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REVIEW

Challenges and advances in the study of gram-negative bacteria

Desafíos y avances en el estudio de las bacterias gramnegativos

Nairobi Hernández Bridon[1]

[1]Instituto de Investigaciones en Microbiología y Parasitología Médica. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Universidad de Buenos Aires. Ciudad Autónoma de Buenos Aires, Argentina.

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Corresponding author: Nairobi Hernández Bridon

ABSTRACT

Introduction: the categorization of a bacteria as gram-negative responds to the staining and coloring it takes during the biochemical identification process using Gram staining. There have been multiple antecedents in relation to the study of bacteria, especially gram-negative ones.
Objective: describe the challenges and advances in the study of gram-negative bacteria.
Method: in relation to the central theme of this research, a bibliographic review was developed. Search terms and their English equivalents were used. They were applied in search engines such as Google Scholar and databases such as Scielo Regional, PubMed and SCOPUS. We worked with 21 investigations to write this work.
Development: Gram-negative bacteria share similar characteristics in terms of their structure in relation to Gram-positive bacteria. However, its main difference lies in the constitution of the wall. Research in the health sciences, especially in the area of microbiology, focuses mostly on the diagnosis of causal agents and the improvement of procedures for their confirmation. At the same time, they provide invaluable information on the basic characteristics of these agents that allow their identification and those that have changed over time. Every scientific advance finds relevant information, as well as new challenges for researchers. These challenges lay the foundations for new questions and unmask new horizons in search of new information on the topic investigated.
Conclusions: in relation to advances, they have focused on determining the resistance capacities of bacteria, in coordination with their behavior in the environment and how these data could influence therapeutic alternatives. For their part, the challenges contribute to the improvement of the processes, especially from a technological point of view, to guarantee greater focus on the research carried out.

Keywords: Bacteria; Gram-negative Bacteria; Investigation; Health Research; Microbiology.

RESUMEN

Introducción: la categorización de una bacteria como gramnegativa responde a la tinción y coloración que toma durante el proceso bioquímico de identificación mediante la tinción de Gram. Múltiples han sido los antecedentes en relación con el estudio de las bacterias, en especial las gramnegativos.
Objetivo: describir los desafíos y avances en el estudio de las bacterias gramnegativos.
Método: en relación con la temática central de la presente investigación, se desarrolló una revisión bibliográfica. Se utilizaron términos de búsqueda y sus equivalentes en inglés. Fueron aplicados en motores de búsquedas como Google Scholar y bases de datos como Scielo Regional, PubMed y SCOPUS. Se trabajó con 21 investigaciones para la redacción del presente trabajo.
Desarrollo: las bacterias gramnegativos comparten características similares en cuanto a su estructura con

relación a las grampositivas. Sin embargo, su principal diferencia radica en la constitución de la pared. Las investigaciones en las ciencias de la salud, en especial en el área de la microbiología se enfocan en su mayoría en el diagnóstico de los agentes causales y el perfeccionamiento de los procederes para su confirmación. A su vez, aportan información de inestimable sobre las características básicas de estos agentes que permiten su identificación y aquellas que se ha modificado a lo largo del tiempo. Todo avance científico encuentra informaciones relevantes, a la vez que nuevos retos para los investigadores. Estos retos sientan las bases de nuevas interrogantes y desenmascara nuevos horizontes en búsqueda de nueva información sobre el tema investigado.

Conclusiones: en relación con los avances se han enfocado en la determinación de las capacidades de resistencia de las bacterias, en coordinación con el comportamiento de estas en el entorno y como estos datos pudieran influir en las alternativas terapéuticas. Por su parte, los desafíos contribuyen al perfeccionamiento de los procesos, en especial desde el punto de vista tecnológico, para garantizar un mayor enfoque en las investigaciones desarrolladas.

Palabras clave: Bacteria; Bacterias Gramnegativas; Investigación; Investigación en Salud; Microbiología.

INTRODUCTION

The study and interest generated by a particular subject in medicine respond to multiple causes and reasons. However, it is valid to emphasize that the impact on the state of health and the development of therapeutic alternatives (mainly preventive or curative) can be considered the main reason for the undertaking of studies in order to achieve the comparison of a certain pathological process and its resolution. There have been many antecedents to studying bacteria, especially gram-negative bacteria. From the dissimilar discoveries throughout history as the main causal agents of specific infections such as tuberculosis and syphilis, among others, to the determination of the first strains resistant to antibiotics and their different mechanisms of action.[1]

The categorization of a bacterium as gram-negative responds to the staining and coloration it takes during the biochemical process of identification by Gram staining. They stain red due to the structural components of their wall, such as lipopolysaccharides and their spatial arrangement.[2] Bacteria are involved in several infections, often in excess compared to other agents such as fungi and viruses. One of the sepsis that plagues humanity the most, especially newborns, as the age group most affected is neonatal sepsis, which is estimated to be responsible for 400 000 deaths per year and is the third cause in children under five years of age. In the particular case of Mexico, it is estimated that 40 % of confirmed cases of neonatal sepsis result from inoculation by gram-negative bacteria.[3]

Another current interest, of a growing and constant nature, is the development of antimicrobial resistance that biological agents, especially bacteria, have been developing. In this regard, gram-negative bacteria play a crucial role in resistance, as they are one of the main causes of infections that are resistant to treatment. Among the drugs with the highest level of resistance are beta-lactam drugs as a result of the production of enzymes such as beta-lactamases of carbapenem-resistant *Enterobacteriaceae* or producers of extended-spectrum beta-lactamases and the chromosomal cephalosporins of the AmpC type of *Pseudomona aeruginosa*, which creates significant resistance to aminopenicillins, 1st and 2nd generation cephalosporins.[4,5] Since 1996, the development of antimicrobial resistance of gram-negative bacteria has been studied from *Klebsiella pneumoniae* strains and others. Bacteria are responsible for producing antibiotic-degrading enzymes such as *Klebsiella pneumoniae carbapenemase*. One of the first countries to publish the isolation of these enzymes in gram-negative bacteria and to confirm their presence as one of the key elements in the development of their resistance was Colombia, a country where these bacteria occupy one of the first places in infections associated with health systems from the epidemiological point of view.[6]

Healthcare-associated infections have grown considerably. In Europe they reach figures between 5,7 and 7,1 %, in the United States with values around 4,5 %, being higher in low-developed countries that can reach 19,2 %. Gram-negative bacteria are responsible for most of these infections; the following stand out *Acinetobacter baumannii* 12,5 %, *Enterobacter spp.* 10,2 % y *Escherichia coli* 9,6 %.[7]

However, gram-negative bacteria play roles in multiple processes in nature, not only in medicine, where they are both necessary and beneficial to the success of the process and the results. Such is the case of their use as hydrocarbon biodegrades thanks to the particularities of their membranes due to the presence of lipopolysaccharides in their outer membrane, which facilitates the formation and stabilization of emulsions, which in turn, contributes to increasing the contact with lipid contaminants.[8] Undoubtedly, the development of research and medical advances imply an increasing number of future problems to be investigated. The behaviors and therapeutic protocols have renewed the medical arsenal against infections; at the same time, they have opened new scenarios for the development of bacteria; therefore, the constant study of the behavior

of these agents is key to the understanding of the pathophysiological changes they generate and the therapeutic variations that are required. Based on the above, the present review describes the challenges and advances in studying gram-negative bacteria.

METHOD

A bibliographic review of the challenges and advantages of the study of gram-negative bacteria was developed to discuss the central theme of this research. To fulfill the objective, the following terms: Bacteria, Gram-negative Bacteria, Research, Health Research, Microbiology, and their equivalents in English were defined from the descriptors DeCS and MeSH related from the Boolean operators. They were applied to search engines such as Google Scholar and databases such as Scielo Regional, PubMed, and SCOPUS. For the selection of articles or sources to be used in the study, the following were defined as inclusion criteria: works or research available in institutional repositories, articles available in full text, articles published in peer-reviewed journals, and research that provided substantial data of novel interest for the development of the study. Research that did not meet the above criteria was excluded. A total of 27 sources were found; after applying the criteria, 21 research studies were used for writing this paper.

DEVELOPMENT

Microbiology, as a medical specialty, studies the different microorganisms that affect human health. Its field of study contemplates an important group of branches, including bacteriology. One of the main milestones of this science was the development of a stain to differentiate bacteria into two large groups. Known as Gram stain, in honor of the Danish bacteriologist Hans Christian Joachim Gramen, who developed it in 1844, this stain acts on the structural components of the bacterial wall, giving it colors for differentiation: gram-positive for those with a violet color and gram-negative for those with a pink color.[9] Gram-negative bacteria share similar characteristics regarding their structure to gram-positive bacteria. Both have a plasma membrane, cell wall, capsule, flagella, and other elements. However, their main difference lies in the constitution of the wall, which is responsible for their coloration. The wall is 10 nm thick and comprises peptidoglycan or murein, periplasmic space, outer membrane, proteins, and lipopolysaccharides. They can be divided into strict aerobic bacteria, strict anaerobic bacteria, and facultative anaerobic bacteria.[9]

Advances in the study of gram-negative bacteria

Research in the health sciences, especially in the area of microbiology, is mostly focused on the diagnosis of causative agents and the improvement of procedures for their confirmation. At the same time, they provide invaluable information on the basic characteristics of these agents that allow their identification and those that have been modified over time. Results and information necessary for the adequacy of therapeutic alternatives. Significant progress has been made in the diagnosis of these agents. These approaches have made it possible to identify the bacteria with the greatest impact on nosocomial and out-of-hospital infections. In turn, it is important to identify those strains with a high capacity for resistance to certain antimicrobials. In the study by Soria-Segarra et al.[10] on infections in patients colonized by gram-negative bacteria with resistance to carbapenems in a medium-sized Spanish city, a predominance of A. baumannii was found, followed by *Enterobacterales, S. maltophilia and P. aeruginosa*, with a gradual increase in the presence of these bacteria by an average of 9 to 12 % approximately per year.

Despite possible false positives in microbiological studies, especially from bacteriological and epidemiological points of view, it is valid to recognize that there are correlations between data and methods that help to solve and consolidate the data shown. An example of the above is the results shown by Soria-Segarra[11] when purchasing two commercial methods for the determination of the minimum inhibitory concentrations of meropenen against bacteria of the genus *Klebsiella pneumoniae* producing carbapenemase type KPC.

The study of bacteria should not only be based on their impact on human health. It should be taken into account that they are living systems in close relationship with the environment and that any alteration of the environment can negatively influence the balance and trigger endemic, epidemic, or pandemic diseases. This is the approach taken by the epidemiology of diseases. Based on the above, the results shown by Barroso González et al.[12] can be considered as advances in the study of gram-negative bacteria. The author and his work team focused on studying the production of antibiotic-resistant enzymes in freshwater ecosystem bacteria in Havana province. To the data shown by the authors, 65 % of the isolated strains of *Escherichia Coli* were resistant to at least one antibiotic, and only 35 % were sensitive to the drugs used for sensitivity tests (families of tetracyclines, quinolones, aminoglycosides, and macrolides). In correlation with the data shown about the antimicrobial resistance capacity of *Escherichia coli*, it is valid to recognize that there are studies similar to the previous one.

One was developed by the author Quiñones Pérez et al.[13] Their research focused on the study of 119 strains of this agent isolated in different samples from 30 hospitals in the country. From them, it could be evidenced

that 23,5 % of the samples corroborate the presence of this pathogen in surgical wound infections, followed by 20,7 % with bacteremia and 17,6 % with respiratory infections. With a predominance of beta-lactam resistance between 61,3 % and 89,1 %. At the same time, 79,8 % and 80,5 % of the isolates were resistant to trimethoprim/sulfamethoxazole and tetracycline, respectively. Based on the above data, this bacterium can be considered one of the bacteria with the highest incidence of infections, especially those associated with health care. The assistance and research component is one of the basic functions all health personnel should develop in their profession. In this sense, there is a close relationship between the results shown in research and their repercussions in updating and adapting diagnostic and therapeutic protocols, commonly known as evidence-based medicine.

Determination from field studies or research using advanced technologies shows increasingly overwhelming results on the high resistance capacity of gram-negative bacteria. For this reason, it is necessary to search for new therapeutic alternatives to counteract the influence and incidence of this phenomenon in health indicators.

Juárez-Cortés et al.[14], in their studies, analyze the application of phage endolysins as one of the therapeutic variants for the treatment of infections mediated by gram-negative bacteria. Endolysins, also known as "phage lytic enzymes," are murein hydrolases encoded by bacteriophages in the final stage of the infection cycle. The function of endolysins is to break down and weaken this wall. The process begins when endolysins are synthesized and accumulated in the cytoplasm. This process is achieved by three well-coordinated mechanisms: holin-endolysin system, translocation, and anchoring and release by pinholes.

Using antibiotic therapeutic niches by combining two or more drugs has been a widely spread and approved practice for treating resistant bacteria. The narrative review developed by Muñoz Angulo et al.[15] on the usefulness of the therapeutic niche of ceftazidime-avibactam shows encouraging results in its application. However, it specifies the importance and the need to continue developing studies on this subject. Other therapeutic alternatives developed are based on natural and traditional medicine applications. Such is the case of the research developed by Carvajal León[16] on the antimicrobial activity of garlic aqueous extract against some gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa,* and *P. fluorescens.*

The results show that the aqueous garlic extract is effective against *Escherichia coli* and *Pseudomonas fluorescens* strains, unlike *Pseudomonas aeruginosa*. Each of these studies focused on therapeutic alternatives, which opens new horizons about the different therapeutic behaviors that can be developed against the antimicrobial resistance generated by this group of bacteria. For this reason, the authors consider that both fields should be correlated to achieve results of greater scientific relevance due to their practical approach, as one is the counterpart of the other; for this, it is necessary to develop treatment protocols.

One of the protocols and treatment categories is the Highly Resistant Microorganisms system, developed by the Dutch Society for Medical Microbiology. Through this system, vital attention should be paid to bacteria that meet the following characteristics: being etiological agents, having an AMR pattern that makes (empirical) therapy difficult, and having the potential to spread if additional measures beyond standard precautions are not taken. Especially when managing severe infections in patients admitted to Intensive Care Units.[17]

Challenges in the study of gram-negative bacteria

Every scientific advance finds relevant information and new challenges for researchers. These challenges lay the foundations for new questions and unmask new horizons in searching for new information on the subject under investigation. There is still much ground to be covered in the study of gram-negative bacteria. One of the main areas where the study of gram-negative bacteria is focused is their high capacity for antimicrobial resistance. There is a wide range of mechanisms for developing antibiotic resistance; however, the production of ß-lactamase enzymes is one of the best-known and greatest interests to researchers.

Particularly noteworthy are the ß-lactamase-type enzymes AmpC serine ß-lactamases belonging to group 1 of the Bush-Jacoby-Medeiros classification, which occur naturally or inducibly in this bacterial group.[18] Although studies such as that of Mora Moreno et al.[19] on the determination of these enzymes in gram-negative bacteria in a third-level hospital in Colombia show that 57,6 % of the variants analyzed were positive for the presence of these enzymes that provide the ability to be resistant to a considerable group of antibiotics. However, developing these tests requires specialized equipment and a high economic investment that hinders their application in routine medical practice.[20]

In correlation with the data previously mentioned and about the existing challenges in the study of the characteristics of this group of bacteria, Soria-Segarra et al.[11] recognize in the introduction of their work that the technical methods dedicated to the study of the resistance capacity of gram-negative bacteria can imply problems in the precision and emission of their results; for this reason, it is indispensable to continue the study in this area. Although the usefulness of field studies with a high epidemiological focus for the analysis of gram-negative bacteria has been expressed in previous paragraphs, certain limitations hinder the complete development of certain investigations. One of the areas investigated is related to the presence of gram-

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negative bacteria on the screen of cell phones. However, Alvarado-Garcia et al.[21] recognize that studies on this subject are scarce. Most of them highlight that the bacterial endowment on cell phone screens mainly differs according to the profession, highlighting that health personnel are at greater risk. It should be recognized that these studies are of considerable importance due to the frequent use of mobile devices. In addition, due to their close relationship and proximity to certain entrance doors of the organism, such as the oral cavity and the auricular pavilion. Likewise, they frequently come into contact with hands in different scenarios, making them carriers of infections.

CONCLUSIONS

The challenges and advances in the study of gram-negative bacteria are varied, and there is a direct and reciprocal relationship between them. About the advances, they have focused on determining the resistance capabilities of bacteria, in coordination with their behavior in the environment, and how these data could influence therapeutic alternatives. On the other hand, the challenges contribute to improving the processes, especially from the technological point of view, to guarantee a greater focus on the research developed.

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The authors declare that there is no conflict of interest.

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Drafting - original draft: Nairobi Hernández Bridon.
Writing - proofreading and editing: Nairobi Hernández Bridon.

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