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Comparative Evaluation of Three Different Methods of Genomic DNA Extraction for *Staphylococcus aureus*

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Abstract: Extraction of DNA from *Staphylococcus aureus* is often difficult and less efficient. In this study, the relative recovery of extraction of staphylococcal DNA from pure culture was compared using rapid lysis, bashing beads and commercial silica column methods. For both methicillin - resistant and sensitive *Staphylococcus aureus* the DNA extracted by rapid lysis method was found to have higher value of DNA concentration than bashing beads and silica column methods. The concentrations of DNA obtained from both methicillin – resistant and sensitive *Staphylococcus aureus* by rapid lysis method were 74 and 98 µg/ml, while DNA obtained by bashing beads method were 35 and 31 µg/ml in that order from the same strains. The DNA concentrations obtained from the commercial silica column method were 5 and 3 µg/ml correspondingly from the same strains. The present study indicated that rapid lysis method might be a suitable choice for extracting DNA from *Staphylococcus aureus* strains. For commercial silica column method, high molecular weight intact DNA bands were not observed. Therefore the use of specific and optimized DNA extraction methods is important for the successful PCR on clinical samples.

Key words: DNA extraction · Genomic · *Staphylococcus aureus* · Methicillin-resistant

INTRODUCTION

Molecular methods have increasingly been used for the detection of staphylococcal DNA. Furthermore, the need for a rapid and easy-to-use on-site molecular detection method is imperative. Many techniques have been used for DNA extraction from clinical samples, such as enzymatic, chemical or thermal lysis, mechanical disruption of the cell wall by beads or sonication, or a combination of the above [1-2]. A method to be chosen should be sensitive, less time consuming and cost-effective in its ability to isolate staphylococcal DNA.

Molecular analysis of clinical samples for *Staphylococcus aureus* can offer various advantages over culture methods, including greater sensitivity and specificity [3]. Time constriction may make conventional phenol chloroform extraction of staphylococcal DNA impractical as additional purification processing may be required to take out carry-over phenols which suppress the polymerase chain reaction [4]. Gram positive cell wall has a thicker and highly cross linked peptidoglycan layer than Gram negative and it lacks the outer membrane [5]. In *S. aureus* cell wall, a pentaglysine inter-bridge links the tetrapeptide units of adjacent glycan strands [6]. The use of appropriate DNA extraction methods is critical for successful PCR studies on clinical samples and it is recommended that the DNA extraction techniques should be carefully selected with particular regard to the specimen type [7]. The purity of DNA extracted from samples is essential in the precision and usefulness of

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biological techniques such as PCR for infectious pathogens. Rapid lysis technique is used for release staphylococcal DNA to be used by PCR to detect different genes in *S. aureus* strains. Moreover, this method has advantage of the overall reduction in time required to obtain results [8]. This study was designed to compare three different techniques for staphylococcal nucleic acid extraction estimated by ultraviolet absorbance spectrophotometry and agarose gel electrophoresis.

MATERIALS AND METHODS

Staphylococcus aureus strains were collected from the laboratory of the Department of Microbiology and Parasitology, School of Medical Sciences, University Sains Malaysia from August 2010 to February 2011. Staphylococcal DNA was extracted from pure culture using three extraction methods.

In *rapid lysis method*, a standard protocol for extraction of DNA was used to provide a source of staphylococcal DNA in PCR experiments [8]. For the rapid lysis procedure, bacteria were harvested from agar plates by taking 1 to 3 colonies from each agar plate and suspended in 15 µl of sterile water. The cells were resuspended again in 50 µl of lysostaphin (100 µg/ml in water; Sigma Chemical Co., St. Louis, Mo.). Cells suspensions were incubated at 37°C. for 10 minutes, then 50 µl of proteinase K solution (100 µg/ml; Sigma) and 150 µl of buffer were added (0.1 M Tris (pH 7.5)). Cells suspensions were incubated at 37°C for an additional 10 minutes and then placed in a boiling water bath for 5 min. This treatment efficiently lysed *S. aureus* cells and prevented DNase activity.

In *bashing beads method*, 1 to 3 colonies of pure staphylococcal cells were suspended to 200 µl of buffer and then transferred to a bashing bead lysis tube and 750 µl lysis solution was added to the tube and fixation in a bead beater was done and running at maximum speed for 5 minutes was performed; followed by centrifugation of the tube at 10,000 x g for 1 minute. Later on 400 µl of the supernatant was transferred to a spin filter within a collection tube and centrifuged at 7,000 x g for 1 minute. After that 1,200 µl of bacterial DNA binding buffer was added to the filtrate in the collection tube. Then 800 µl of the mixture was transferred to column in a collection tube and centrifuged at 10,000 x g for 1 minute, the flow through was discard, 500 µl DNA wash buffer was added to the column in a new collection tube and centrifuged at 10,000 x g for 1 minute. Lastly the column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl DNA elution buffer was added directly to the column matrix and centrifuged at 10,000 x g for 30 seconds to elute the DNA. In commercial *silica column method* (QIA amp DNA Mini Kit), 1 to 3 colonies of pure staphylococcal broth was centrifuged for 10 minutes at 5000 x g. The bacterial pellet was resuspended in 180 µl of enzyme solution (200 µg/ml lysostaphin; 20 mM Tris HCl, pH 8.0; 2 mM EDTA; 1.2% Triton) and incubated for 30 minutes at 37°C. Then 20 µl proteinase K and 200 µl buffer were added and mixed by vortexing, after that they were incubated at 56°C for 30 minutes. Again the tube was centrifuged for a few seconds and 200 µl buffer was added and mixed well, later 200 µl ethanol 96% was added to the sample and mixed well. Carefully the mixture was transferred to mini spin column inside a 2 ml collection tube and centrifuged at 6000 x for 1 minute and the mini spin column was placed to a clean 2 ml collection tube. Finally 200 µl buffer AE was added and incubated at room temperature for 1 min and then centrifuged at 6000 x g for1 minute to elute the DNA.

Purified genomic DNA was visualized by 0.8-1.0% agarose gel electrophoresis. Five microlitres of genomic DNA was loaded on agarose gel for 30 min and ran at 60 volt. Measurement of DNA concentration was made by ultraviolet absorbance spectrophotometry. DNA absorbance was measured at 260 nm. Distilled water was used as blank. The dilution factor was set at 1:50 ratio. The DNA was diluted accordingly by adding one microliter of the DNA to 49 µl of distilled water in the cuvette and mixed well by pipetting up and down the mixture. Reading of the absorbance was performed by spectrophotometer and the reading was automatically converted to the concentration of DNA and was expressed in µg/ml.

RESULTS AND DISCUSSION

In this study three different DNA extraction methods were assessed in order to determine the best method to extract staphylococcal DNA from clinical samples. The comparison of three DNA extraction methods in terms of DNA concentration for both methicillin-resistant and sensitive *S. aureus* strains is shown in Figure 1. The DNA extracted by rapid lysis lysostaphin method was found to have higher values (74, 98 µg/ml) DNA concentration of both methicillin-resistant and sensitive *S. aureus* than in two other methods.

Table 1: Concentration and purity ratio of extracted genomic DNA from both methicillin-resistant and sensitive *S. aureus* demonstrated by three different methods of DNA extraction

	S. aureus type concentration (µg/ml)		Absorbance ratio (A260/A280 nm)	
Methods	MRSA	MSSA	MRSA	MSSA
Rapid lysis lysostaphin method	74	98	1.8071	1.7828
Bashing beads method	35	31	1.8023	1.7360
Commercial silica column method	5	3	1.7072	1.7219

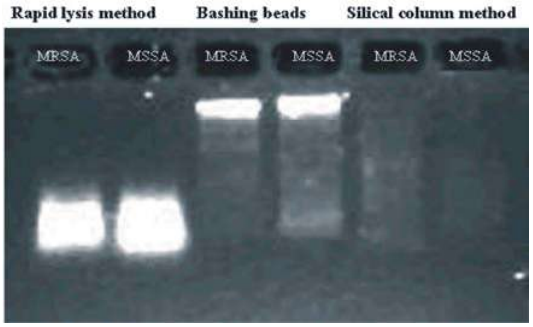

Fig. 1: Agarose gel showing high yield DNA is achieved form both MRSA and MSSA using rapid lysis method in comparing to bashing beads and silica column methods.

This study showed that rapid lysis lysostaphin and bashing beads methods of extracting DNA from pure culture were more successful than commercial silica column method. Quality and quantity of the DNA were found better in rapid lysis lysostaphin and bashing beads DNA extraction methods. Bright DNA bands were observed punctually in those specimens which were extracted by using rapid lysis lysostaphin and bashing beads methods. In rapid lysis lysostaphin DNA method cells were disrupted quickly, efficiently and safely [8]. DNA extraction efficiency is restricted by type of bacterial species [9]. Ultraviolet absorbance of the extracted DNA was shown in Table 1. In the silica column DNA extraction method, too much reagents used which were too much costly than reagents used in rapid lysis lysostaphin and bashing beads DNA extraction methods. While in rapid lysis lysostaphin DNA extraction method needed only lysostaphin and proteinase K. Rapid lysis lysostaphin and bashing beads DNA extraction methods were less time consuming than silica column DNA extraction method. Thirty minutes were needed for rapid lysis lysostaphin method whereas, silica column DNA extraction method required approximately twice time than rapid lysis lysostaphin method and bashing beads DNA extraction method needed only forty minutes.

The constitution of the bacterial cell wall may impede cell lysis and the liberation of DNA from the cell. Silica column method was unable to extract DNA properly for producing DNA bands. This loss of DNA could be happened due to inability of this method to properly lysis the thick cell wall. With a pure sample of genomic DNA, the ratio of absorbance at 260 nm and 280 nm (A260/A280) is 1.8 to 2.0 while a reading that is less than 1.7 and higher than 2.0 suggests that the DNA extracted is contaminated with protein and RNA, respectively [10].

In conclusion the rapid lysis method extracted a higher value of DNA concentration and the extracted DNA was of high molecular weight for both MRSA and MSSA strains. From this study we could be suggested that rapid lysis method might be a suitable choice for extracting DNA from both MRSA and MSSA strains because it's less time consuming, cost-effective and easy-to-use. Therefore, this study indicated that DNA extraction methods should be cautiously selected with particular concern to the specimen type. Additional comparative studies are necessary to ensure that staphylococcal DNA extraction techniques are optimized for each particular application. Following the results of this study, we recommend rapid lysis method as a method of choice for staphylococcal DNA extraction, based on the processing time, easiness of use and DNA extraction efficiency.

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