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Research Article

EXTRACTION AND PURIFICATION OF LPS FROM CATHETER-ASSOCIATED URINARY TRACT INFECTION (CAUTI) OF GRAM-NEGATIVE ORGANISMS

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ABSTRACT

Globally, one of the most prevalent health-related illnesses is catheter-associated urinary tract infection (CAUTI). A leading cause of nosocomial infection is commonly associated with the use of catheters, leading to high morbidity and mortality rates worldwide. MDR bacteria are the main reason to leading to longer hospital stays and increased costs for treatment. Uropathogens are responsible for half of the CAUTI infections. In the pathophysiology of gram-negative bacteria, lipopolysaccharide (LPS), usually called endotoxin, is essential for colonization and invasion. It plays a crucial role in gram-negative bacteria-induced diseases and septic shock. It has broad applications in different cell stimulation experiments, providing a conceptual basis for studies directly related to the isolation and purification of LPS. This study aims to extract and purify the LPS from the Gram-negative organisms of CAUTI bacterial strains such as *E. coli* and *P. aeruginosa*. The primary approach, which is based on the hot phenol-water method extraction protocol, is used to purify LPS from bacterial strains, with high purity and minimal contamination of proteins and nucleic acids. Finally, to check the purity of the extracted LPS, it was evaluated by silver Coomassie blue staining of SDS-PAGE. The result showed that high purity of *the E. coli* range is 10-25 kDa; similarly, *Pseudomonas aeruginosa* demonstrated a banding pattern characteristic of varying O-antigen chain length. HPLC analysis reveals that the purity of the extracted LPS was 100% compared with the standard LPS.

Keywords: Urinary Tract Infection, Gram-Negative Bacteria, Multidrug Resistance, Lipopolysaccharide.

INTRODUCTION

The urinary tract is the most common site of hospital-acquired infection, mainly CAUTI, accounting for more than 40% of the total number reported by acute care hospitals and affecting approximately 600,000 patients. Annually 66% to 86% of these infections usually follow instrumentation of the urinary tract, mainly catheterization (Sunzida Arina *et al.*, 2021). Hospitalized and catheterized individuals are at the highest risk for catheter-associated urinary tract infections (CAUTIs). CAUTI occurs when microbes enter the urinary tract via a urinary catheter, and because infections are associated with longer hospital stays, higher healthcare expenses, and morbidity (Mohamed *et al*., 2022). Though essential for many medical procedures, indwelling urinary catheters provide a conduit for microbial invasion, leading to biofilm formation and subsequent infection. The risk of infection rises significantly with the duration of catheterization, emphasizing the need for timely intervention and appropriate antibiotics therapy (Kawalec *et al.,* 2023). The alarming resistance rate has led to a global challenge in selecting appropriate empiric treatments, making routine surveillance and susceptibility profiling crucial.

Gram-negative organisms are the major cause of CAUTI. The most common organism is *Escherichia coli*. The common significant risk factors are the duration of catheter use every day; a catheter that remains increases the risk of infection for patients (Jacobsen *et al*., 2008). Gram-negative bacilli, particularly those belonging to the Enterobacteriaceae family and non-fermenting organisms like *E. coli*, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii*, are the leading causative agents of CAUTIs.

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These organisms are not only efficient colonizers but are also notorious for developing multidrug resistance (MDR) through various mechanisms, including extended-spectrum β-lactamase (ESBL) production, efflux pumps, and target site mutations (Ndomba *et al*., 2022) Major infections in hospitals and healthcare facilities are also caused by multidrug-resistant (MDR) microorganisms, particularly in the intensive care unit. An extended hospital stay, increased burden, higher care expenses, and higher mortality are linked to MDR organism infection (Alberto F. Monegro *et al*., 2023).

The formation of a cell surface permeability barrier by LPS results in inherent resistance to many antimicrobial treatments in most gram-negative bacteria. LPS can trigger the innate response through Toll-like receptor 4 (TLR4), which occurs in many immune cells (Aisha Farhana *et al.,* 2025). The Enterobacteriaceae is characterized and to confirm the purity of LPS by its high molecular weight ranging from 10-25 kDa and *E.coli* has a smooth strain reach up to 23 kDa characterized by the presence of variable O-antigen polysaccharide chain extending from the core region (Al-Aalim *et al., 2022).* This study aims to identify and characterize Gram-negative bacterial strains isolated from CAUTI cultures and extract and purify the LPS from the Gram-negative organisms.

MATERIALS AND METHODS

Extraction and Purification of Lipopolysaccharide (LPS)

The formation of a cell surface permeability barrier by LPS results in inherent resistance to many antimicrobial treatments in most gram-negative bacteria. LPS can trigger the innate response through Toll-like receptor 4 (TLR4), which occurs in many immune cells (Aisha Farhana *et al.,* 2025). The Enterobacteriaceae are characterized, and to confirm the purity of LPS, by their high molecular weight, ranging from 10 to 25 kDa, and E. coli has a smooth strain that reaches up to 23 kDa, characterized by the presence of a variable O-antigen polysaccharide chain extending from the core region (Al-Aalim *et al., 2022).* This study aims to identify and characterize Gram-negative bacterial strains isolated from CAUTI cultures and extract and purify the LPS from the Gram-negative organisms.

Preparation of Bacterial Culture

E. coli and *P.aeruginosa* isolates were inoculated into 5 mL of brain heart infusion broth and incubated at 37°C for 24 hours. The cultures were adjusted to 0.5 McFarland standard and transferred to 750 mL of LB broth (adjusted from the original 1000 mL as per the current study's requirement) for large-scale growth. Final bacterial turbidity was standardized to 2×10^9 CFU/mL using spectrophotometry ($OD_{600} = 1.84$).

Extraction Procedure

The bacterial cultures were centrifuged at 4100 rpm for 15 minutes at 4°C. The resulting pellets were washed with phosphate-buffered saline (PBS, pH 7.2) containing 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and resuspended in 10 mL PBS. To lyse the cells and release LPS: The suspension was sonicated at 29 MHz for 10 minutes and treated with 100 µg/mL proteinase K at 65°C for 1 hour.

LPS Separation

An equal volume of hot (65–70°C) 90% phenol was added, and the mixture was incubated at 70°C for 15 min with shaking. It was then cooled on ice and centrifuged at 10000 rpm for 15 minutes. The aqueous layer was collected and re-extracted. LPS was precipitated by adding 0.5 M sodium acetate and 10 volumes of 95% ethanol, then stored at –20°C overnight. Following precipitation, LPS was collected by centrifugation (4100 rpm, 10 min, 4°C), resuspended in distilled water, and dialyzed against double-distilled water at 4°C for 48 hours using a dialysis tube (cutoff: 3500 Da). The dialyzed extract was lyophilized at –55°C under 0.001 mbar pressure for 24 hours and stored at 4°C.

ANALYSIS AND CHARACTERIZATION OF LPS

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The extracted LPS was subjected to SDS-PAGE to assess purity and molecular weight. To detect impurities of protein and nucleic acid, gels were stained with both Coomassie Blue (for protein contamination) and silver nitrate stain (for LPS band visualization). Distinct bands ranging from 15 to 32 kDa confirmed the presence of both core and O-antigen components.

High-Performance Liquid Chromatography (HPLC) Analysis

For purity assessment, High-Performance Liquid Chromatography (HPLC) was performed using a Supelco™ C18 column (25 cm × 4.6 mm, 5 µm) with a mobile phase of acetonitrile:water (5:95), a flow rate of 0.8 mL/min, and detection at 210 nm. The ELPS exhibited a single sharp peak with a retention time of 1.650 min, indicating 100% purity compared to standard LPS.

RESULT AND DISCUSSION

Additionally, the visual observation of the purified lipopolysaccharide (LPS), as shown in the image of the centrifuge tubes, provides compelling qualitative confirmation of the effectiveness of the extraction and precipitation procedures. Both tubes exhibit a prominent, dense, white, cottony pellet at the bottom, a well-recognized indicator of successful LPS precipitation, typically seen following the addition of ethanol and sodium acetate. This visual cue is significant, as it suggests that a substantial quantity of LPS was recovered from the bacterial cell lysates. The white, fibrous appearance of the pellet is consistent with LPS aggregates, which form as the polysaccharide and lipid components come out of solution during alcohol-induced precipitation. Moreover, the

supernatant in both tubes appears clear and free of any visible turbidity or coloration. This clarity strongly indicates that non-LPS cellular components, such as proteins, nucleic acids, and other soluble impurities, were effectively removed during the multiple centrifugation and washing steps. The absence of any residual debris further confirms the specificity and efficiency of the purification process, minimizing contamination and improving the reliability of downstream analyses. Beyond the physical appearance, this qualitative assessment gains further credibility when supported by electrophoretic profiling, such as SDS-PAGE, where the purified LPS typically presents as distinct bands devoid of protein or nucleic acid smearing. This combined evidence not only verifies the high purity of the final LPS preparation but also reinforces the reproducibility of the extraction method employed (*D.G. Kalambhe et al., 2017*). Such purity is particularly important in experimental contexts where LPS is used for immunological studies, receptor-binding assays, or structural characterizations, as any residual contaminants could interfere with biological responses or skew analytical results. The successful isolation of high-purity LPS ensures that any observed biological activity or molecular interaction can be confidently attributed to the LPS itself, rather than to unintended co-purified substances. Consequently, the visual observation, alongside biochemical validation, confirms that the extracted LPS is of excellent quality, making it suitable for sensitive downstream applications, including endotoxin quantification, macrophage activation assays, and vaccine formulation studies (Richard 1983).

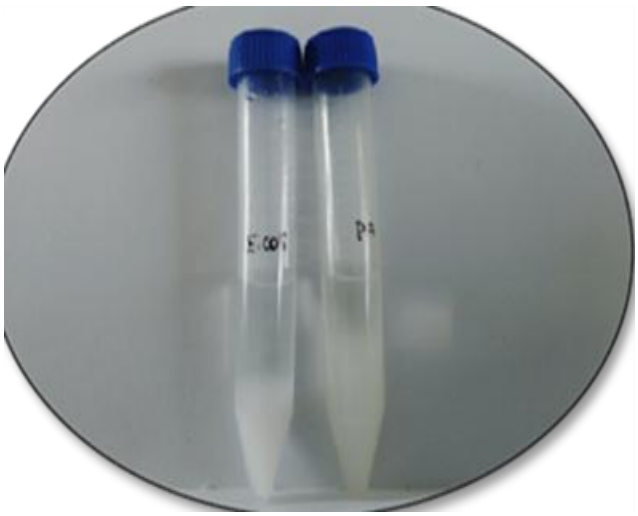

Figure 1. Purified LPS.

SDS-PAGE analysis was conducted on the purified lipopolysaccharide (LPS) extracted from *Pseudomonas aeruginosa* and *Escherichia coli* (E. coli) to evaluate molecular integrity and check for the presence of protein contamination. The standard values are considered in the previous reference as 15-25 kDa (Al-Aalim *et al., 2022).* The gel, stained with Coomassie Brilliant Blue, displayed well-defined bands representing a range of molecular weights between approximately 10 kDa and 100 kDa. The LPS sample obtained from *E. coli* showed distinct banding primarily between 10 and 23 kDa, which corresponds with the expected molecular weight range of LPS components, notably lipid A and the core oligosaccharide. Finally, the result showed that the enzymatic treatment of the hot phenol method gave high purity of LPS molecular weight of *E.coil a* range is 10-25 kDa. Similarly, the *Pseudomonas aeruginosa* LPS sample demonstrated a banding pattern characteristic of varying O-antigen chain lengths, reflecting the structural diversity of LPS across different Gram-negative bacteria. Crucially, there were no smeared bands or additional stained regions that would indicate residual protein contamination. This suggests that the enzymatic treatments used during purification, specifically proteinase K, DNase, and RNase, were effective in eliminating protein and nucleic acid impurities. These results confirm the high level of purity achieved in the LPS samples and support the reliability of the hot phenol-water extraction method used in this study.

The banding patterns observed in the SDS-PAGE gel not only confirm the structural integrity of the extracted LPS but also provide insight into the molecular composition and heterogeneity of the samples. In the *E. coli* LPS, the prominent bands between 18 and 68 kDa are indicative of the core oligosaccharide and lipid A regions, which are typically conserved within Enterobacteriaceae. These components play a critical role in the endotoxic activity of LPS and are essential in host-pathogen interactions, particularly in triggering immune responses. The uniformity and clarity of these bands further imply minimal

degradation during the extraction process, highlighting the gentle yet effective nature of the hot phenol-water method in preserving key molecular features of LPS.

In the case of *P.aeruginosa* LPS, the broader range of bands and the presence of multiple higher-molecular-weight regions reflect the natural variability in O-antigen chain lengths, which is a well-documented feature of *Pseudomonas aeruginosa*. The O-antigen is a highly variable polysaccharide component that contributes to the antigenic diversity and immune evasion capabilities of Gram-negative bacteria. The ability to resolve these variations in chain length through SDS-PAGE analysis confirms that the extraction method maintained the structural complexity of the LPS, which is vital for further functional studies. The absence of protein or nucleic acid contamination, as evidenced by the clean band profiles and the lack of non-specific staining, also ensures that these LPS samples are suitable for downstream applications such as immunogenicity testing, receptor-binding assays, or structural analysis using advanced techniques like mass spectrometry or NMR spectroscopy (Nilofer QureshiSQ *et al*., 1988).

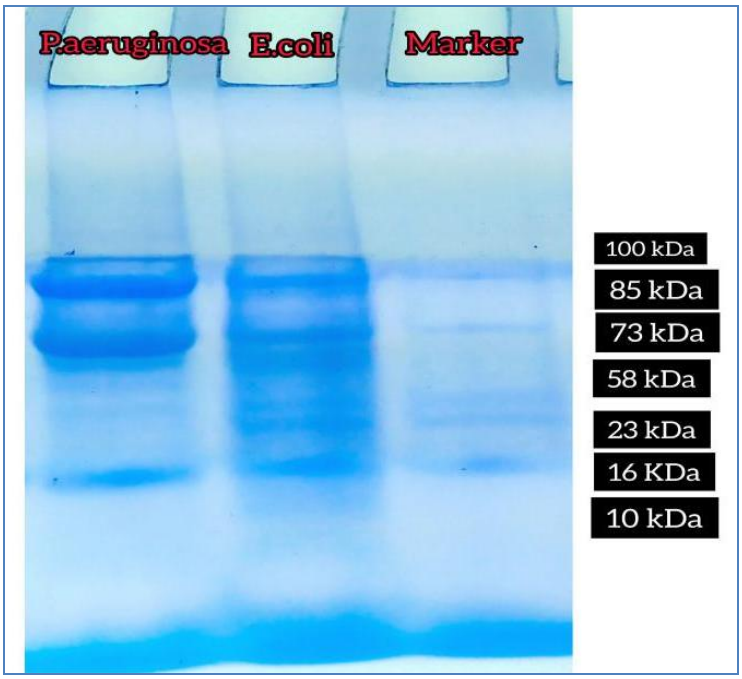

Figure 2. Electrophoresis of LPS on SDS PAGE (*E.coli*, *P.aeruginosa* and Marker).

High-performance liquid chromatography (HPLC) was utilized as an analytical tool to assess and confirm the purity of lipopolysaccharide extracted from various bacterial sources. This method offers high sensitivity and resolution, making it an ideal technique for evaluating the structural composition and potential contaminants in complex biological samples like LPS. Chromatographic detection was performed at a UV wavelength of 210 nm, a standard detection range for identifying LPS-associated components due to the absorbance properties of their polysaccharide and lipid moieties (Heba M. Hassan *et al*., 2020). The first chromatogram, representing the purified LPS extracted from *Escherichia coli*, displayed a distinct, sharp, and symmetrical peak at a retention time of approximately 3.8 minutes. The clarity and singularity of this peak, without any noticeable secondary peaks, shoulders, or baseline drift, serve as strong indicators of sample purity. Such a chromatographic profile strongly suggests that the extraction and purification steps particularly the combined enzymatic digestion and solvent precipitation—were effective in eliminating interfering substances like proteins, nucleic acids, and residual solvents. This level of purity is critical for applications in immunological assays, structural studies, and pharmaceutical formulations, where even trace levels of contaminants can significantly affect biological responses.

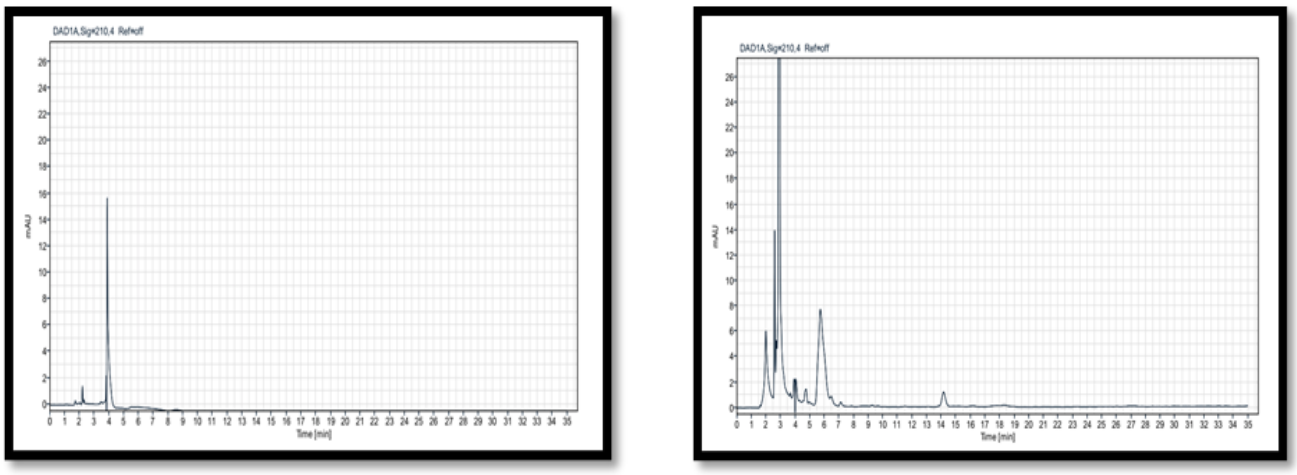

Figure 3. HPLC for the sample.

The second chromatogram corresponded to a commercially available standard LPS from E. coli O55:B5, which is often used as a benchmark in experimental settings due to its well-characterized composition. Similar to the purified sample, a dominant peak was observed near the 4-minute mark, reinforcing the structural and compositional similarity between the extracted and standard LPS. However, an additional broader secondary peak was noted between 12 and 13 minutes. This minor peak may represent residual excipients, stabilizing agents, or naturally co-occurring structural variants such as different lengths of the O-antigen polysaccharide. The presence of this secondary signal, while not necessarily indicative of contamination, highlights the inherent complexity of standard preparations and the possible presence of batch-dependent variations in industrial LPS products. In contrast, the third chromatogram, which illustrates the profile of a crude or minimally processed LPS extract, revealed a complex array of peaks dispersed across the full 35-minute runtime. This heterogeneous elution pattern, featuring multiple peaks of varying heights and shapes, reflects the presence of numerous co-extracted biomolecules. Likely contributors to this complex chromatographic signature include proteins, DNA, RNA, lipids, and residual phenol from the extraction process. Such a profile is characteristic of insufficiently purified samples and underscores the need for rigorous processing steps to isolate high-quality LPS. This includes enzymatic treatments (e.g., proteinase K, DNase, and RNase) and multiple organic solvent washes that are essential for removing non-LPS substances that may compromise the functionality or safety of the final product.

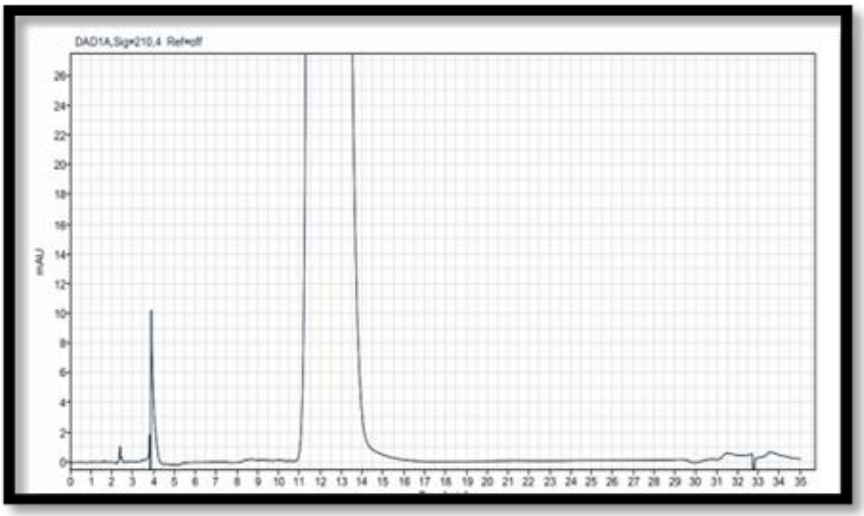

Figure 4. HPLC for *E.coli* standard and sample.

In contrast, the third chromatogram, which depicts a crude or inadequately purified LPS sample, displays multiple peaks dispersed throughout the entire 35-minute run time. This complex and scattered elution pattern, characterized by peaks of varying intensity, reflects the presence of numerous co-extracted substances. These likely include contaminants such as residual proteins, nucleic acid fragments, and phenolic compounds from the extraction process. The intricate nature of this chromatographic profile highlights the critical importance of employing thorough enzymatic treatments and solvent-based purification steps to remove these impurities. Achieving such high purity is essential to ensure that the LPS preparation is of sufficient quality for sensitive downstream biological and immunological applications, where contaminants could interfere with experimental outcomes or clinical efficacy (Heba Hassan 2019).

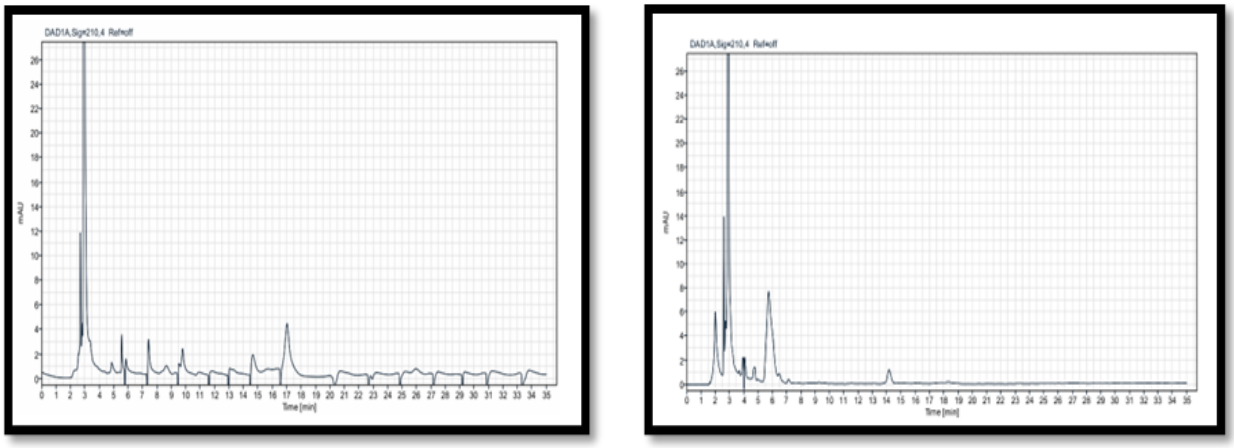

Figure 5. HPLC for *P.aeruginosa* of standard and sample.

CONCLUSION

Among UTIs acquired in the hospital approximately 75% are associated with a urinary catheter. CAUTI is the most common type of HAI, accounting for more than 1 million cases annually. Globally, CAUTI is the main contributor to the grave problems of morbidity and mortality. The primary obstacles to creating novel urinary catheters include the development of biofilms, encrustation, treatment cost, and the emergence of antibiotic resistance in microbes. However, catheterization is associated with an increased rate of infection, and more research trials and prevention are required to reduce the incidence of CAUTI in hospitals and healthcare centers. (Manaf A1-Qahtani, et al*., 2019*). Finally, without any protein or DNA contamination, the LPS was extracted and purified with high purity by using the hot phenol method.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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