# COMPARISON OF THREE METHOD OF DNA EXTRACTION FOR METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

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## ABSTRACT

**Background:** MRSA is a gram positive bacteria that has a peptidoglycan in the cell wall which is not easy to lysis during the DNA extraction procces.
**Objectives:** This study aimed to compare three DNA extraction method, using a conventional method, chelex method, and commercial kit in MRSA.
**Methods:** This is a descriptive study with laboratory experiments. The sample was swabs of purulent wounds from inpatients in Jambi that were randomly selected. Swab sample identified as MRSA was extracted with three methods and tested using UV-Vis spectrophotometer.
**Results:** The study results showed that the best DNA purity was found by DNA extraction using commercial kit (1.43), followed by chelex (1.14) and conventional (1.07) methods. Likewise, the highest concentration of DNA was DNA extraction using commercial kit (330 g/ml), followed by the chelex (78 g/ml) and conventional (54 g/ml) methods.
**Conclusion:** A conventional method can be used as an extraction method that is easy to do and more economical. The best DNA purity and concentration was obtained in the kit extraction method, followed by chelex and conventional methods.

**Keywords**: MRSA, extraction, conventional, chelex, kit

## ABSTRAK

**Latar belakang:** MRSA adalah bakteri gram positif yang memiliki dinding peptidoglikan yang tidak mudah lisis selama proses ekstraksi DNA.
**Tujuan:** Penelitian ini bertujuan membandingkan tiga metode ekstraksi DNA, menggunakan metode konvensional, metode chelex dan kit komersial pada MRSA.
**Metode:** Penelitian ini merupakan studi desktiptif dengan eksperimen di laboratorium. Sampel merupakan swab dari luka purulent pasien yang di rawat inap di Jambi yang dipilih secara random. Sampel swab yang teridentifikasi sebagai bakteri MRSA diekstraksi dengan tiga metode dan dites dengan UV-Vis spektrofotometer.
**Hasil:** Hasil penelitian menunjukkan bahwa kemurnian DNA yang paling baik diperoleh pada ekstraksi DNA dengan kit komersial (1,43), diikuti metode chelex (1,14) dan metode konvensional (1,07). Sedangkan konsentrasi DNA tertinggi ada pada DNA yang diekstraksi menggunakan kit komersial (330 g/ml), diikuti dengan chelex (78g/ml) dan metode konvensional (54 g/ml).
**Simpulan:** Metode konvensional dapat digunakan sebagai metode ekstraksi yang mudah dan murah. Kemurnian DNA dan konsentrasi DNA didapatkan melalui metode ekstraksi dengn kit komersial, diikuti chelex dan metode konvensional.

**Kata kunci :** MRSA, ekstraksi, konvensional, chelex, kit

## INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an infection caused by *Staphylococcus aureus* (*S. aureus*), which is resistant to methicillin and other beta-lactam antibiotics, such as penicillin, cephalosporins, monobactams, and carbapenems. MRSA is resistant to beta-lactam antibiotics and non-beta-lactam antibiotics such as macrolides (erythromycin), protein synthesis inhibitors (tetracyclines, chloramphenicol), and quinolones.[1,2,3] MRSA is resistant due to genetic changes caused by exposure to irrational antibiotic therapy. At the hospital level, the mechanisms of bacterial resistance are thought to be through exposure of bacterial population by resistant organisms, spontaneous mutation of sensitive strains or genetic transfer, expression of resistance in bacteria that were previously present in the population, and the spread of resistant organisms through cross-transmission mechanisms.[4]

The prevalence of MRSA infection is increasing. Data show that from 94,000 cases of infection in America, the morbidity rate due to MRSA infection is 18,650 cases.[5] The prevalence of MRSA infection in Indonesia reach approximately 30-40%.[6,7] Also in Jambi, a previous study in 2107 showed a relatively high incidence of MRSA, 11 samples (45.83%) from a total of 24 samples of *S. aureus*.[8]

MRSA diagnosis using the Polymerase Chain Reaction (PCR) method is an alternative examination that is more time-efficient despite the high cost. The cost depends on some component, one of them is DNA extraction method. MRSA is a gram positive bacteria that has a peptidoglycan in the cell wall. Peptidoglycan is a non-covalent binding protein which is contributed to the immobilize proteins on its surface so that gram positive wall become resitant to chemical treatment and not easy to lysis.[9] This is a reason for bad quality DNA in the DNA extraction procces. Conventional DNA extraction methods are generally cheaper than extraction using commercial kits, although kits usually provide more accurate results. In addition, there is also an isolation method using chelex as a lysis buffer which is not as cheap as the conventional method but cheaper than using a commercial kit. This study compared DNA extractions using a conventional method, chelex method, and commercial silica column method for MRSA.

## METHOD

MRSA bacteria was found from clinic isolate on inpatients festering wound at hospitals in Jambi. Wound swabs taken using a sterile disposable swab covered aseptically. Swab samples were rubbed on Mannitol Salt Agar (MSA) media surfaces and incubated for 16 -18 hours at 35 °C. Pure colonies were harvested from MSA agar and confirmed as gram-positive cocci through gram staining. Then *S. aureus* was identified using Staphytect plus Test DR 850 M (Oxoid) by means of latex agglutination to detect

clumping factors, protein A and capsule polysaccharide types 5 and 8.

Bacterial DNA extraction with conventional methods was by using bacteria on MSA media after an 18-24-hour growth and then dissolved in sterile 0.9% NaCl solution until the turbidity reaches a standard of 0.5 Mc. Farland. The bacterial suspension was ready to use as a template for PCR.[10]

DNA extraction carried out using chelex method was done using five bacterial colonies placed in a tube containing 500 µL of phosphate buffer saline (PBS) and centrifuged at 5,000 rpm for 5 minutes. The supernatant was removed, and the pellet obtained was resuspended with lysis buffer, i.e. saponins. It was then incubated on ice for 10 minutes and then centrifuged at 12,000 rpm for 10 minutes. As much as 50 µL Chelex resin 20% and 100 µL sterile distilled water were added, boiled in boiling water for 10 minutes, and centrifuged at 12,000 rpm for 10 minutes. DNA would be in the supernatant. A total of 2–3 µL of liquid containing DNA could be used as a Polymerase Chain Reaction (PCR) template.[11]

Bacterial DNA extraction carried out using commercial silica column method (Qiagen 69534 Mercon DNA Bacteria Plus Kit) was done according to the instructions. Furthermore, the DNA isolates were analyzed for the DNA concentration and purity using a UV-Vis spectrophotometer.

## RESULTS

In this research, three DNA extraction methods were assessed in order to determined the best method based on time, cost and the concentration and the purity of DNA isolate of MRSA. Total time and cost needed on each DNA extraction method were shown on **Table 1.** This result shows that conventional method (5 minutes) need shortest time than chelex (45 minutes) and commercial silica column method (25 minutes). The conventional method also cheaper than other DNA extraction method.

**Table 1.** Total time and cost of three DNA extraction method

| Extraction Method | Time (minute) | Price of each sample (Rupiah) |
|---|---|---|
| Conventional | 5 | 10.000,- |
| Chelex | 45 | 50.000,- |
| Commercial silica column | 25 | 200.000,- |

**Table 2** shows that the best DNA purity is DNA extraction using commercial silica column method (1.43) and then followed by chelex (1.14) and conventional methods (1.07). Similarly, the highest DNA concentration was DNA extraction using commercial silica column method (330 µg/ml), followed by chelex method (78 µg/ml) and conventional method (54 µg/ml). This is in line with a hypothesis that DNA extraction using kits is better because of its ability to remove DNA inhibitors than the chelex and conventional methods. In addition, the chelex method is also better than the conventional method because, in direct dilution with sterile 0.9% NaCl, there are still many DNA inhibitors.[12]

**Table 2.** Concentration and Purity of DNA Isolate with Three Different Extraction Methods

| Extraction Method | DNA Purity (A260/280) | DNA Concentration (µg/ml) |
|---|---|---|
| Conventional | 1.07 | 54 |
| Chelex | 1.14 | 78 |
| Commercial silica column | 1.43 | 330 |

The conventional method is the simplest method to obtain DNA. The process is easy to organize and economical. However, some enzyme inhibitors, protein, and bivalent cations are still present in the final extraction result, which might become inhibitors for further investigations, such as PCR or sequencing.[13] Chelex 100 (Bio-Rad Laboratories, CA, USA) or Chelating Ion Exchange Resin contained chelex resin that can extract DNA by heating at certain temperatures, usually at 95-100 ºCelsius for 30-45 minutes. During the extraction process, the chelex resin was able to protect the sample from DNA-se enzymes through binding ions and cations, such as magnesium ion ($Mg^{2+}$). Through this binding, the chelex resin makes DNA-se unreacted, thereby protecting the DNA, which greatly affects the success of the PCR process. After the extraction process, the cellular components and DNA and RNA will be dissolved in the chelex solution. As a result, the DNA is on the surface (supernatant), and the cellular components are at the bottom (sediment).[11]

The extraction process using chelex has an easy process, even need more time then other method. Chelex is also a choice of reagent that is economical and safe to use because it does not contain harmful solvents. However, chelex reagent has a weakness in DNA extraction that relatively little DNA or RNA is produced since the heating step can damage the DNA structure (denaturation); thus, a freeze shock is needed after the heating process to normalize the DNA structure. It also requires reliable skills to take DNA extracts

so that they are not contaminated with other molecules or compounds. In addition, DNA molecules produced by this method are less stable in storage for a long time.

Between the two methods described above, the extraction method using a kit called a double spin column offers a different situation, namely the use of a silica membrane to trap DNA released from the cells. In addition, the use of kits with special reagents usually used in this method can increase the quantity and quality of the DNA produced. The enzymatic reaction of proteinase K used in this method can lyse DNA in the cells, thus facilitating the DNA extraction process. Special reagents were used to bind and clean residual alcohol and other impurities, such as some enzyme inhibitors, proteins, and bivalent cations remaining from the sample preservation process. More than once rinsing processes using special reagents and high-speed centrifugation process (8000 rpm to 14000 rpm) applied in the method were able to produce DNA extracts that were cleaner, purer, and with higher concentrations. Thus, the resulting DNA extract is expected to support the success of the following process (DNA amplification and DNA sequencing).[14]

In addition, the use of several special buffer solutions in the kit, which work by binding to DNA, can maintain the quality of DNA extracts for more extended storage (more than 2-3 years). These are some of the advantages of this method. On the other hand, the main drawback of using this method is that in general, the extraction process requires a relatively long time, which is about 2 hours and maybe even more than 24 hours, starting from the cell splitting process to obtaining DNA extract, so it is considered less practical. In addition, the price of the kit, which is still expensive compared to the cost of the reagents used in other methods, is a separate obstacle in using this method.

## CONCLUSION

The conventional method need less time and cost than chelex and commercial silica column method. The highest concentration of DNA was found in the kit extraction method, followed by chelex and conventional methods. Likewise with DNA purity, the kit extraction method yielded higher results, followed by chelex and conventional methods. A conventional extraction method can be used as an extraction method that is easy to do and more economical to get MRSA isolate. However, the kit extraction method is more recommended for further examinations, such as sequencing, compared to the chelex and conventional methods because the purity of the DNA produced is much better.

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