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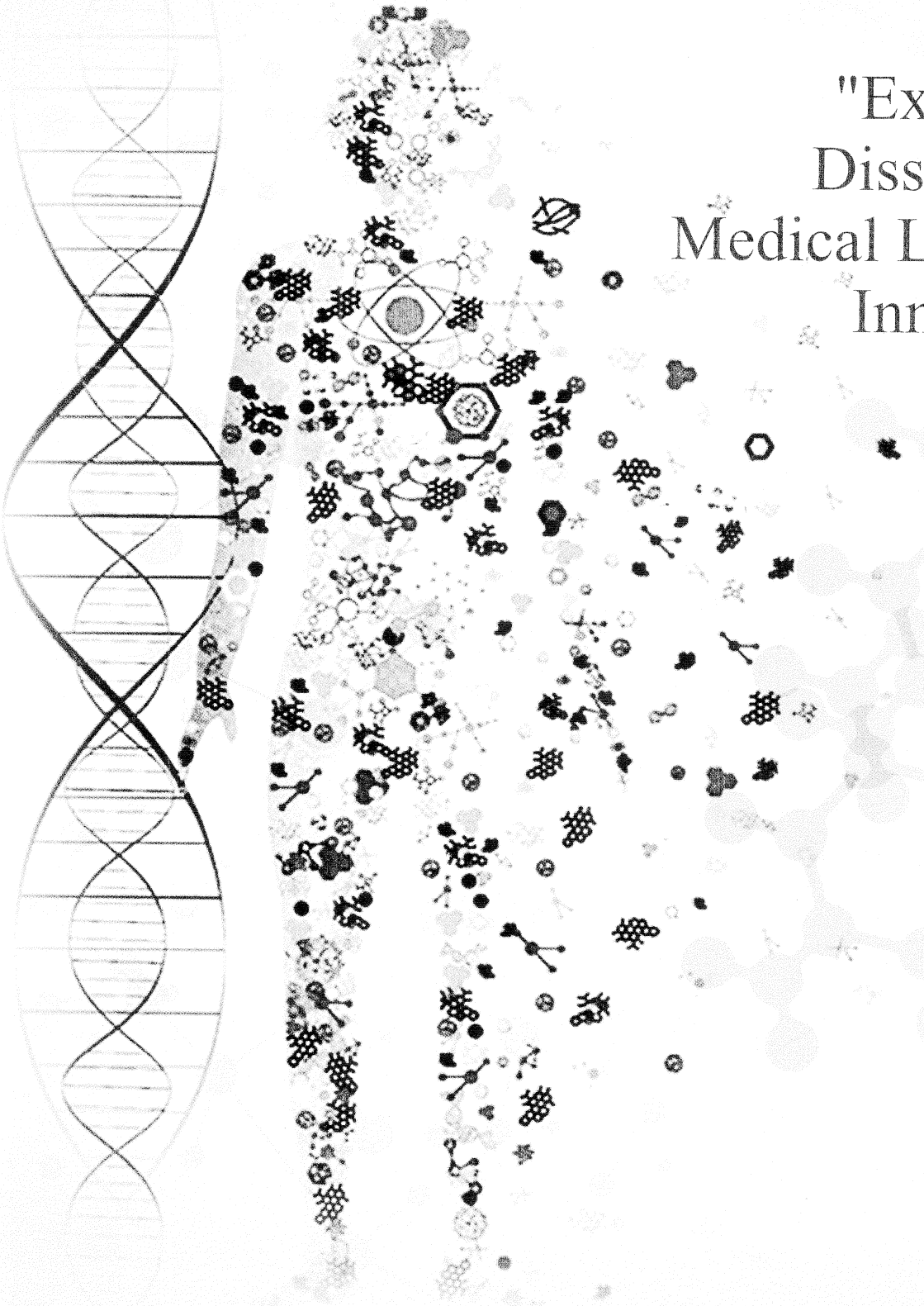

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COMPARISON OF SIX DIFFERENT DNA EXTRACTION METHODS FOR POLYMERASE CHAIN REACTION BASED DENATURING GRADIENT GEL ELECTROPHORESIS (PCR-DGGE)

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Introduction:
Culture-independent molecular fingerprinting method: Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) is identified as one of best methods to profile microbial communities in complex ecosystems. PCR-DGGE requires a good quality and a higher yield of DNA, therefore DNA extraction protocol should be carefully selected.

Objectives:
The study aimed to compare different DNA extraction methods to evaluate a simple DNA extraction protocol for profiling eubacteria in chronic wounds using PCR-DGGE.

Methods:
Wound tissue debris specimens were obtained from chronic ulcers of ten patients presenting to Colombo South Teaching Hospital for routine debridement. The specimens were weighed, minced and used for DNA extraction. Total DNA was extracted from each specimen using six DNA extraction methods: heat treatment in distilled water, heat treatment in NaOH, Bead beater–phenol chloroform method using STES buffer, bead beater–phenol chloroform method using TN150 buffer, salting out method and QIAGEN DNeasy blood and tissue kit. All extractions were performed in duplicate. The yield, purity and quality of DNA was measured. PCR amplification was done targeting V2-V3 region of eubacterial 16S rRNA gene. The resulting PCR products were subjected to DGGE using a 30-55% denaturing gradient. The DGGE gels were stained and visualized by a UV trans-illuminator.

Results:
QIAGEN DNeasy Blood and Tissue Kit produced good quality genomic DNA compared to the other five DNA extraction methods and gave a broad diversity of bacterial communities in chronic wounds. Among five conventional methods, bead beater/phenol–chloroform method with STES buffer gave a yield of DNA with a high purity and resulted in a higher DGGE band diversity. Although DNA extraction using heat and NaOH had lowest purity, DGGE revealed a higher bacterial diversity.

Conclusions:
DNeasy Blood and Tissue Kit produced good quantity and quality genomic DNA with a broad microbial diversity. Bead beater/phenol–chloroform method with STES buffer was the best among five conventional methods tested.

Key words: Bacteria, Chronic wounds, DNA extraction methods, Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE)