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APPLICATIONS OF IMAGING TECHNIQUES IN THE DETECTION OF BIOFILMS AND RESISTANCE MECHANISMS IN PATHOGENIC BACTERIA

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ABSTRACT

Bacterial resistance, a critical public health challenge, is driven by mechanisms such as biofilms, which protect bacteria against antimicrobials, being studied by imaging techniques such as electron microscopy and fluorescence. These tools reveal morphological and structural details, such as cell wall thickening and efflux pumps, aiding in the understanding of the pathophysiology of infections and the development of targeted therapies. The rise of multidrug-resistant bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, highlights the urgency of strategies to contain this global threat.

Keywords: Bacterial resistance. Biofilms.

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INTRODUCTION

The problem of bacterial resistance to antimicrobials is one of the greatest challenges faced by global public health1,2. One of the main mechanisms associated with this resistance is the formation of biofilms, which are complex structures that protect bacteria against antimicrobial agents3,4. The use of advanced imaging techniques, such as scanning electron microscopy and fluorescence microscopy, has allowed a more in-depth study of these biofilms and other structural mechanisms of resistance1,5,6, such as the thickening of cell walls and the presence of efflux pumps6-8. These technologies offer a detailed view of the morphological changes that are linked to resistance, helping to better understand the pathophysiology of bacterial infections and to develop more targeted therapies8. Bacterial resistance was identified shortly after the introduction of antibiotics in the 1940s9,10, when penicillin, which transformed the treatment of infections, began to lose its efficacy due to bacteria that developed defense mechanisms, such as the production of enzymes that neutralize the antibiotic5,11. Since then, the number of multidrug-resistant bacteria has increased, including important pathogens such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa[9,12,13].

Bacterial resistance is seen as a significant threat to global public health14, as it leads to infections that are more difficult to treat, increases morbidity and mortality, and generates a major economic impact due to the need for more prolonged and complex treatments, often resorting to state-of-the-art antibiotics that are less effective and more expensive10, 15,16. The World Health Organization (WHO) points out that, without urgent action, drug-resistant infections could cause more than 10 million deaths per year by 2050, surpassing cancer deaths17,18. In view of this alarming scenario, the study of bacterial resistance has become a public health priority9,11. Understanding the molecular mechanisms of resistance, identifying risk factors for the spread of resistant strains, and developing strategies for the responsible use of antibiotics are fundamental steps to control this threat14. In addition, research into new therapies, including the development of new antimicrobials and alternatives to the use of antibiotics, is crucial to contain the advance of bacterial resistance8,9,11.

OBJECTIVE

This work aims to explore the use of imaging techniques, such as electron microscopy and fluorescence, in the identification and analysis of bacterial biofilms and

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other structural mechanisms associated with antimicrobial resistance. The research aims to demonstrate how these tools contribute to the understanding of the pathophysiology of nosocomial infections and help in the development of more effective therapeutic strategies.

METHODOLOGY

The methodology adopted consisted of a review of scientific literature, focusing on studies that used imaging techniques applied to the analysis of bacterial biofilms and structural mechanisms of antimicrobial resistance. Searches were carried out in databases such as MEDLINE/PUBMED, LILACS, and SciELO, prioritizing studies that addressed biofilm visualizations, cell wall thickening, and the presence of efflux pumps, especially in strains isolated from clinical samples and hospital settings. Specifically, studies that used imaging techniques for the analysis of biofilms, cellular structures and mechanisms associated with antimicrobial resistance observed in bacterial strains isolated from clinical samples were selected. Research that addressed the expression of efflux pumps and changes in the cell wall in the context of hospital infections was also evaluated.

DEVELOPMENT

IMAGING METHODS IN MICROBIOLOGY

Electron and fluorescent microscopy techniques have been shown to be indispensable in the identification and characterization of bacterial biofilms and structures associated with antimicrobial resistance1. Scanning electron microscopy (SEM) allows the visualization of the external morphology of bacteria and the three-dimensional organization of biofilms, evidencing the thickness of the extracellular matrix and the interaction between cells1–4. Transmission electron microscopy (TEM) reveals ultrastructural details of the interior of bacterial cells, including changes in the cell wall, membrane thickening, and accumulation of substances associated with resistance4–6. Studies have shown that pathogens such as Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli form biofilms with complex architectures that hinder the penetration of antibiotics[7]. Biofilms are one of the most common and effective mechanisms of bacterial resistance. Biofilms consist of aggregates of microorganisms adhered to surfaces, surrounded by a polymeric extracellular matrix (EPS) that protects them against antimicrobial agents and the host's immune response.

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Scanning electron microscopy (SEM) is widely used to visualize biofilms, making it possible to observe their three-dimensional organization and the thick layer of EPS that surrounds bacterial cells.[1] Studies indicate that the biofilm structure acts as a physical barrier, limiting antimicrobial penetration and directly contributing to resistance. SEM analysis shows these organized structures and densely protected by extracellular matrix, while TEM complements it by showing internal modifications related to bacterial adaptation in environments of high antimicrobial pressure8,9. In hospital settings, where selective pressure is high, these techniques are essential to investigate the persistence of infections and treatment failure10. Fluorescence microscopy, in turn, offers the advantage of analyzing live cells and structures labeled with specific fluorophores, enabling real-time observation of mechanisms such as the expression of efflux pumps of the RND and MFS[types 11]. These systems are activated in the presence of antibiotics and have been associated with high levels of resistance, especially in chronic nosocomial infections10,12,13. Fluorescence also allows multiple labeling, which facilitates the study of interactions between cellular components and antimicrobial agents10.

The combination of these approaches significantly expands the understanding of the pathophysiology of resistant bacterial infections, allowing not only the identification of biofilms and structural mechanisms of resistance, but also the targeting of more effective therapies14. The ability to correlate morphological data with genetic and functional information contributes to personalized medicine and the development of drugs that act directly on specific structural targets7,15. In addition, studies have shown that the images obtained help to correlate morphological data with genetic and phenotypic information, offering a broader approach to microbiological diagnosis11,16. The combined use of these techniques expands the capacity to detect structural mechanisms of resistance and guides the development of targeted therapies, focusing on the destructuring of biofilms and inhibition of mechanisms such as efflux pumps10,17,18.

These techniques not only broaden the understanding of bacterial biology, but also help identify specific cellular targets for new drugs. By clarifying how microorganisms interact with different compounds, TEM and SEM contribute to the development of effective strategies in the fight against bacterial infections, especially those caused by resistant strains, and thus be the key to addressing the challenges of resistant infections and promoting public health.

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However, both methods have their limitations. Electron microscopy requires complex sample preparation, which can alter the natural morphology of bacteria, and has high operating costs, in addition to the need for specialized equipment19,20. Samples must generally be subjected to a vacuum, limiting the observation of living organisms11. Fluorescence microscopy, on the other hand, has a lower resolution than electron microscopy, which limits the observation of very small structures. In addition, it relies on specific markers that may not be available for all bacteria and may face autofluorescence problems in some samples, interfering with the interpretation of the results21.

IDENTIFICATION OF BACTERIAL RESISTANCE

Bacterial resistance is linked to factors that corroborate the action of antibiotics, which is often a consequence of the misuse of antimicrobials22. In this way, it is determined that bacteria, as the main focus of the organism, develop the ability to survive the action of antibiotics that were previously effective against them. This means that infections that were previously treatable may become difficult or impossible to treat, leading to higher morbidity and mortality rates23. The excessive use of antibiotics, and often inappropriate ones, can cause major problems for humans1. When antibiotics are used inappropriately, such as in viral infections, bacteria are more likely to develop resistance, and many people use antibiotics without a prescription, which contributes to resistance1,13,23,24. One of the most favorable occasions is within the hospital environment, where hospital-acquired infections (HAIs) often involve resistant bacteria due to the intensive use of antibiotics in hospital environments19. Genetic factors may contribute to some bacteria incubating, as they have genes that make them naturally resistant to certain antibiotics25. In addition, the transfer of resistance genes between bacteria can occur, increasing resistance in bacterial populations26,27.

MECHANISMS OF RESISTANCE OBSERVED IN IMAGES

Microbial resistance to antimicrobial agents represents a critical and growing challenge in the areas of microbiology and public health2,3. Several mechanisms are responsible for the ability of microorganisms to resist treatments, among which the formation of biofilms, changes in the structure of the cell wall and the production of enzymes that inactivate antimicrobials stand out. Imaging techniques, such as electron microscopy and fluorescence microscopy, play a crucial role in the visualization and

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elucidation of these resistance mechanisms28. Scanning electron microscopy (SEM) is widely used to visualize biofilms, making it possible to observe their three-dimensional organization and the thick layer of EPS that surrounds bacterial cells. Studies indicate that the biofilm structure acts as a physical barrier, limiting the penetration of antimicrobials and directly contributing to resistance26,27.

In addition, confocal fluorescence microscopy is essential for the dynamic study of biofilms in real time10,29. The use of fluorophores to mark living cells and matrix components allows the visualization of biological processes, such as the formation and maturation of biofilms, as well as the analysis of internal bacterial interactions. Confocal microscopy also reveals heterogeneity within biofilms, showing areas of reduced bacterial growth or the presence of dormant cells, which are particularly resistant to antimicrobial treatments[15]. Changes in the structure of the cell wall constitute another critical mechanism of resistance, especially in Gram-negative bacteria, which have an external barrier of lipopolysaccharides (LPS) that hinders the entry of antimicrobials30. Transmission electron microscopy (TEM) is indispensable for the detailed visualization of cell wall layers, allowing the identification of morphological changes associated with resistance15. Mutations that modify the pores (porins) of the outer membrane can be detected on a nanometric scale, showing a reduction in permeability to antibiotics such as penicillins and cephalosporins31,32.

In Gram-positive bacteria, the thickening of the cell wall, which confers resistance to agents such as vancomycin, can also be evidenced by means of electron microscopy images. These images provide a detailed understanding of the structural changes that hinder the effective action of antimicrobials, revealing crucial adaptations for bacterial survival in hostile environments33. In addition to biofilms and changes in the cell wall, microscopy has made it possible to visualize other resistance mechanisms, such as the presence of efflux pumps and the production of antibiotic-inactivating enzymes[1,15,34]. Fluorescence microscopy allows the identification of the location and activity of these efflux pumps, which act by exporting antimicrobials out of the cell29. The study of their distribution in different regions of the cell membrane is crucial to understand how they contribute to multidrug resistance21,35. Electron microscopy has also been effective in detecting the production of enzymes, such as beta-lactamases, that degrade beta-lactam antibiotics. The observation of enzymatic aggregates and their association with specific cell

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regions is facilitated by the use of markers, offering an accurate and detailed view of this resistance mechanism[28,33,36].

CLINICAL ASPECTS AND FUTURE DIRECTIONS

The use of techniques in the evaluation of immunological diseases is a new phenomenon with great impact today, the options for diagnosis and treatment of these diseases by viruses are more appropriate to culture and technology37. of prevention, which is based on the culture and technology of prevention, which has a significant effect. It can be time-consuming and uneventful in rapidly changing diseases. New technologies that include high-resolution images with artificial intelligence are able to provide a clearer picture of the immune system21. This system can have a direct impact on care, allowing doctors to take care of themselves better and faster. The demonstration of protective mechanisms or the change of bacterial targets can facilitate the selection of treatment methods, avoiding the use of broad-spectrum antibiotics. This not only improves patient outcomes, but also reduces the risk of further attacks due to inappropriate antibiotic use19,20. In addition, the ability to detect sporadic bacterial resistance to antibiotics may allow for improved treatment. For example, the technology can be used to monitor the effects of different combinations or changes in anti-inflammatory drugs, to improve treatment control during severe illnesses or diseases such as clinical infections and osteomyelitis38.

6 FINAL CONSIDERATIONS

While technology for immunological imaging has advanced, there is a need to expand research in many areas. First, more clinical research should be done to validate these methods in different hospitals to increase their effectiveness and feasibility. In addition, it is important that the study investigates the potential of this technology to detect low resistance or new mutations in new diseases. Another area that deserves more attention is the development of image analysis software with artificial intelligence to streamline the image interpretation process. Advanced algorithms can be trained to correctly identify different immune systems, improving diagnostic accuracy and reducing human error. The development of AI technology for the analysis of advanced microscopy images in the clinical setting is important for the future of this method.

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It is also important that future research examines the integration of these new technologies into existing medical systems. The combination of clinical and imaging data can enable rapid and coordinated infection control. Additionally, the development of easy-to-use devices can provide new solutions for real-time diagnostics, especially in remote or hard-to-reach areas. In short, the investigation of antibiotic resistance may be evolutionary. There are immediate results in the development of epidemiology and personal care, while the development of this technology and its contents continues. Imaging techniques are fundamental for the detection and analysis of the structural mechanisms that contribute to bacterial resistance. The identification of biofilms, cell wall changes, and efflux pumps by means of electron and fluorescent microscopy represents a significant advance in the field of clinical microbiology. Such tools allow not only a better understanding of the structural organization associated with resistance, but also the proposition of new therapeutic strategies aimed at the control of resistant infections, especially those sustained by the formation of biofilms.

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