10 minutes. Following incubation, 200 µl ethanol (95-100%) was added to the lysate and mixed thoroughly by vortexing for a few seconds. The lysate obtained was put into HiElute Miniprep Spin Column and centrifuged at 8000 × g for 1 minute. The flow through was discarded and the spin column was placed in the same collection tube. This process was repeated until entire lysate was passed through the column.

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Table 1

Primers used for detection of various genes in *S. aureus*

Gene	Oligonucleotide sequence (5'-3')	Amplicon size (Base pairs)	Reference
16S *r*DNA	F: CAG CTC GTG TCG TGA GAT GTR: AAT CAT TTG TCC CAC CTT CG	420	Strommenger *et al.* (2003)
*mec*A	F: AAA ATC GAT GGT AAA GGT TGG CR: AGT TCT GCA GTA CCG GAT TTG C	532	
*fem*A	F: AAA AAA GAC CAT AAC AAG CGR: GAT AAA GAA GAA ACC AGC AG	132	Duran *et al.* (2012)

Column was then rinsed with 500 µl of prewash solution by centrifuging at 8000 × g for 1 minute. The flow through was discarded and the column was rinsed with 500 µl of diluted wash solution followed by centrifugation at 12,000 × g for 3 minutes. The column was then transferred to a new collection tube and centrifuged again at same speed for 1 minute to dry the column. The column was transferred to a fresh collection tube. The bound DNA in column was eluted by adding 200 µl of elution buffer into the column without spilling to the sides. The tube was kept at room temperature for 5 minutes. The tube was then centrifuged at 8000 × g for 1 minute to elute the DNA. The spin filter was discarded and eluted DNA was stored at -20 °C until further used.

Phenol Chloroform isoamyl alcohol method: DNA was isolated as per the method described by Sambrook and Russell (2001) with slight modifications. 600 µl TSB culture was taken in a 2 ml microcentrifuge tube and centrifuged at 3381×g for 10 minutes. This was followed by discarding the supernatant and resuspending the pellet in 600 µl nuclease free water. To it, 10 µl (250 µg/ml) of Proteinase K and 12 µl of 10% Sodium dodecyl sulphate (SDS) was added and further incubated at 56 °C for 1 h. Equal volume (600 µl) of PCI (25:24:1) was added followed by vortexing for 15 seconds and further centrifugation at 15871×g for 15 minutes. Top most aqueous layer was dispensed into another microcentrifuge tube and 600 µl PCI was added to it followed by vortexing for 15 seconds and then centrifugation at 15871 × g for 15 minutes. The supernatant was taken and dispensed into another tube. To it, chloroform: isoamyl alcohol was added and vortexed for 15 seconds followed by centrifugation at 15,871 × g for 15 minutes. Supernatant was taken and dispensed into another tube to which equal volume of ethanol, isopropanol and one-tenth volume of sodium acetate was added and further incubated at -80 °C for 30 minutes followed by centrifugation at 15,871×g for 10 minutes. The supernatant was discarded without disturbing the pellet. The pellet was washed using 500 µl of 70% chilled ethanol. This was followed by centrifugation at 15,871×g for 10 minutes and supernatant was discarded. One more washing was given and finally the pellet was suspended in sterile nuclease free water and stored at -20 °C until further used.

The concentration and purity of DNA was checked using nanodrop. Further DNA was diluted with nuclease free water in such a way that the final concentration was between 50-100 ng/µl. *S. aureus* strain ATCC 33591 was used as positive control for *mec*A resistance gene identification. The multiplex PCR amplification was carried out for the detection of 16S *r*DNA, *fem* and *mec*A genes as per the method optimized by Zehra *et al.* (2017). According to this method, the amplification was carried out in a total reaction volume of 25 µl containing 12.5 µl EmeraldAmp® GT PCR Master Mix (Takara Clontech, Japan), 2.5 m MMgCl2, 0.4 m MdNTP, 10 pmol/µl of each primer set containing forward and reverse primers (Table 1), 0.01 µg- 0.2 µg template and sterilized nuclease free water to make up the reaction volume. Mastercycler Gradient Thermocycler (Applied Biosystems, USA) was used to perform the amplification reaction. The cycling conditions included an initial denaturation at 94 °C for 4 minutes; followed by 30 cycles each of denaturation at 94 °C for 1 minute; annealing at 55 °C for 2 minutes and extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 5 minutes and hold at 4 °C. The amplified PCR product was visualized through agarose gel electrophoresis. Agarose gel (1.5%) was prepared in 1X TBE buffer. Ethidium bromide was added to a final concentration of 0.5 µg/ml when the gel cooled to 50 ºC. DNA ladder (100bp), positive control, negative control and the PCR products (5 µl) were loaded into the wells. The electrophoresis was carried out at 80 v/cm for 1 h. Gel documentation system (Syngene, U.S.A) was used for the visualization of the bands.

RESULTS AND DISCUSSION

DNA was isolated using these three techniques. The concentration and purity of DNA was checked using nanodrop (Thermofisher scientific). The concentration of DNA was highest in PCI method followed by kit method and lowest for the snap chill method. However, the purity was almost equal for kit and PCI method. The concentration of the DNA isolated by boiling and chill method was

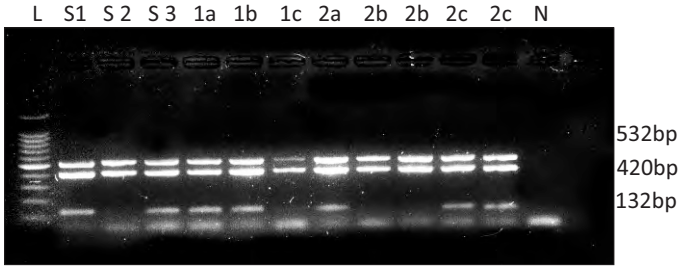

Fig. 1. Gel electrophoresis picture of 16SrDNA (420bp), femA (132bp), mecA (532bp)
L-Gene ruler; S1- Positive control , DNA isolated by kit method; S2- Positive control, DNA isolated by Boiling and snap chill method; S3- Positive control, DNA isolated PCI method; 1a, 2a- DNA from kit method; 1b, 2c-DNA from PCI method; 1c,2b-DNA from boiling and snap chill method; N-No template control

between 80 to 200 ng/µl with A260/A280 ratio between 1.6-1.7. By PCI method, the concentration of DNA was between 700-1000 ng/µl with A260/A280 ratio 1.8 to 1.9. DNA isolated by kit method had a concentration of 180-300 ng/ µl with A260/A280 ratio 1.8 ± 0.5. After amplification in the thermalcycler, the product was run on agrose gel electrophoresis (AGE). On AGE in which DNA from kit and PCI method was used, all the three bands indicating the presence of 16S *r*DNA, *fem* and *mec*A genes were visible (Fig. 1). However, fem gene was absent when DNA from snap chill method was used.

The results of PCR inhibition are in concordance with the other studies that have reported varied level of PCR inhibition from the environmental samples (Rock *et al.*, 2010; Jane *et al.*, 2014). Salt, DNase, RNase are common impurities in the nucleic acid samples (Smaraon and Smith, 2003). In order to proceed for the further processes and analysis, it is important to increase the purity of nucleic acids by removing these factors (Sambrook and Russel, 2001). For DNA isolation by kit method or PCI method, chemicals are used in stepwise manner to neutralize these impurities, but in DNA isolation by boiling and snap chill method, only the extremes of temperature and no chemicals are used for DNA isolation. Thus, the DNA isolated by boiling and snapchill method contain many impurities which may inhibit the activity of the components important for the amplification of the DNA, thus giving the negative results even in the presence of that particular gene.

CONCLUSION

Boiling and Snap chill method although cost and time efficient when compared to PCI and kit method, however, as per this study, the technique was not completely reliable to conclude the mere absence of the band as negative for that particular gene.

ACKNOWLEDGEMENT

This work was financially supported at School of Public Health and Zoonoses, GADVASU under Rashtriya Krishi Vikas Yojana (RKVY).

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