

## Enhancing Bacterial DNA Extraction from Urine Tract Infection using Antibiotics

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**Received:** 2024, 15, Nov  
**Accepted:** 2024, 21, Nov  
**Published:** 2024, 23, Dec

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**Abstract:** Optimizing the extraction of Escherichia coli DNA from urine samples while keeping costs low is crucial for research and diagnostic purposes. Urine is a convenient and non-invasive sample for studying the urogenital microbiota and diagnosing sexually transmitted infections. However, challenges like low bacterial DNA quantities, PCR inhibitors, and crystal precipitation at cold temperatures can impede efficient DNA extraction and PCR amplification. To address these issues, researchers explored the effectiveness of adding different, The implementation of penicillin treatment as a method to enhance bacterial DNA extraction showed promising results. Here are the key findings: Concentration Variability: The concentration of penicillin solution used for treating bacterial cultures varied based on experimental conditions. Common concentrations ranged from 50 to 1000 units per milliliter (U/mL) or 0.1 to 10 milligrams per milliliter (mg/mL), with low, moderate, and high concentrations yielding varying degrees of cell wall disruption. Treatment Efficacy: Bacterial cultures treated with

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penicillin showed increased susceptibility to cell wall disruption, leading to enhanced DNA extraction efficiency. The duration of incubation with penicillin varied depending on factors such as bacterial species and desired level of cell wall weakening. Incubation Conditions: Optimal incubation conditions, including temperature and agitation, were maintained during penicillin treatment to ensure effectiveness. These conditions were adjusted based on the specific requirements of the bacterial species under study. Control Groups: Control groups consisting of untreated bacterial cultures or cultures treated with solvent alone were included in the experimental setup to compare the effectiveness of penicillin treatment. This allowed for the assessment of the specific contribution of penicillin to the enhancement of DNA extraction. DNA Extraction Efficiency: Following penicillin treatment, DNA extraction was performed using standard methods such as phenol-chloroform extraction, silica column-based purification, or commercial DNA extraction kits. The weakened cell walls resulting from penicillin treatment facilitated the release of DNA during the extraction process, leading to improved DNA yield and quality.

Further validation involved spiking urine samples with DNA from bacterial isolates, specifically targeting *Escherichia coli*. Median concentrations of *Escherichia coli* 16S rRNA gene copies were found to be significantly higher in urine processed with Tris-EDTA, indicating the effectiveness of this method in extracting *Escherichia coli* DNA. By maximizing bacterial DNA yield

from urine, especially targeting *Escherichia coli*, researchers can achieve more accurate assessments of bacterial populations and enhance the detection of specific bacteria in the genital tract. This approach offers a practical and economical solution for extracting *Escherichia coli* DNA from urine samples, advancing research and diagnostic capabilities in microbiology.

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## Introduction

DNA Extraction is the technique used to isolate DNA from a biological sample.

Extraction of nucleic acids is the starting point in any molecular biology study and hence is considered as a crucial process.

The first crude extraction of DNA had been performed by the Swiss physician Friedrich Miescher in 1869. He had accidentally purified DNA from the nucleus while investigating proteins from leukocytes and found that the property of this substance was fundamentally different than proteins, hence coined the term “nuclein”

However, it was only in 1958 that Meselson and Stahl developed a routine laboratory procedure for DNA extraction. They performed DNA extraction from bacterial samples of *Escherichia coli* using a salt density gradient centrifugation protocol (Sameer et al ,2023).

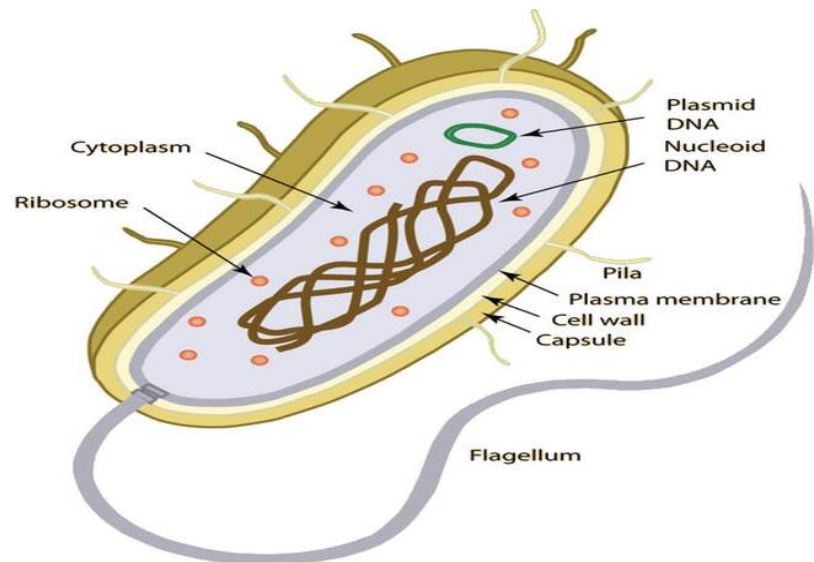
The bacteria *Escherichia coli* was discovered by German pediatrician Theodor Escherich (1857–1911), who isolated it from babies' feces in 1885 (Feng P et al 2007). *E. coli* is a gram-negative, non-sporulating, rod-shaped, facultative anaerobic, and coliform bacterium pertaining to the genus *Escherichia* that commonly inhabits the environment, foods, and warm-blooded animals' lower gut (Campbell NA et al 2002). In the domains of biotechnology and microbiology, it is the most widely studied prokaryotic model organism. It can live for long periods of time in feces, soil, and water, and is frequently used as a water contamination indicator organism. For 2–3 days, the bacterium multiplies rapidly in fresh feces under aerobic circumstances, but its numbers gradually fall after that. *E. coli* is gram-negative, straight, rod-shaped, non-sporing, non-acid fast, and bacilli that exist in single and pairs. Cells are typically rod-shaped, with 1–3  $\mu\text{m} \times 0.4\text{--}0.7 \mu\text{m}$  (micrometer) in size around 1  $\mu\text{m}$  long, 0.35  $\mu\text{m}$  wide, and 0.6–0.7  $\mu\text{m}$  in volume (Karger, R. 2003). It is motile due to peritrichous flagellar arrangement, and very few strains are non-motile. The optimal growth of *E. coli* occurs at 37°C (98°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F). It takes as little as 20 min to reproduce in favorable conditions

(M Basavaraju.2022). Fimbriated strains exist both as motile and non-motile. A polysaccharide

capsule has. been discovered in some *E. coli* strains isolated from extraintestinal infections. The *E. coli* capsules can be clearly seen using negative staining procedures, which produce a bright halo over a dark backdrop. They have a thin cell wall with only one or two layers of peptidoglycan (Köhler CD .2011)as shown in

Figure (1).

Figure (1). (Enger ED et al .2003)



It colonizes a newborn's gastrointestinal (GI) tract within hours after birth and even helps to keep our digestive tract healthy. Several strains of *E. coli* have been identified as good and effective probiotics and are currently employed in pharmaceuticals. It truly is a facultative anaerobic chemoorganotrophic capable of both respiratory and fermentative metabolism (Blount ZD.et al 2015). Although most strains of *E. coli* are safe, some serotypes can induce diarrhea when consumed through contaminated food or drink, while others might cause urinary tract infections (UTIs), anemia, and respiratory or kidney infections (BS Gunashree.2011). However, certain strains have developed into pathogenic *E.coli* by using plasmids, transposons, bacteriophages, and/or pathogenicity islands to acquire virulence factors (Kaper J.et al 2004). Serogroups, pathogenicity mechanisms, clinical signs, and virulence factors can all be used to classify the pathogenic strain of *E. coli* (Sarowska J.et.al 2019).

The bacterium can be grown easily and inexpensively in a laboratory setting under appropriate conditions. It takes as little as 20 min to reproduce and has been intensively investigated for over 60 years (MS Erjavec .et.al 2014). *E. coli* is the most widely studied prokaryotic model organism and an important species in the field of biotechnology and microbiology, where it serves as the host organism for recombinant DNA and experimental workhorse for DNA manipulation and protein production (Idalia VN.et.al 2017).

### The Aim of the Study

- 1- **Clearly state the goal of the proposal:** is to enhance bacterial DNA extraction from UTI samples using antibiotics to increase the yield and quality of DNA for downstream analysis.
- 2- **Evaluation of Antibiotic Effects on DNA Extraction:** Conducting experimental studies to assess the impact of antibiotic treatment on bacterial DNA extraction efficiency.
- 3- **Assessment of DNA Integrity and Purity:** Implementing quality control measures to evaluate the integrity and purity of extracted DNA samples. This will include assessing DNA fragmentation, presence of PCR inhibitors, and other factors that may affect downstream molecular analysis.
- 4- **Validation of Enhanced DNA Extraction Methodology:** Validating the efficacy of the proposed methodology for enhancing bacterial DNA extraction from UTI samples. This will involve comparing the sensitivity, specificity, and accuracy of molecular detection methods using DNA extracted from antibiotic-treated versus untreated UTI samples.
- 5- **Optimization of Downstream Molecular Analysis Techniques:** Investigating the applicability of the enhanced DNA extraction methodology for various downstream molecular analysis techniques, including qPCR, NGS, and other molecular assays. This may involve optimizing

PCR conditions, sequencing parameters, and data analysis pipelines to maximize the utility of the extracted DNA for UTI diagnosis and characterization.

### 1.1. The Urinary tract infection.

Urinary tract infections (UTI) are some of the most common bacterial infections, affecting 150 million people each year worldwide (Stamm et al., 2001).

In 2007, in the United States alone, there were an estimated 10.5 million office visits for UTI symptoms (constituting 0.9% of all ambulatory visits) and 2- 3 million emergency department visits. (Schappert et al.,2007).

UTIs are a significant cause of morbidity in infant boys, older men and females of all ages, serious sequelae include frequent recurrences, pyelonephritis with sepsis, renal damage in young children, pre-term birth and complications caused by frequent antimicrobial use, such as high-level antibiotic resistance and *Clostridium difficile* colitis. (Foxman et al., 2010)

### 2.1. Bacteria Causing Urinary Tract Infections:

#### 2.2.1. *Pseudomonas aeruginosa*:

*P. aeruginosa* is an opportunistic Gram-negative pathogen that is frequently isolated in urinary tract infections (UTI) in the elderly and catheterized patients and is associated with ineffective antibiotic treatment and poor clinical outcome. Invasion has been shown to play an important role in UTIs caused by *Escherichia coli* but has only recently been studied with *P. aeruginosa*. The ability of *P. aeruginosa* to adapt and evolve in chronic lung infections is associated with antibiotic resistance but has rarely been studied in *P. aeruginosa* UTI populations (Newman et al., 2022).

*P. aeruginosa* is responsible for 12% of all nosocomial urinary tract infections (UTIs), making it the third most common organism after *Escherichia coli* and enterococci isolated from UTI patients in the hospital setting (Kunin et al., 1994)

*P. aeruginosa* produces numerous virulence determinants associated with pathogenicity, including exotoxins, proteases, other enzymes, pigments, secretion systems, alginate capsules, flagella, and pili (Sultan et al., 2021).

#### 2.2.2. Enterobacter:

Is a gram-negative, rod-shaped, facultative anaerobic bacteria of the Enterobacteriaceae family community-acquired infections, including urinary tract infections (UTI), respiratory infections, soft tissue infections, osteomyelitis, and endocarditis, among many others (Ramirez et al., 2022)

Enterobacter has become increasingly resistant to many previously effective antibiotics for this reasons it need to develop new antibiotics (Ramirez et al., 2022).

#### 2.2.3. *Escherichia coli* (E. coli)

Is a group of bacteria that can cause infections in your gut (GI tract), urinary tract and other parts of your body. Most of the time, it can live in your gut without hurting you. But some strains can make you sick with watery diarrhea, vomiting and a fever. Shiga toxin-producing *E. coli* (STEC) is most likely to cause severe illness (James R. Johnson et al., 2002).

The Common types of *E. coli* infection include gastrointestinal and urinary tract infections (UTIs). Other types of *E. coli* infections include:

- Bloodstream infections.
- Prostatitis (prostate infection).
- Pelvic inflammatory disease (PID).
- Gallbladder infection (cholecystitis).
- Wound infections.

- Pneumonia (rare).
- Meningitis (rare).

The symptoms of E.coli in the urinary tract, if E.coli infects parts of your urinary tract, you might have:

- Abdominal or pelvic pain.
- Pain or burning sensation when you pee.
- An urgent need to pee frequently.
- Cloudy, foul-smelling pee. (James R. Johnson et al., 2002).

### **2.3. The E. coli Genome and Proteome**

The full genome of E. coli K12 was published by Science in 1997, making it one of the first species to have its genome completely sequenced. E. coli has a circular DNA molecule with 4288 annotated protein-coding genes (arranged into 2584 operons), 7 ribosomal RNA (rRNA) operons, and 86 transfer RNA (tRNA) (data for the E. coli laboratory strain K-12 derivative MG1655) (Kaper J. et al 2004). However, E. coli core genome (i.e., genes found in all strains) accounts for less than 20% of the pan genome's genes or nearly all (90%) of the genomes, leaving only a tiny fraction of genes found in roughly half of the genomes (Touchon M et.al 2004). The E. coli core genome is estimated to have less than 1500 genes, while it has a huge pan-genome with more than 22,000 genes (Nanda Kafle G et.al,2017). According to genomic analysis many of the genes of the pan-genome could be not yet unidentified but crucial virulence factors (Robins-Browne R Met.al.2016). There are 27,621 E. coli genome assemblies and annotation sequences available to date and each genome comprises between 4000 and 5500 genes (Mueller M. et al. 2021). The E. coli genome as a whole is remarkably ordered in terms of local replication direction and oligonucleotides that may be involved in replication and recombination (Blattner FR. et.al. 1997).

The diverse behavior of this species is explained by its enormous genetic and phenotypic diversity. With a mean distance between genes of only 118 base pairs, the coding density was found to be extremely high. A multitude of factors contribute to the higher gene density: a. bacterial genes lack introns throughout the genome, and neighboring genes are fairly near together, i.e., there are no many large non-coding DNA sections between genes. There are several transposable genetic elements, repetitive elements, cryptic prophages, and bacteriophage remnants in the genome and a variety of additional patches with unique compositions, showing genome plasticity due to horizontal gene transfer (Blattner FR. et.al. 1997 & Zhaxybayeva O. et.al. 2011 ).

### **2.4. Bacteria Resistance to Antibiotics:**

Antibiotics are medicines used to prevent and treat bacterial infections, Antibiotic resistance occurs when bacteria change themselves in response to the use of these medicines (Son et al., 1997) Bacteria show resistance to antibiotics and may cause infections in humans and animals that are more difficult to treat than those caused by non- antibiotic-resistant counterparts (Son et al., 1997).

Antibiotic resistance is the ability of bacteria to develop mechanisms that enable them to resist the effects of antibiotics. (Shariff et al., 2000).

Resistance arises either naturally through genetic mutations or through the transfer of resistance from one species that has acquired it to another that has not yet acquired it (Shariff et al., 2000).

### **2.5. Mechanism of Antibiotic Resistance:**

**Antibiotic resistance occurs in four ways:**

**1-**Inhibiting or changing the antibiotic: such as the enzymatic inhibition of Penicillin G in some penicillin-resistant bacteria by manufacturing lactamases (Lihan et al.,2014).

**2-** Changing the target site (the site of antibiotic activity): such as changing the PBP the site of

activity of penicillin in a type of bacteria called MRSA, as well as in other bacteria that are resistant to penicillin (Rodgers et al., 2009).

**3- Metabolic pathway change:** Aminobenzoic acid (PABA) is an important factor for the synthesis of folic acid and nucleic acids in bacteria. This factor can be inhibited by sulfonamides, However, some sulfonamide-resistant bacteria dispense with this essential factor by using ready-made folic acid (by taking it directly from their surroundings), just like animal cells (Zulkifli et al., 2009).

**4-Reducing antibiotic aggregation:** by reducing the permeability of the antibiotic into the cell and/or accelerating active flow (pumping to the periphery) of drugs across the bacterial cell membrane (Lihan et al., 2014).

## Material and Methods

### Reagents and equipment

#### Reagents

All reagents have been mentioned in the text, which should include information about the suppliers and manufacturers.

#### Equipment

Application	Device	Manufacturer
Agarose Gel Imaging	G:BOX Trans-illuminator	Syngene, A Division of Synoptics Ltd., Cambridge, UK
Anaerobic Incubator	Bug Box Plus	Ruskinn Technology Ltd., Bridgend UK
Bench-top Centrifugation	MiniSpin	Eppendorf UK Ltd., Stevenage, UK
Centrifuge	Laborzentrifugen model 3K10	SciQuip Ltd., Shropshire, UK
Incubation	Water bath	Grant Instruments, Cambridge, UK
Determination of Nucleic Acid Absorption	NanoDrop ND-100	Thermo Fisher Scientific Ltd., Loughborough, UK
DNA Electrophoresis	Gel Tank Power Pac 300	Apollo Instrumentation Bio-Rad, Hertfordshire, UK
Gradient PCR	T100 PTC-200	Bio-Rad Laboratories Ltd., Hertfordshire, UK
Microplate Reader	Synergy HT Microplate Reader	Bio Tek UK, Swindon, UK
Autoclave	Benchttop	Astell, Kent, UK
Heat shock	Heating block	Flowgen bioscience Ltd., Nottingham, UK

#### Sample collection

#### Maintaining and preparing cultures of bacteria

#### Bacteria used in this study

**Table 1: Strains used in this study.**

Strains	Source
<i>Escherichia coli</i>	This study

## Routine culture of oral bacteria

### Gram stain

### DNA extraction and analysis

To purify chromosomal DNA from *Escherichia coli* the Masterpure™ DNA purification kit (Epicenter, Cat. No. MCD85201, supplied by Cambio Ltd., Cambridge, UK) was used according to the manufacturer's instructions, with an extra step to ensure efficient disruption. It was implemented with the following modifications: *S. gordonii*, and *A. oris* were cultured in BHYG broth and *V. parvula* in BHYGL for 18 h at 37°C. While *F. nucleatum* was cultured in FAB for 24 – 48 h. Cells were harvested and the supernatant discarded. The pellet was re-suspended in 150 µl (37°C pre-warmed) spheroplasting buffer (20 mM Tris-HCl, pH 6.8; 10 mM MgCl<sub>2</sub>; 26% w/v raffinose.5H<sub>2</sub>O). After addition of 250 µg/mL lysozyme (Sigma Aldrich) and 5 µg mutanolysin (Sigma Aldrich, reconstituted at 10,000 U mL<sup>-1</sup>), the bacteria were incubated at 37°C for 30 min. Following incubation, cells were placed in screw-cap Eppendorf tubes with 150 µl of 2x T&C Lysis Solution (Epicenter) and placed into a bead-lysis machine (Qiagen Tissue Lyser Ltd., UK) at 50 Hz, for 5 min, using 25-50 mg of acid washed glass beads (0.1 mm). The MasterPure™ Gram Positive DNA Purification Kit (Epicentre® Biotechnologies) contains all of the reagents needed to purify genomic DNA from Gram Positive bacteria and was used according to the manufacturer's instructions. After extraction, DNA was suspended in 25 µl elution buffer (10 mM Tris pH 8.5). DNA concentrations were determined using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific), using the DNA-50 setting. DNA was stored at -20°C for use in downstream applications.

### Analysis of DNA concentration by Nanodrop

The quantity and purity of the chromosomal DNA, plasmid DNA and RNA were determined by measuring the optical density (OD) ratios at 260/280 and 260/230 nm and by 1% agarose gel electrophoresis (see section 0). NanoDrop ND-1000, UK spectrophotometer was used to measure DNA and RNA concentration in the samples. DNA or RNA samples were then loaded in 1-2 µL volumes onto the NanoDrop stage. The NanoDrop system has a program that can automatically calculate concentrations and purity. DNA and RNA concentration is estimated by measuring the absorbance at 260 nm as ng/µl, each 1 OD<sub>260</sub> unit = 50 µg/mL for DNA while for RNA 1 OD<sub>260</sub> unit = 40 µg/mL. The ratio of 260 /280 nm absorbance was used to estimate the DNA and RNA purity (a ratio of DNA and RNA 1.8-2.0 was considered acceptable). While the ratio of absorbance at 260/230 was used as a secondary measure of RNA purity between ~2.0 -2.2 that was acceptable as pure for RNA from contamination with guanidine thiocyanate.

### DNA gel electrophoresis

Agarose gel electrophoresis was used to check the size of DNA (FMC Bioproducts, Rockland, USA). Agarose gels were prepared between 1–2% dissolved in an electrophoresis buffer depending on the DNA size of the products being examined. Agarose gels between 1-2% (w/v) were prepared for standard PCR and restriction enzyme digest products, whereas RT-qPCR products were analyzed using 2% agarose gels. Agarose was dissolved in TAE buffer (40 mM Tris-acetate, 1.0 mM EDTA, pH 8.0) and supplemented with 0.1 µg/mL (5 µl per 50 mL) Gel Red Nucleic Acid Gel Stain (10,000x in water; Biotium) in order to visualize DNA. The following DNA ladders (or DNA molecular weight markers) were used in gel electrophoresis in order to determine the products size on the gel: HyperLadder 25 bp (25-500 bp; Biotium); HyperLadder 100 bp (100-1013 bp; Bio-line, UK), HyperLadder 1 kb Plus (250-12,007 bp; Biotium). Loading dye [40% (w/v) sucrose, 100 mM EDTA, pH 8.0, 0.01% (w/v) bromophenol blue] and DNA sample were added to the well after mixing. Gels were run at 85-100 V and 250-300 mA for 120-180 min. After the electrophoresis processes were completed, the molecules in the gel were visualized using exposure to long-wave ultraviolet (UV) light from a transilluminator. DNA was imaged and recorded at 5.51 Mpixel using a gel documentation system (G: BOX Transilluminator, Syngene).

## Primer design

Primers were designed from genomic DNA sequences obtained from National Center for Biotechnology Information (NCBI). Primer3Plus (Untergasser et al., 2007) was used to design PCR primers for use in RT-qPCR. Most of the primers were designed to be between 15-25 bp in length, guanine:cytosine content (40-60 %), and melting temperature between 50-60°C, and to generate a product between 50-150 bp in length. SnapGene software (GSL Biotech LLC, Chicago, UK) was utilized to design primers for other PCR applications. Oligonucleotide primers were purchased and synthesized by Eurogentec. Stock solutions were diluted to a final concentration of 10 µM and stored at -20 °C. The list of the primers sequences is found in table

IDs Gene		Primer Sequence	bp	Source
acrA	F	CGACAAACAGGCCCAACAAG	468	This Study
	R	CCGCTAATCGGAGAGGTGAC		

## Conventional polymerase chain reaction

The ReddyMix™ (ABgene) and Expand High Fidelity PCR System (Roche) kit were used typically in 50 µL reactions. Firstly, the ReddyMix™ kit was used to amplify short fragments of DNA. Alternatively, the Expand High Fidelity PCR System was employed for efficient PCR reactions with lower chances of incorporating erroneous bases because it contains Tgo DNA polymerase enzyme, isolated from *Thermococcus gorgonarius* and with proofreading activity. Standard polymerase chain reaction (PCR) was performed on DNA samples according to manufacturer's protocols. The general protocols for Expand High Fidelity PCR are shown in Table 2.4 and 2.5 while ReddyMix™ is demonstrated in Table 2.7 and 2.8. Annealing temperatures were optimized depending on the primers used. All polymerase chain reactions experiments were carried out on DNA Engine PTC-200 (MJ Research) thermocycler or T100 Thermal Cycler (Bio-Rad) machines.

## PCR product purification

PCR product purification was carried out to remove primers, nucleotides, enzymes, and short-failed PCR products. The QIAquick PCR purification kit (QIAGEN) was used according to the manufacturer's protocols. 100 µl of Buffer PB was added to 20 µl PCR sample (5/1 volumes) and mixed well. The final volume (120 µl) was added to QIAquick column that was already placed in a 2 mL collection tube, and centrifuged for 30-60 seconds at 10,000 g. 0.75 mL Buffer PE was added to the QIAquick spin column to wash DNA that had attached to the spin column and centrifuged for 30-60 seconds at 10,000 g then the spin column was placed in a clean 1.5 mL microcentrifuge tube. The elution step was achieved by adding 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the centre of QIAquick spin column and centrifuged for 1 min at 10,000 g. Samples were stored at -20 until use.

**Table: Expand high fidelity PCR thermal cycling.**

Reaction Mix	Thermal Cycler Protocol		
50 µl	Initial denaturation	94°C / 2 min	1x
	Denaturation	94°C / 15 s	10 cycles
	Annealing	45- 65°C / 30 s	
	Elongation	68 or 72°C / 2 min	
	Denaturation	94°C / 15 s	20 cycles
	Annealing	45-68°C / 30 s	
	Elongation	72°C / 2 min <sup>A</sup>	
	Final Elongation	7 min	1x

## Enhancing Bacterial DNA Extraction by penicillin.

To enhance bacterial DNA extraction using penicillin, the following methods can be employed:

**Preparation of Penicillin Solution:** Prepare a penicillin solution by dissolving the appropriate concentration of penicillin in a suitable solvent, such as sterile water or phosphate-buffered saline (PBS). The concentration of penicillin solution used for treating bacterial cultures can vary depending on factors such as the specific bacterial species being studied, the desired extent of cell wall weakening, and the experimental conditions. However, typical concentrations of penicillin solution range from 50 to 1000 units per milliliter (U/mL) or 0.1 to 10 milligrams per milliliter (mg/mL). Here are some common concentrations of penicillin solution used in research:

- A. Low Concentration: 50 to 100 U/mL or 0.1 to 0.2 mg/mL
- B. Moderate Concentration: 200 to 500 U/mL or 0.4 to 1.0 mg/mL
- C. High Concentration: 500 to 1000 U/mL or 1.0 to 2.0 mg/mL

Penicillin may vary depending on the bacterial species and the desired level of cell wall disruption.

**Treatment of Bacterial Cultures:** Add the penicillin solution to the bacterial cultures and incubate them for a specified period. The duration of incubation may vary depending on factors such as the bacterial species, the concentration of penicillin, and the desired extent of cell wall weakening.

**Incubation Conditions:** Maintain appropriate incubation conditions during treatment, including temperature and agitation. Optimal conditions for penicillin treatment may vary depending on the specific requirements of the bacterial species being studied.

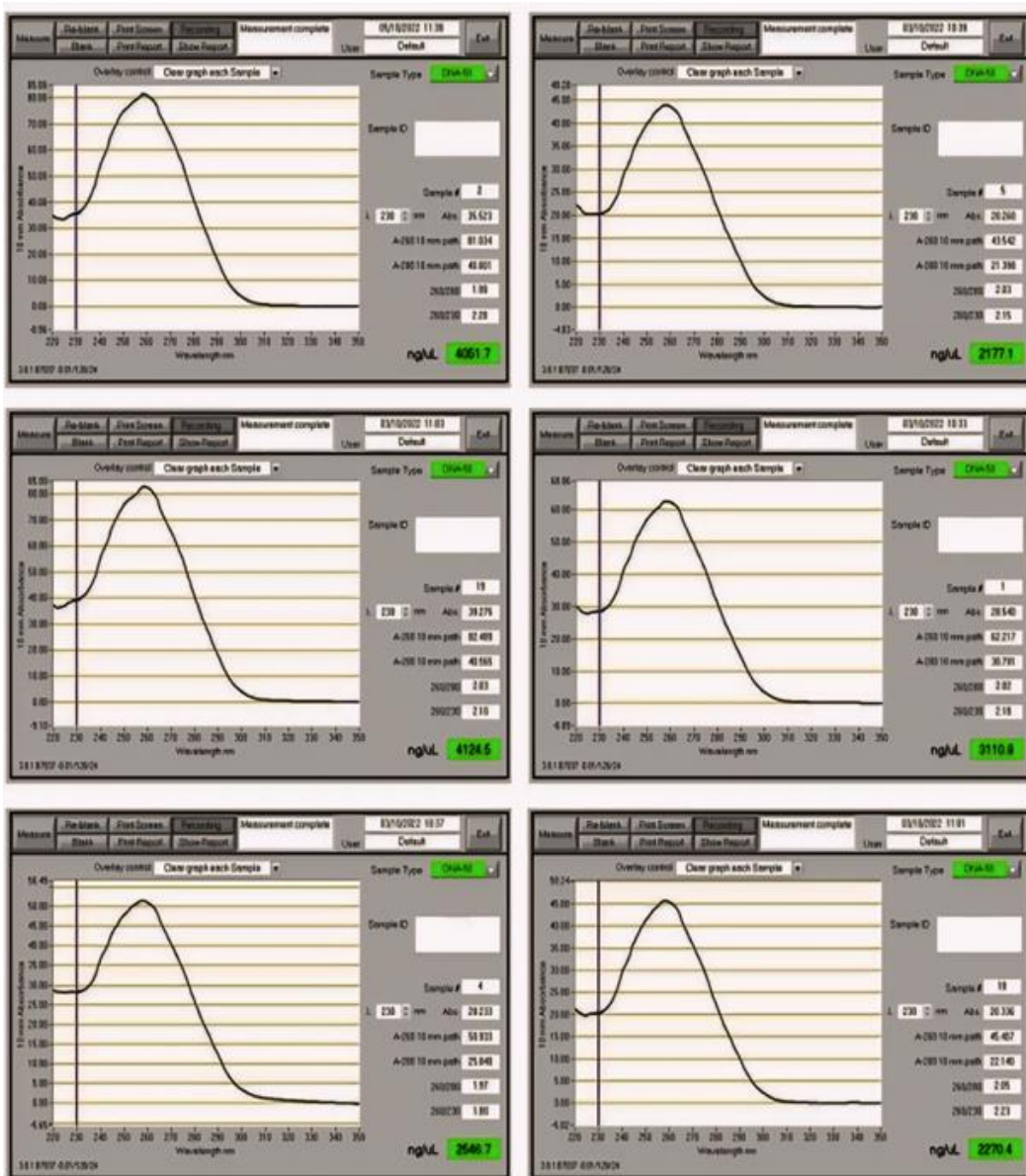
**Control Groups:** Include appropriate control groups in the experimental setup to compare the effectiveness of penicillin treatment. Control groups may consist of untreated bacterial cultures or cultures treated with solvent alone.

**DNA Extraction:** After penicillin treatment, proceed with DNA extraction using standard methods such as phenol-chloroform extraction, silica column-based purification, or commercial DNA extraction kits. The weakened cell walls resulting from penicillin treatment facilitate the release of DNA during the extraction process.

## Results and Discussion

### Analysis of DNA concentration by Nano-drop

Evaluating the quality of a DNA sample is crucial, and the A<sub>260</sub>/A<sub>280</sub> ratio obtained from the NanoDrop test is a key indicator. A ratio of approximately 1.8 suggests purity, while deviations may hint at contaminating substances like proteins. Similarly, an A<sub>260</sub>/A<sub>230</sub> ratio above 2.2 may signal the presence of RNA or other impurities, necessitating additional purification steps. When all ratios fall within the 1.8-2.2 range, it implies high-quality samples suitable for various molecular biology techniques, like gene expression profiling or RNA sequencing. This range also reflects careful sample preparation, minimizing interference from organic compounds. However, while the A<sub>260</sub>/A<sub>280</sub> ratio is vital for assessing nucleic acid sample quality, it shouldn't be the sole consideration. Factors like DNA or RNA integrity, purity, and concentration also influence sample quality and suitability for downstream applications. Consequently, researchers should evaluate multiple criteria to ensure samples meet their specific experimental needs.



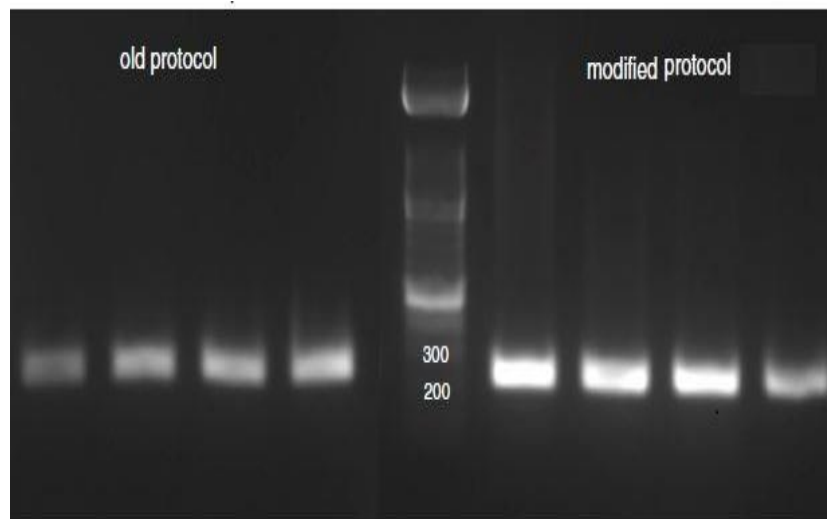


Fig: Comparison between PCR products obtained from *E. coli* samples using the old and modified penicillin method. a Clear bands were obtained using the old (left) and modified (right) protocols to analyses the product from *acrA* primer pairs in *E.coli* samples from patients.

The study has demonstrated that this method not only improved the quality of DNA (a 6.3-fold improvement in the 260/230 ratio) but also enhanced the yield by concentrating the DNA in solution (a 20-fold improvement in the yield compared to the old protocol). The study has also shown that minimal quantities of bacteria samples, that is, a fraction of 5  $\mu\text{l}$  of suspension taken yield ample genomic DNA for multiple PCR reactions. This method also works well for trace quantities (100 microliter) are able to yield 1000 ng/ $\mu\text{l}$  of purified DNA. For comparison, 300  $\mu\text{l}$  of bacteria yields 5–15  $\mu\text{g}$ , whereas 110 microliter of bacteria yields 15–20  $\mu\text{g}$  of DNA using commercially available kits from Promega Genomic DNA Purification Kit Technical Manual TM050]. Similarly, 200  $\mu\text{l}$  of bacteria yields 4–12  $\mu\text{g}$  whereas 25 mg of staphylococcus yields 15-30  $\mu\text{g}$  of DNA using the QiaAmp kit . The study also performed Trizol based DNA extraction and obtained a yield of 127.9 ng/ $\mu\text{l}$  (260/280 = 1.7; 260/230 = 1.12) from 140 microliter of bacteria, when the DNA was resuspended in 200  $\mu\text{l}$  of 8 M NaOH solution. . However, the modified method is consistent across all trials of these experiments for different samples. This method uses less than half the volume of bacteria normally using from the tail for genotyping, and results in a purified DNA sample with a yield for multiple PCR reactions. Similarly, this ethod may find applications in determining specific mutations that may have resulted in speciation as well as point mutations that may result in disease. Besides its use in forensic medicine, our protocol may be utilized for genotypic screening and sequencing of specific genes, following the detection of SNPs.

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