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MTT ASSAY TO ASSESS THE EFFICACY OF ANTIMICROBIAL AGENTS AGAINST UPEC ECOLI

Medical Microbiology

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KEYWORDS

Urinary catheters, being the most commonly applied invasive medical devices, quite unsurprisingly also appear among the most common causes of nosocomially acquired infections.Among them, E. coli is the most frequent agent causing about 80% of urinary tract infections in humans, as well as bacteremia associated with Gram-negative bacteria in hospitalized patients .The above bacteria generally form rigid biofilms on both the inner and outer surfaces of implanted catheters, as well as on the epithelium of the urinary tract; in this way, they represent a common cause of chronic infectious diseases(1)Biofilm formation by UPEC is considered a crucial factor in the persistence and recurrence of urinary tract infections. Biofilms can adhere to the surfaces of urinary tract tissues, making them more resistant to antibiotics and the host immune response.Regarding the prevalence of UPEC biofilm formation include:Clinical Isolates, Variability, Association with Recurrent Infections Biofilms can protect bacteria from antibiotics and the host immune response, contributing to the chronic and recurrent nature of some UTIs(5).Methodological Differences Standardization of methods is essential for accurate comparisons.Genetic Factors.Community-acquired UTIs occur in individuals outside of healthcare settings and are often associated with factors such as sexual activity, urinary tract abnormalities, and the use of certain contraceptives. Women are more prone to community-acquired UTIs due to factors like shorter urethral length, proximity of the urethral opening to the anus, and hormonal influences(7). Epidemiologic studies in adults and children over many years have demonstrated that papG are predominantly present in strains causing acute pyelonephritis causing recurrent UTIs in women.The global emergence of antimicrobial resistance (AMR) in uropathogens during the last decade has complicated the UTI treatment. Hence, there is an urgency to develop an alternate treatment strategy. Anti-virulence therapeutic strategies can selectively target uropathogens sparing the commensal bacteria(2)

It should be noted that some conventional antibiotics used conventionally to fight UTIs, such as fluoroquinolones or trimethoprim/ sulphamethoxazole, may no longer be used for empiric treatment due to high resistance rates . Additionally, fluoroquinolones are characterized by significant toxicity, manifested by disorders of the gastrointestinal tract and inflammation of the connective tissue (permanent damage to tendons and cartilage)(3)

In order to assess the effect of the antimicrobials on the viability of the biofilm-embedded cells, bacterial biofilms were grown under static conditions in the BM broth for 48 h at 37 °C, washed, and exposed to 1, 4, 16× MBCs antimicrobials (see MBC values in Table S1) for 24 h, either in the presence or in the absence of Longidaza® (750 IU/mL) in fresh BM. The viability of the cells was assessed by MTT assay [65]. Briefly, the wells were washed twice with 0.9% NaCl to remove non-adherent cells. The MTT solution (1 mg/mL in PBS) was added into the wells with the biofilm, followed by the mechanical removal of the biofilm from the surface and incubation at 33 °C until formazan crystals could be observed in the control (non-treated) wells. Next, the samples were centrifuged for 5 min at 4400 rpm, and the liquid was replaced with dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 15 min at 33 °C to dissolve the formazan crystals. The absorption was measured on a Tecan Infinite 200 Pro at 570 nm.(1)

In order to assess qualitatively the viability of the bacteria in the biofilm and cell-clumps after their exposure to the antimicrobials, the resazurin assay was performed. Briefly, mature biofilms were treated with antibiotics and Longidaza® as described earlier. After 24 h of incubations, the detached cells in the culture fluid were transferred to new plates, harvested by centrifugation for 5 min at 4400 rpm, and resuspended in 160 µL 0.9% NaCl. The biofilms were washed once with 0.9% saline and destroyed mechanically in 160 µL 0.9% NaCl. After that, 40 mL resazurin solution (0.1 mg/mL) was added to the samples and incubated for 15 min at 30 °C until the pink color could be observed in the non-treated samples. The blue color indicated the death of the bacterial cells.(1).Combination of treatment that includes the administration ceragenins (CSAs), will reinforce the effect of antimicrobial LL-37 peptide continuously produced by urinary tract epithelial cells. Such treatment might be an innovative approach to enhance innate antibacterial activity against multidrug-resistant E. coli.(3)the Cri-dots editing strategy to target virulence factor, papG in UPEC, important for adhesion to host. The establishment and application of Cri-dots editing strategy would improve the genetic engineering in bacteria and provide insights for the development of anti-virulence strategy in other bacterial pathogens.(2)

The studies demonstrate that combinations of LL-37 with ceragenins possess the potential to be used as novel strategy to treat UTIs caused by antibiotic-resistant E.coli strains. Extra- and intracellular activity, and importantly, anti-biofilm activity of these combinations appear well suited for treatment of recurrent UTI (3)

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is commonly used to assess cell viability and proliferation. While it is more commonly applied in eukaryotic cell cultures, it can be adapted for certain bacterial studies as well. However, it's essential to note that the MTT assay is primarily designed for eukaryotic cells, and alternative methods may be more suitable for assessingthe efficacy of antimicrobial agents against bacteria.(8,10)

Types (4,7)

Standard MTT Assay for Biofilms:
Grow biofilms in the desired system (e.g., microtiter plates or other biofilm models). Treat biofilms as needed (e.g., with antimicrobial agents or experimental compounds). Add MTT solution to the biofilm and incubate. Solubilize the formazan crystals formed by the viable cells in the biofilm. Measure the absorbance at the appropriate wavelength.

Crystal Violet-MTT Assay:
Grow biofilms in a microtiter plate. Fix the biofilms with methanol. Stain the biofilms with crystal violet to visualize biomass. Add MTT solution to assess metabolic activity. Solubilize the formazan crystals and measure absorbance.

Combination with Other Techniques:
Combine the MTT assay with other biofilm assessment techniques, such as the crystal violet assay or live/dead staining, for a more comprehensive analysis.

Time-Course MTT Assay:
Conduct MTT assays at different time points during biofilm development to observe changes in metabolic activity over time.

Fluorescence-Based MTT Assay:
Use a fluorescence-based MTT assay variant, such as the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) or MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay, for enhanced sensitivity.

Incorporating Enzymatic Treatments:
Introduce enzymatic treatments to assess the impact of biofilm matrix-degrading enzymes on the metabolic activity of biofilm cells.

Procedure (7)
Bacterial Culture:
Grow and maintain your UPEC cultures under standard conditions.

Treatment with Antimicrobial Agents:
Treat the bacterial cultures with varying concentrations of the antimicrobial agents want to test.

Incubation Period:
Allow the treated bacteria to incubate for a specific period.

MTT Addition:
After the incubation, add MTT to the bacterial culture. MTT is typically reduced by mitochondrial enzymes in eukaryotic cells, producing a purple formazan product. In bacteria, this reduction may be less specific.

Incubation of MTT with Bacteria:
Incubate the MTT-treated bacterial cultures under appropriate conditions.

Harvesting and Solubilization:
Harvest the bacterial cells by centrifugation and solubilize the formazan crystals in an appropriate solvent (e.g., DMSO).

Spectrophotometric Measurement:
Measure the absorbance of the solubilized formazan at a specific wavelength using a spectrophotometer. In eukaryotic cell studies, this is usually around 570 nm.

Data Analysis:
Compare the absorbance values of the treated samples with untreated controls. A decrease in absorbance may indicate reduced bacterial viability.

Considerations:
Controls: Include appropriate positive and negative controls for comparison.

Viability vs. Proliferation: Understand that the MTT assay primarily assesses viability, which might be different from the growth inhibition of bacteria.

Interpretation: Interpret results cautiously, considering the differences between bacterial and eukaryotic cell metabolism.

Advantages of MTT Assay in Biofilm Studies:
Metabolic Activity Assessment:
MTT primarily measures the metabolic activity of cells. In biofilms, assessing metabolic activity can be indicative of the overall health and viability of the biofilm.

Non-Destructive:
MTT is a non-destructive assay, allowing for longitudinal studies on the same biofilm. This is particularly useful when monitoring changes in biofilm viability over time.

Quantitative Results:
The MTT assay provides quantitative results, allowing for the comparison of different treatments and conditions.

Versatility:
The MTT assay is versatile and can be adapted for different microorganisms, including bacteria, fungi, and even some types of algae.

Ease of Use:
The MTT assay is relatively simple and can be easily performed in a laboratory setting with basic equipment.

Disadvantages of MTT Assay in Biofilm Studies:
Limited Specificity:
MTT was originally designed for eukaryotic cells, and its use in bacteria, especially in biofilm studies, may lack specificity. The metabolic pathways in bacteria may not produce the same formazan product as in eukaryotic cells, potentially leading to misinterpretation.

Extracellular Matrix Interference:
Biofilms are composed of microbial cells embedded in an extracellular matrix. The matrix components may interfere with the MTT assay, affecting the accuracy of the results.

Heterogeneity:
Biofilms are often heterogeneous, with cells in different metabolic states and locations within the biofilm. MTT measures an overall metabolic activity, but it may not capture the variability present in different regions of the biofilm.

Interference from Matrix Components:
The extracellular polymeric substances (EPS) in biofilms may interfere with the solubilization step of the MTT assay, affecting the ability to accurately measure formazan production.

Limited Information on Biofilm Structure:
While MTT provides information on metabolic activity, it does not offer insights into the structural characteristics of the biofilm, such as thickness, composition, or architecture.

Considerations:
Combining Techniques:
Researchers often use a combination of assays and microscopy techniques to obtain a comprehensive understanding of biofilm properties.

Alternative Methods:
Other assays, such as crystal violet staining, live/dead staining, or quantitative polymerase chain reaction (qPCR) for specific gene expression, may be used in conjunction with or as alternatives to the MTT assay for biofilm studies(5,8)

In conclusion, while the MTT assay has advantages in assessing metabolic activity in biofilms, it should carefully consider its limitations and may need to use it in conjunction with other techniques for a more comprehensive analysis of biofilm properties.The MTT assay has proven to be a valuable tool in assessing the metabolic activity of Uropathogenic Escherichia coli (UPEC) biofilms, shedding light on their viability and response to specific treatments. The study reveals [the impact of antimicrobial agents or experimental compounds on biofilm metabolic activity].

The observed [increases/decreases] in metabolic activity, as indicated by MTT assay results, suggest a dynamic response of UPEC biofilms to [specific conditions/treatments]. These findings emphasize the importance of understanding the metabolic status of biofilm-embedded UPEC cells, which play a crucial role in the persistence and recurrence of urinary tract infections.

Furthermore, the performed in conjunction with the MTT assay corroborate the results and provide a more comprehensive view of UPEC biofilm behavior. The study contribute to the growing body of knowledge regarding UPEC biofilm dynamics.

These insights have implications for the development of targeted therapeutic strategies aimed at disrupting UPEC biofilms. The [identification of potential targets highlighted in the study may pave the way for novel approaches to combat biofilm-associated urinary tract infections, ultimately improving treatment outcomes and reducing the risk of recurrence.

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